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Ocena ryzyka w zastosowaniu biowęgla do gleb w kontekście trwałości i biodostępności WWA w glebach

Rozprawa doktorska
wykonana w Katedrze Radiochemii i Chemii Środowiskowej
pod kierunkiem prof. dr hab. Patryka Oleszczuka

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Spis treści

Wykaz stosowanych skrótów

AC	Węgiel aktywny
BC	Biowęgiel z osadów ściekowych
BCW	Biowęgiel z osadów ściekowych i wikliny
C_{acc}	Zawartość potencjalnie biodostępnej frakcji zanieczyszczeń
CEC	Pojemność kationowymienna (ang. *cation exchange capacity*)
C_{free}	Zawartość biodostępnej frakcji zanieczyszczeń
C_{tot}	Całkowita zawartość zanieczyszczeń
d	Średnica porów
DMF	Dimetyloformamid
DOC	Rozpuszczalny węgiel organiczny (ang. *dissolved organic carbon*)
EI	Jonizacja elektronów
GC-MS	Chromatograf gazowy sprzężony ze spektrometrem mas
GI	Zahamowanie kiełkowania (ang. *germination inhibition*)
HPCD	(2-hydroksypropylo)-β-cyklodekstryna
PHE	Fenantren (ang. *phenanthrene*)
POM	Polioksymetylen
POPs	Trwałe zanieczyszczenia organiczne (ang. *persistent organic pollutants*)
PYR	Piren (ang. *pyrene*)
RGI	Zahamowanie wzrostu korzenia (ang. *root growth inhibition*)
SIM	Tryb monitorowania wybranych jonów podczas analizy GC-MS
SL	Osad ściekowy (ang. s*ewage sludge*)
TOC	Węgiel organiczny (ang. *total organic carbon*)
TTB	1,3,5-tri-*tert*-butylbenzen
V_{tot}	Całkowita objętość porów
V_{macro}	Objętość makroporów (o średnicy większej niż 50 nm)
V_{micro}	Objętość mikroporów (o średnicy mniejszej niż 2 nm)
WWA	Wielopierścieniowe węglowodory aromatyczne

1. Publikacje będące przedmiotem rozprawy

D1 P. Oleszczuk, **P. Godlewska**, D. D. Reible, P. Kraska, Bioaccessibility of polycyclic aromatic hydrocarbons in activted carbon or biochar amended vegetated (*Salix viminalis*) soil, *Environmental Pollution* 227 (2017) 406-413. (**$IF_{5\text{-letni}}$: 10,366; MEiN: 100 pkt.**)

D2 P. Oleszczuk, M. Rakowska, T. D. Bucheli, **P. Godlewska**, D. D. Reible, Combined effects of plant cultivation and sorbing carbon amendments on freely dissolved PAHs in contaminated soil, *Environmental Science and Technology* 53 (2019) 4860-4868. (**$IF_{5\text{-letni}}$: 8,827; MEiN: 140 pkt.**)

D3 **P. Godlewska**, A. Siatecka, M. Kończak, P. Oleszczuk, Adsorption capacity of phenanthrene and pyrene to engineered carbon-based adsorbents produced from sewage sludge or sewage sludge-biomass mixture in various gaseous conditions, *Bioresource Technology* 280 (2019) 421-429. (**$IF_{5\text{-letni}}$: 9,658; MEiN: 140 pkt.**)

D4 **P. Godlewska,** Y.S. Ok, P. Oleszczuk, The dark side of black gold: Ecotoxicological aspects of biochar and biochar-amended soils, *Journal of Hazardous Materials* 403 (2021) 123833. (**$IF_{5\text{-letni}}$: 12,505; MEiN: 200 pkt.)**

D5 **P. Godlewska,** P. Oleszczuk, Effect of biomass addition before sewage sludge pyrolysis on the persistence and bioavailability of polycyclic aromatic hydrocarbons in biochar-amended soil, *Chemical Engineering Journal* 429 (2022) 132143. (**$IF_{5\text{-letni}}$ = 14,66; MEiN: 200 pkt.)**

D6 **P. Godlewska,** I. Jośko, P. Oleszczuk, Ecotoxicity of sewage sludge- or sewage sludge/willow-derived biochar-amended soil, *Environmental Pollution* 305 (2022) 119235. **($IF_{5\text{-letni}}$ = 9,988; MEiN: 100 pkt.)**

D7 **P. Godlewska**, P. Oleszczuk, Effect of carrier gas during sewage sludge or sewage sludge-willow co-pyrolysis on the persistence and bioavailability of polycyclic aromatic hydrocarbons in biochar-amended soil, *Environmental Pollution* 314 (2022) 120145. **($IF_{5\text{-letni}}$ = 10,366; MEiN: 100 pkt.)**

D8 **P. Godlewska**, M. Kończak, P. Oleszczuk, Effect of carrier gas change during sewage sludge or sewage sludge and willow pyrolysis on ecotoxicity of biochar-amended soil, *Ecotoxicology and Environmental Safety* 247 (2022) 114224. (**$IF_{5\text{-letni}}$ = 6,68; MEiN: 100 pkt.**)

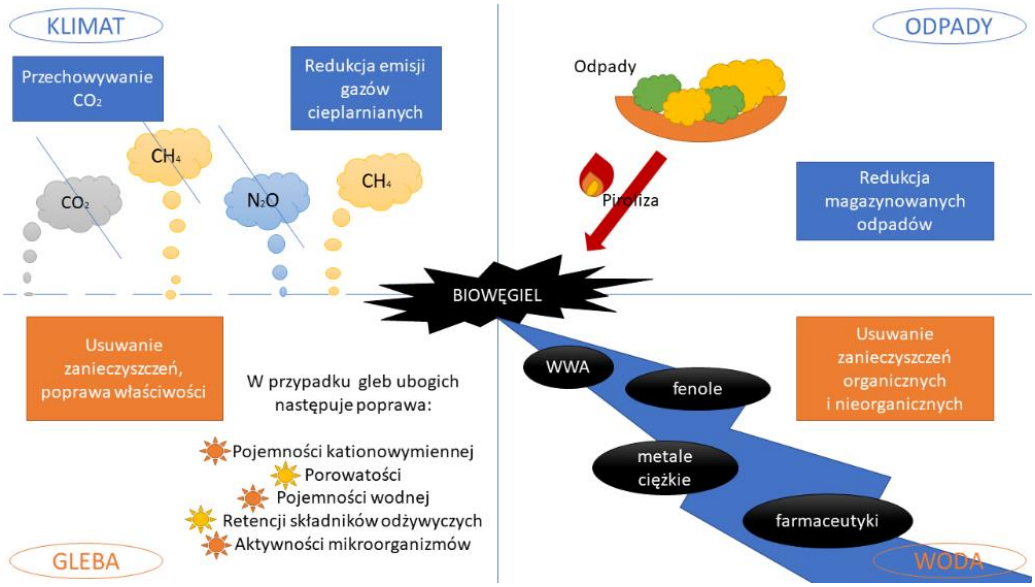
Sumaryczny IF: 83,05

Sumaryczna liczba punktów MEiN: 1080

2. Aktualny stan wiedzy

2.1. Wprowadzenie

Biowęgiel jest stałym produktem pirolizy biomasy lub innych substancji organicznych [1] w temperaturze <1000°C przy braku lub niewielkiej dostępności tlenu [2,3]. Do otrzymywania biowęgla mogą być również używane odpadowe części roślin, odpady odzwierzęce czy nawet przemysłowe [1]. Struktura biowęgla jest porowata, a w składzie znajduje się głównie węgiel. Biowęgiel może **(Rys. 1)**: (1) pełnić rolę adsorbenta zanieczyszczeń [1–3], (2) poprawiać gleby słabej jakości (wpływając na ich, istotne dla rolnictwa, właściwości, takie jak pH, pojemność wodna, pojemność kationowymienna i struktura populacji mikrobiologicznej) [2,4], a także (3) wiązać węgiel w glebie w ujęciu długoterminowym, co przyczynia się do obniżenia stężenia ditlenku węgla (CO_2) w atmosferze [2].



Rysunek 1. Wpływ biowęgla na różne aspekty ochrony środowiska

Poprzez adsorpcję różnorodnych zanieczyszczeń (organicznych i nieorganicznych) i jednoczesną poprawę właściwości fizycznych, chemicznych i biologicznych gleb, biowęgiel może być doskonałym materiałem stosowanym w remediacji gleb zanieczyszczonych zarówno związkami organicznymi, jak i nieorganicznymi [5]. Efektywność adsorpcji danej grupy zanieczyszczeń przez biowęgiel zmienia się w zależności od jego właściwości, które determinowane są głównie warunkami pirolizy (temperatura, gaz nośny i czas pirolizy), użytym surowcem i ewentualnymi dalszymi czynnościami mającymi na celu zmianę właściwości

biowęgla zwanymi modyfikacją lub aktywacją [3,6]. Podobnie zróżnicowany jest wpływ biowęgla na fizyko-chemiczne oraz mikrobiologiczne właściwości gleb [7]. Szczególnie gleby ubogie w składniki odżywcze są podatne na znaczącą poprawę właściwości i zwiększenie plonowania dzięki nawożeniu biowęglem [8].

Obok „tej pozytywnej", biowęgiel ma również „drugą twarz", przez którą nie pozostaje bez wad. Największą z nich jest obecność w biowęglu substancji toksycznych. Substancje te można podzielić ze względu na pochodzenie, tzn. tworzące się w trakcie pirolizy oraz obecne w surowcu użytym do otrzymania biowęgla. Zanieczyszczenia powstające podczas pirolizy to m.in. wielopierścieniowe węglowodory aromatyczne (WWA) [9,10], lotne zanieczyszczenia organiczne (głównie kwasy: octowy, mrówkowy, masłowy i propionowy oraz metanol, fenole i krezole [11,12]) oraz dioksyny i furany [9,13]. W tym ostatnim przypadku niezbędna jest obecność chloru w pirolizowanym surowcu. Drugą grupę mogą reprezentować metale ciężkie [14] oraz związki perfluorowane [15], znajdujące się w materiale zarówno przed, jak i po procesie pirolizy.

Zawartość zanieczyszczeń w biowęglu nie jest jednak jedynym powodem, dla którego może on być toksyczny dla organizmów. Biowęgiel to materiał, który zasadniczo wpływa na warunki środowiska (np. właściwości gleby). Zmiany, które biowęgiel wprowadza we właściwościach fizycznych, chemicznych lub biologicznych gleb mogą pośrednio wywierać szkodliwy wypływ na organizmy. Negatywne działanie biowęgla na organizmy nie musi więc wynikać bezpośrednio z jego szkodliwego działania bądź obecności w nim szkodliwych zanieczyszczeń, ale może być związane ze zmianą warunków środowiska, które dla niektórych organizmów są niekorzystne (np. zwiększenie pH, obniżenie dostępności składników odżywczych, itp.). Ponadto biowęgiel pod wpływem warunków środowiskowych z czasem podlega zmianom chemicznym, fizycznym i biologicznym [16–18], które również mogą wpływać na jego toksyczność w stosunku do różnych organizmów [4]. Z jednej strony wpływ ten może być związany ze zmniejszeniem się powinowactwa zanieczyszczeń do biowęgla w wyniku tzw. starzenia biowęgla. Na skutek tego procesu uwolnione z biowęgla zanieczyszczenia stają się toksyczne dla organizmów. Z drugiej jednak strony właściwości biowęgla mogą ulegać zmianom, które wpływają na właściwości gleb, a to niekorzystnie oddziałuje na organizmy. Ocena bezpieczeństwa stosowania biowęgla, jak widać z przedstawionych powyżej informacji, jest procesem bardzo złożonym, który wymaga uwzględnienia wielu czynników zarówno środowiskowych, jak i związanych z właściwościami biowęgla.

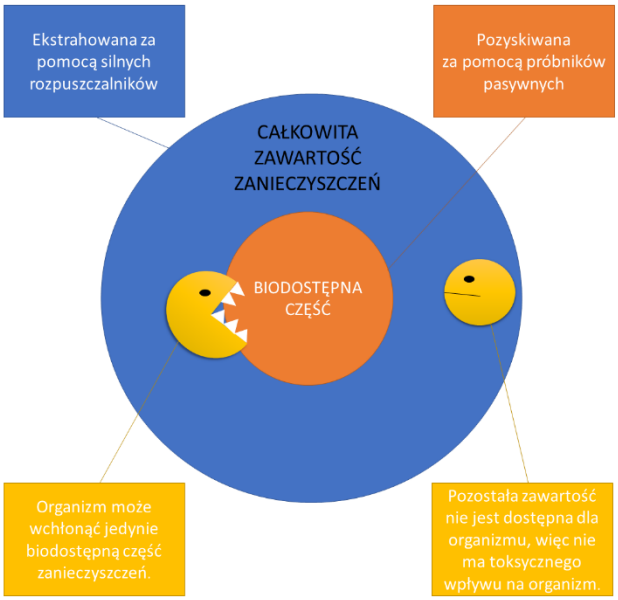
2.2. Biowęgiel z osadów ściekowych

Ilość otrzymywanego rocznie osadu ściekowego (SL), czyli pozostałości po oczyszczaniu ścieków, rośnie. W Polsce odnotowano, że w 2018 roku wytworzono 583 tys. ton tego typu odpadu [19], czyli o 23% więcej w porównaniu z rokiem 2003. Ze względu na objętość, odory oraz substancje toksyczne, osad ściekowy jest uciążliwy w składowaniu. Z drugiej jednak strony osad ściekowy może posiadać potencjalne zastosowanie w rolnictwie, związane z zawartością w nim składników odżywczych (zwłaszcza węgla, azotu i fosforu) [20]. Ze względu na właściwości nawozowe osad ściekowy może być również używany do rekultywacji gleb zdegradowanych [21–23]. Bezpośrednie stosowanie osadu ściekowego na pola uprawne jest jednak ryzykowne, ponieważ wiąże się z wprowadzeniem do gleb zarówno szkodliwych substancji, takich jak metale ciężkie oraz zanieczyszczenia organiczne (np. WWA, farmaceutyki, związki wpływające na układ hormonalny człowieka), jak i materiału biologicznego (np. jaja pasożytów czy nasiona chwastów) [24]. Przekształcenie osadów ściekowych do biowęgla w procesie pirolizy może eliminować niektóre z powyższych jego wad [14,25–30].

Piroliza osadu ściekowego do biowęgla może obniżyć biodostępność metali ciężkich oraz innych zanieczyszczeń i zmniejszyć objętość odpadów [14], jednak biowęgiel otrzymany z osadu posiada niezadowalające właściwości (np. w porównaniu do biowęgli otrzymanych z biomasy roślinnej). Dla przykładu, biowęgiel z osadu ściekowego posiada zwykle, małą powierzchnię właściwą i niską zawartość węgla oraz wysoką zawartość frakcji mineralnej [31]. W celu poprawy właściwości biowęgla proponowane są rozwiązania polegające na pirolizie osadu ściekowego z biomasą lignocelulozową [20,32–34]. Współpiroliza osadu ściekowego z biomasą zmniejsza zawartość WWA i metali ciężkich w otrzymanym biowęglu oraz zwiększa jego pojemność kationowymienną i powierzchnię właściwą [35,36]. Inne działanie, która może wpłynąć na poprawę właściwości biowęgla to zmiana gazu nośnego podczas pirolizy ze standardowo używanego azotu (N_2) na ditlenek węgla (CO_2). Badania dowodzą, że biowęgiel z SL otrzymany w atmosferze CO_2 posiada bardziej rozwiniętą strukturę porów, tzn. pory o bardziej zróżnicowanym kształcie i zwiększonej powierzchni, oraz niższe zasolenie i pH [36], co może istotnie zmniejszyć negatywne oddziaływanie biowęgla na środowisko.

2.3. Biodostępność wielopierścieniowych węglowodorów aromatycznych

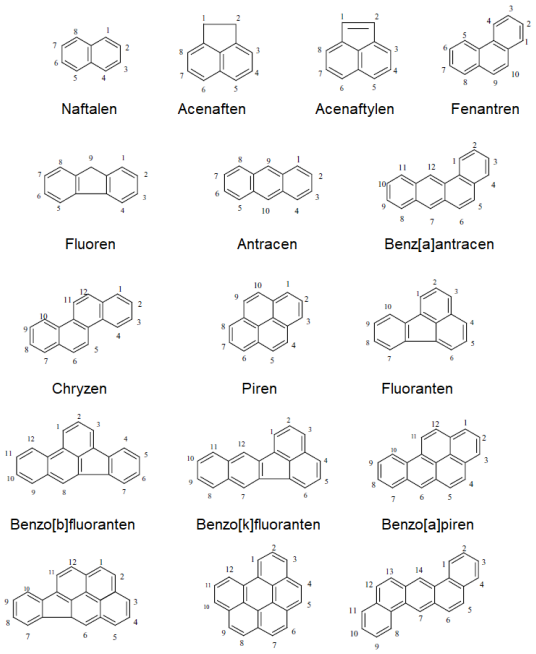
Istotnym zagadnieniem odnoszącym się do obecności zanieczyszczeń w różnych matrycach środowiskowych (gleba, osad denny) lub antropogenicznych matrycach środowiskowych (osad ściekowy, biowęgiel) jest ich dostępność dla organizmów, a przez to potencjalny efekt toksyczny [13,37,38]. Stężenie całkowite zanieczyszczeń w matrycy nie jest bowiem jednoznaczne z zawartością, która może wywołać efekt toksyczny [39]. Część zanieczyszczeń jest na tyle mocno związana z matrycą, że nie może zostać pobrana i wchłonięta przez rośliny, mikroorganizmy lub zwierzęta. Przez to efekt toksyczny pochodzący od tej frakcji danego zanieczyszczenia jest marginalny. Tylko ta frakcja zanieczyszczenia, w przypadku której istnieje możliwość przeniknięcia do organizmu, może wywołać efekt toksyczny. Dlatego też coraz częściej oprócz frakcji całkowitej (ekstrahowanej przy pomocy silnych ekstrahentów organicznych) **(Rys. 2)** oznaczana jest frakcja biodostępna (ang. *bioavailable*) zanieczyszczeń [9,38,40]. Frakcja ta jest bezpośrednio dostępna dla organizmów.



Rysunek 2. Całkowita i biodostępna część zanieczyszczeń

WWA są najczęściej występującym zanieczyszczeniem w biowęglu [10,37]. WWA posiadają 2 lub więcej skondensowanych pierścieni aromatycznych w cząsteczce **(Rys. 3)**. WWA powstają podczas pirolizy, na skutek aromatyzacji i karbonizacji materii organicznej, a także poprzez przyłączanie rodników węglowodorowych i syntezę w cięższe aromatyczne cząsteczki [10]. WWA zaliczane są do trwałych zanieczyszczeń organicznych (ang. *persistent organic pollutants*, POPs) [41] oraz posiadają właściwości kancerogenne, mutagenne i teratogenne [10].

Na zawartość WWA w biowęglu ma wpływ szereg czynników związanych z procesem przekształcania materii organicznej w biowęgiel, takich jak czas przebywania substratów w piecu, szybkość nagrzewania pieca i temperatura pirolizy [10]. Badania pokazują, że podczas wolnej pirolizy (ang. *slow pyrolysis*) otrzymywane są biowęgle o niższej zawartości WWA niż podczas tzw. szybkiej pirolizy (gdy czas przebywania substratów w piecu wynosi poniżej kilku sekund) [10]. Zakłada się [10], że piroliza w zakresie temperatur 400-500°C prowadzi do otrzymania biowęgla o większej zawartości WWA niż w procesie niskotemperaturowym (<400°C) lub wysokotemperaturowym (>500°C). Wyniki pojedynczych badań są jednak często sprzeczne z tym założeniem [10].



Rysunek 3. Wielopierścieniowe węglowodory aromatyczne z listy Agencji Ochrony Środowiska Stanów Zjednoczonych (US EPA)

Na biodostępność WWA w glebie mają wpływ ich stężenie, zawartość innych zanieczyszczeń, rodzaj gleby (zwłaszcza w kontekście zawartości materii organicznej) oraz warunki środowiskowe (pH, temperatura, wilgotność). Ze względu na dużą powierzchnię właściwą oraz aromatyczny charakter powierzchni (a więc silne zdolności sorpcyjne) zakłada się, że biowęgiel dodany do gleby nie zwiększa biodostępnej zawartości WWA w glebie, ale wręcz tę zawartość obniża. Dochodzi do tego nawet jeżeli całkowita zawartość WWA ulega zwiększeniu. Badania dotyczące zawartości frakcji biodostępnej w glebach z dodatkiem biowęgla są nieliczne w porównaniu do badań dotyczących całkowitej zawartości WWA [42–44].

2.4. Testy ekotoksykologiczne a biowęgiel

Wpływ biowęgla na toksyczność gleby może wynikać z bezpośredniego oddziaływania zanieczyszczeń zawartych w biowęglu na organizmy [3,7,30], ale również z pośredniego jego oddziaływania poprzez zmianę pewnych parametrów gleby [4]. W tym kontekście istotne jest przeprowadzenie testów z udziałem organizmów (mikroorganizmów, roślin, stawonogów czy skąposzczetów) [46], które w sposób kompleksowy zobrazują potencjalne ryzyko [47],

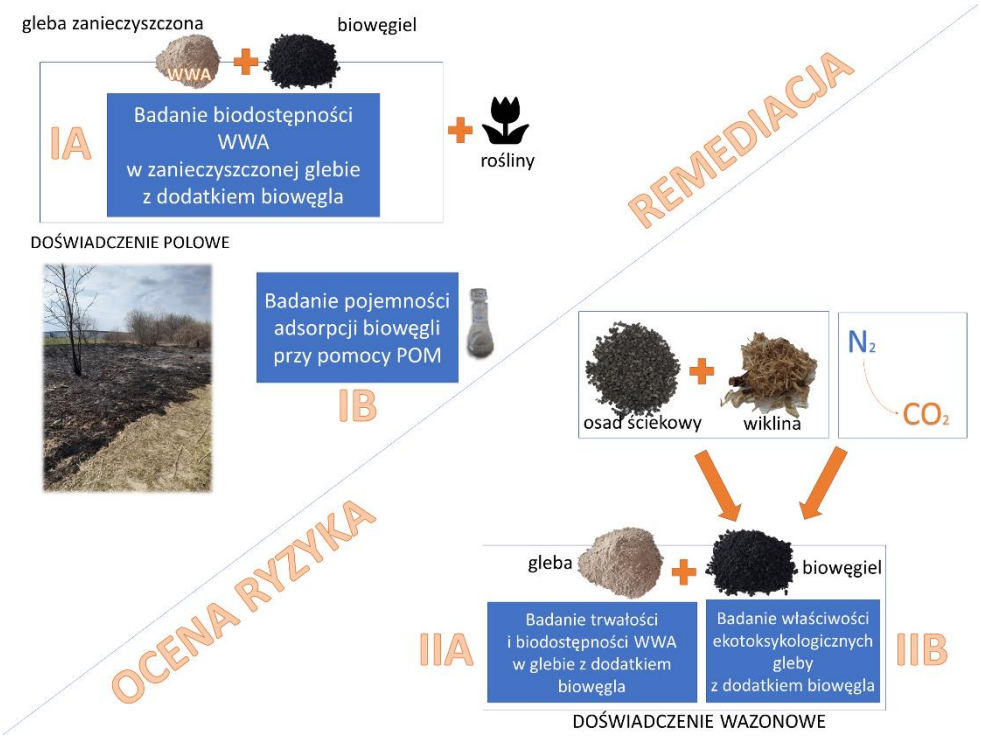
a poprzez zastosowanie metod statystycznych pozwolą na wskazanie potencjalnego czynnika odpowiedzialnego za efekt toksyczny. Badania obejmujące odpowiedź różnych grup troficznych są najbardziej pożądane ze względu na różną wrażliwość gatunków testowych. Ryzyko związane z wykorzystaniem biowęgla w kontekście zawartości zanieczyszczeń oraz toksyczności przedstawiono w publikacji przeglądowej opublikowanej w ramach cyklu prac wchodzących w skład rozprawy doktorskiej (Publikacja D4), której ogólne streszczenie zostało zawarte poniżej.

P. Godlewska, Y.S. Ok, P. Oleszczuk, *The dark side of black gold: Ecotoxicological aspects of biochar and biochar-amended soils*, Journal of Hazardous Materials 403 (2021) 123833.

Biowęgiel, jako produkt pirolizy biomasy, charakteryzuje się znaczną powierzchnią, porowatością, wysoką pojemnością wodną i trwałością środowiskową. Jest postrzegany jako materiał, który przeciwdziała zmianom klimatu ze względu na stabilność węgla w nim zawartego oraz jest uważany za odpowiedni do poprawy gleby (nawożenie i rekultywacja). Jednakże biowęgiel może mieć toksyczny wpływ na organizmy ze względu na zawarte w nim substancje szkodliwe i ich potencjalne działanie negatywne w stosunku do organizmów z różnych poziomów troficznych. W pracy opisano wpływ biowęgla na zawartość i toksyczność substancji szkodliwych w glebie z jego dodatkiem. Dodatek biowęgla do gleby zwykle nie ma toksycznego wpływu i stymuluje rośliny, bakterie i bezkręgowce. Ostateczny efekt zależy jednak od rodzaju biowęgla (surowca do produkcji i warunków pirolizy) oraz zawartości zanieczyszczeń. Za toksyczność biowęgla zazwyczaj odpowiadają pH, wysokie zasolenie, zawartość WWA i metali ciężkich.

3. Cel i zakres badań

Przeprowadzone badania będące podstawą rozprawy doktorskiej składały się z dwóch głównych etapów **(Rys. 4).** Pierwszy etap miał na celu analizę potencjału biowęgla i węgla aktywnego (AC), jako materiałów wykorzystywanych do remediacji gleby zanieczyszczonej przez wielopierścieniowe węglowodory aromatyczne (WWA). W drugim etapie oceniano ryzyko wprowadzania biowęgla (otrzymanego w różnych warunkach) do gleb w kontekście zawartości w nim WWA.



Rysunek 4. Schemat przedstawiający doświadczenia i badania opisywane w rozprawie doktorskiej

W ramach przeprowadzonych badań założono następujące hipotezy badawcze:

1) Biowęgiel lub węgiel aktywny obniży biodostępność (C_{free}) WWA w zanieczyszczonej glebie (D1),
2) Różne gatunki roślin w połączeniu z biowęglem lub węglem aktywnym będą miały zróżnicowany wpływ na straty C_{free} WWA w glebach zanieczyszczonych tymi związkami (D2),

3) Dodatek wikliny do osadu ściekowego podczas pirolizy zwiększy zawartość węgla oraz obniży zawartość frakcji mineralnej, co wpłynie na poprawę zdolności adsorpcyjnych otrzymanego biowęgla w stosunku do WWA (D3),
4) Zwiększenie pola powierzchni w wyniku zamiany gazu nośnego stosowanego podczas pirolizy z N_2 na CO_2 zwiększy powinowactwo biowęgli do WWA (D3).
5) Aplikacja biowęgli z osadu ściekowego współpirolizowanych z biomasą lub otrzymanych w CO_2 zwiększy trwałość i obniży biodostępność WWA w glebie w porównaniu do biowęgli z samego osadu ściekowego lub otrzymanych w N_2 (D5 i D7).
6) Aplikacja biowęgli z osadu ściekowego współpirolizowanych z biomasą lub otrzymanych w CO_2 w wyniku obniżenia C_{free} WWA (hipoteza 5) zmniejszy toksyczność gleby w porównaniu do zastosowania biowęgli z samego osadu ściekowego lub otrzymanych w N_2 (D6 i D8).

4. Ogólny opis metod zastosowanych w badaniach

4.1. Doświadczenie polowe

Doświadczenie polowe (Część I pracy: remediacja) realizowano w stacji doświadczalnej Bezek zlokalizowanej w województwie lubelskim (51º11'49.7"N 23º15'01.2"E). Poletka o wymiarach 2 m x 2 m x 0,2 m wypełniono zanieczyszczoną glebą (kontrola) lub zanieczyszczoną glebą z dodatkami (AC, biowęgiel) w ilości 2% w/w. Dawkę wybrano na podstawie wcześniejszych badań, w których uzyskano najlepszą efektywność immobilizacji zanieczyszczeń po zastosowaniu biowęgla lub węgla aktywnego [48]. Zanieczyszczona gleba pochodziła z terenu koksowni „*Przyjaźń*" znajdującej się w Dąbrowie Górniczej (50º33'99.5"N 19º33'16.1"E). Na tak przygotowanym podłożu wysiano lub posadzono następujące rośliny: wiklina, mieszanka traw i koniczyna. Kontrolę w stosunku do eksperymentu z roślinami stanowiła gleba bez uprawy. Próbki pobierano na początku eksperymentu (0 dni), a następnie po 3, 6, 12 i 18 miesiącach. Doświadczenie poletkowe realizowano w trzech powtórzeniach. Szczegóły dotyczące pobierania próbek, warunków środowiskowych prowadzonego doświadczenia i jego schematu oraz charakterystyki fizyko-chemicznej próbek przedstawiono szczegółowo w publikacjach D1 i D2.

4.2. Badanie adsorpcji

Badanie adsorpcji przeprowadzono w oparciu o metodę zaproponowaną przez Hale i in. [16]. Biowęgle (50 mg) umieszczono w kolbie Erlenmeyera o objętości 50 mL, następnie dodawano 40 mL wody (ultra-czysta (< 0,08 µS/ cm), Hydrolab) zawierającej NaN_3 (200 mg/ L). W kolbie umieszczano 2 paski (4 cm x 4 cm x 0,76 µm, około 0,35 g) polimeru - polioksymetylen (POM). Do kolb dodano fenantren (PHE) i piren (PYR) rozpuszczone w metanolu, uzyskując stężenia w zakresie od 20 do 800 µg/ L. Ilość dodanego metanolu (100 µL) stanowiła mniej niż 0,25% objętości wody, dlatego nie wpłynął on na badany układ eksperymentalny [49]. Początkowe stężenie PHE i PYR w biowęglach określono w kolbach bez dodatku WWA. Kolby wraz z zawartością mieszano przez 28 dni na mieszadle obrotowym z prędkością 10 obrotów/ min. Po 28 dniach polimer wyjmowano, oczyszczono za pomocą ultra-czystej wody, a pozostałe widoczne zanieczyszczenia usuwano. Oczyszczone paski POM ekstrahowano mieszaniną aceton : heptan (20:80, v/v) przez 48 h na mieszadle horyzontalnym

ELPIN 358A (Polska). Następnie rozpuszczalnik odparowano do 1 mL stosując wyparkę rotacyjną RVC 2-25 CD plus (Martin Christ, Niemcy). Zatężone próbki analizowano techniką chromatografii gazowej.

Stężenie PHE lub PYR na pasywnym próbniku (C_{POM}) obliczano według równania (1):

$$C_{POM}(ng/\,kg) = \frac{M_{WWA}\,(ng)}{M_{POM}\,(kg)} C_{POM}(ng/\,kg) = \frac{M_{WWA}\,(ng)}{M_{POM}\,(kg)} \quad (1)$$

gdzie: M_{WWA} (ng) to ilość danego WWA oznaczona za pomocą GC-MS, a M_{POM} (kg) odpowiada masie użytego polimeru.

Stężenie początkowe PHE i PYR biowęgli obliczono poprzez odjęcie $C_{POM\text{-}kontrola}$ (stężenie w kolbie bez dodatku biowęgla) od C_{POM} (stężenie w kolbie z dodatkiem biowęgla, bez PHE/PYR).

Stężenie PHE i PYR w wodzie po 28 dniach mieszania (C_e) obliczono z następującego równania (2):

$$C_{e\,/\,free}\,(\mu g/\,kg) = \frac{C_{POM}\,(\mu g/\,kg) - C_{POM-control}\,(\mu g/\,kg)}{K_{POM}\,(L/\,kg)} \quad (2)$$

gdzie C_{free} (ng/ L) jest stężeniem WWA w fazie wodnej (i jest to stężenie zanieczyszczenia, które jest biodostępne), K_{POM} (L/ kg) jest współczynnikiem podziału WWA pomiędzy POM i wodę wyznaczonym przez Hawthorne i in. [47], C_{POM} to wspomniane wyżej mierzone bezpośrednio stężenie WWA na polimerze w badanej próbce oraz $C_{POM\text{-}control}$ – w próbce kontrolnej.

4.3. Doświadczenie wazonowe

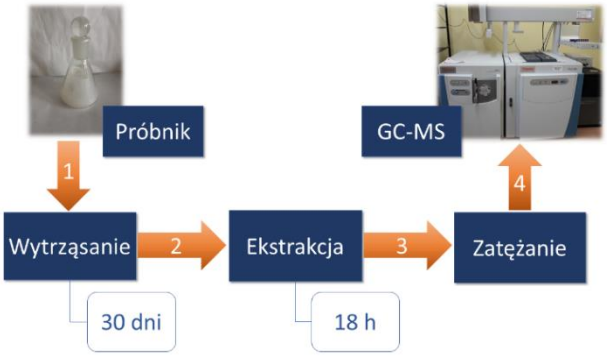
Doświadczenie wazonowe (Część II pracy: ocena ryzyka) prowadzono w pojemnikach plastikowych o objętości 20 L w warunkach kontrolowanych (stała temperatura i wilgotność) i przy zachowaniu dobowych zmian oświetlenia. Gleba zastosowana w doświadczeniu pochodziła ze stacji doświadczalnej Bezek o minimalnej presji antropogenicznej (51°11’49.7”N 23°15’01.2”E). Przed umieszczeniem w pojemnikach, glebę dokładnie mieszano z osadem ściekowym lub biowęglami w dawce 2% wagowych (sucha masa). Próbki pobrano na początku eksperymentu (0 dni) a następnie po 30, 90 i 180 dniach.

4.4. Oznaczanie całkowitej zawartości WWA

Analizowany materiał suszono w temperaturze pokojowej (23±2ºC). Glebę lub glebę z osadem lub biowęglem (pobraną z doświadczenia wazonowego) (10 g) z 10% dodatkiem Na_2SO_4 (w celu chemicznego wysuszenia próbki) oraz 5% dodatkiem Cu (w celu związania siarki) umieszczano w gilzie celulozowej i ekstrahowano w aparacie Soxhleta (BEHR, Niemcy) przez 24 h. Deuterowany standard (PAH Mix 9 deuterated standard (Dr Ehrenstorfer GmbH, Niemcy) – 100 ng/ µL) stosowany był jako wzorzec wewnętrzny. Ekstrakcję prowadzono za pomocą heksanu w temperaturze 100ºC. Otrzymany ekstrakt odparowano w wyparce rotacyjnej RVC 2-25 CD plus (Martin Christ, Niemcy) do objętości 1 mL i poddano oczyszczaniu metodą ekstrakcji ciecz-ciecz przy użyciu dimetyloformamidu (DMF) [48]. Następnie próbki zatężono do objętości 1 mL i analizowano techniką chromatografii gazowej sprzężonej ze spektrometrem masowym.

4.5. Oznaczanie potencjalnie biodostępnej frakcji WWA

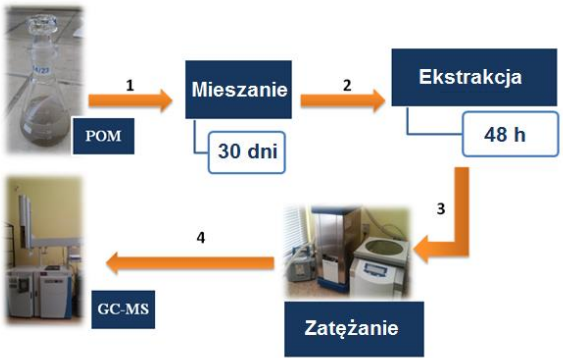
Zawartość potencjalnie biodostępnej (C_{acc}) frakcji WWA oznaczono metodą w oparciu o silikonowe próbniki zgodnie z procedurą zaproponowaną przez Gouliarmou i Mayer [49]. Cała procedura została szczegółowo opisana w pracy D1 oraz schematycznie przedstawiona na **Rys. 5**. Czysty i suchy silikonowy próbnik umieszczano w butelce Pyrex (100 mL), a następnie dodawano badaną próbkę (100 mg) oraz zalewaną roztworem (2-hydroksypropylo)-β-cyklodekstryny (HPCD) (50 mL). Tak przygotowany materiał wytrząsano z prędkością 200 rpm w temperaturze pokojowej (23±2ºC). Po 30 dniach próbniki silikonowe wyjmowano z roztworu, oczyszczono z pozostałości próbki i ekstrahowano acetonem (2 x 100 mL) przez 18 h. Ekstrakty odparowano do 1 mL, a następnie analizowano techniką chromatografii gazowej.



Rysunek 5. Schemat oznaczania potencjalnie biodostępnej frakcji WWA

4.6. Oznaczanie biodostępnej frakcji WWA

Zawartość biodostępnej frakcji (C_{free}) WWA oznaczono metodą w oparciu o polimer- polioksymetylen (POM). Cała procedura przedstawiona została we wcześniejszych pracach oraz schematycznie na **Rys. 6**. Do próbki (1 g) umieszczonej w kolbie Erlenmeyera (o objętości 50 mL) dodawano 40 mL wody (ultra-czysta (< 0,08 µS/ cm), Hydrolab) zawierającej NaN_3 (200 mg/ L). Następnie, do kolby wprowadzano 2 paski POM (4 cm x 4 cm x 0,76 µm, około 0,35 g). Kolby wraz z zawartością mieszano przez 28 dni na mieszadle obrotowym z prędkością 10 obrotów/ min. Po 28 dniach polimer wyjmowano i oczyszczano. Oczyszczone paski POM poddano ekstrakcji mieszaniną aceton : heptan (20:80, v/v) w trakcie 48 h na mieszadle horyzontalnym ELPIN 358A (Polska). Rozpuszczalnik odparowano do 1 mL stosując wyparkę rotacyjną RVC 2-25 CD plus (Martin Christ, Niemcy). Zatężone próbki analizowano techniką chromatografii gazowej.



Rysunek 6. Schemat oznaczania biodostępnej frakcji WWA

Stężenie poszczególnych związków z grupy WWA na pasywnym próbniku (C_{POM}) obliczono według równania (1). Stężenie frakcji C_{free} (ang. *freely dissolved*) obliczono z równania (2).

4.7. Jakościowa i ilościowa identyfikacja WWA za pomocą GC-MS

Jakościową i ilościową analizę WWA ekstrahowanych silnym rozpuszczalnikiem organicznym (C_{tot}), potencjalnie biodostępnych (C_{acc}) oraz biodostępnych (C_{free}) przeprowadzono przy użyciu chromatografu gazowego (Trace 1300) sprzężonego ze spektrometrem masowym z pojedynczym kwadrupolem (ISQ LT) (GC-MS, Thermo Scientific). Spektrometr mas pracował w trybie SIM – monitorowania wybranych, charakterystycznych dla danego związku jonów. Jonizacja cząsteczek w spektrometrze była wywołana strumieniem elektronów o energii 70 eV (jonizacja elektronowa, EI). Do analiz wykorzystano kolumnę kapilarną (5 % polisiloksan difenylu i 95 % polisiloksan dimetylu) o długości 30 m, przekroju 0,25 mm i grubości fazy stacjonarnej 0,25 µm firmy Restek (typ Rxi-5ms Column, USA).

WWA oznaczono metodą, charakteryzującą się następującymi parametrami:

- Temperatura dozownika – 310°C;
- Temperatura linii transferowej – 280°C;
- Temperatura źródła jonów – 250°C;
- Program temperaturowy pieca: 75°C przez 0,5 min, narost 25°C/ min– 245°C, narost 4°C/ min - 300°C przez 1 min;
- Przepływ helu przez kolumnę – 1,5 mL/ min;

4.8. Badanie ekotoksykologiczne gleby

W celu oceny toksyczności gleby (gleba bez dodatków i gleba z dodatkiem osadu ściekowego lub biowęgla) wykonano testy ekotoksykologiczne z wykorzystaniem zróżnicowanych grup organizmów (Tabela 1): mikroorganizmy – *Aliivibrio fischeri* (Microtox®) [50], rośliny – *Lepidium sativum* (Phytotoxkit F® [51] i test wzrostu korzeni fazy ciekłej [52]) oraz stawonogi – *Folsomia candida* (Collembolan test) [53]. Badano toksyczność fazy stałej gleby (Phytotoxkit i Collembolan test) lub wodnego ekstraktu z badanej matrycy (Microtox® i test z *L. sativum*). Wodne ekstrakty przygotowano zgodnie z procedurą EN 12457-2 [54].

Tabela 1. Testy ekotoksykologiczne wykorzystywane w ramach pracy

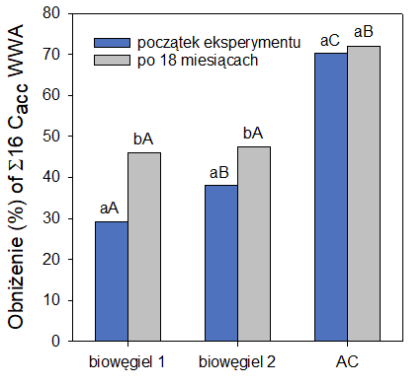
Nazwa testu	Gatunek	Badana faza	Badany parametr
Microtox	*Allivibrio fischeri*	Wodny odciek	Bioluminescencja
	Lepidium sativum		Długość korzeni
Phytotoxkit F	*Lepidium sativum*	Gleba	Długość korzeni
	Folsomia candida		Śmiertelność
	Folsomia candida		Reprodukcja

5. Badania własne

5.1. Biodostępność WWA w glebie z dodatkiem węgla aktywnego lub biowęgla (publikacja D1)

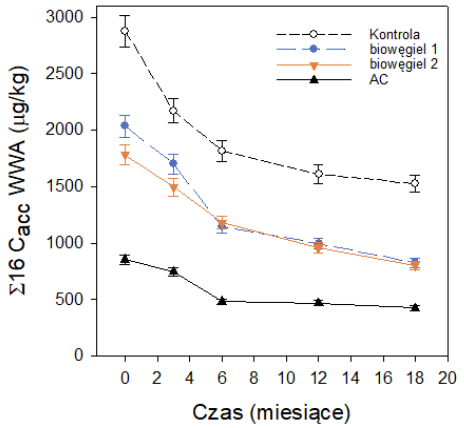
Celem badań było określenie wpływu węgla aktywnego (AC) lub biowęgli na potencjalną biodostępność (C_{acc}) wielopierścieniowych węglowodorów aromatycznych (WWA) w zanieczyszczonej tymi związkami glebie. Określono również wpływ uprawy *Salix viminalis* (wiklina) na zawartość C_{acc} WWA oraz wpływ badanych dodatków na zmiany rozpuszczalnego węgla organicznego, plon roślin i zawartość w nich WWA.

AC i biowęgiel powodowały obniżenie frakcji C_{acc} WWA w zanieczyszczonej glebie. Bezpośrednio po dodaniu do gleby AC oraz biowęgli zmniejszenie sumy 16 (Σ16) C_{acc} WWA wynosiła odpowiednio 70, 38 i 29% **(Rys. 7)**. Największe obniżenie C_{acc} WWA obserwowano w przypadku 5- i 6-pierścieniowych związków (od 54 do 100%), podczas gdy najsłabszą dla 2-pierścieniowych WWA (od 8 do 25%).



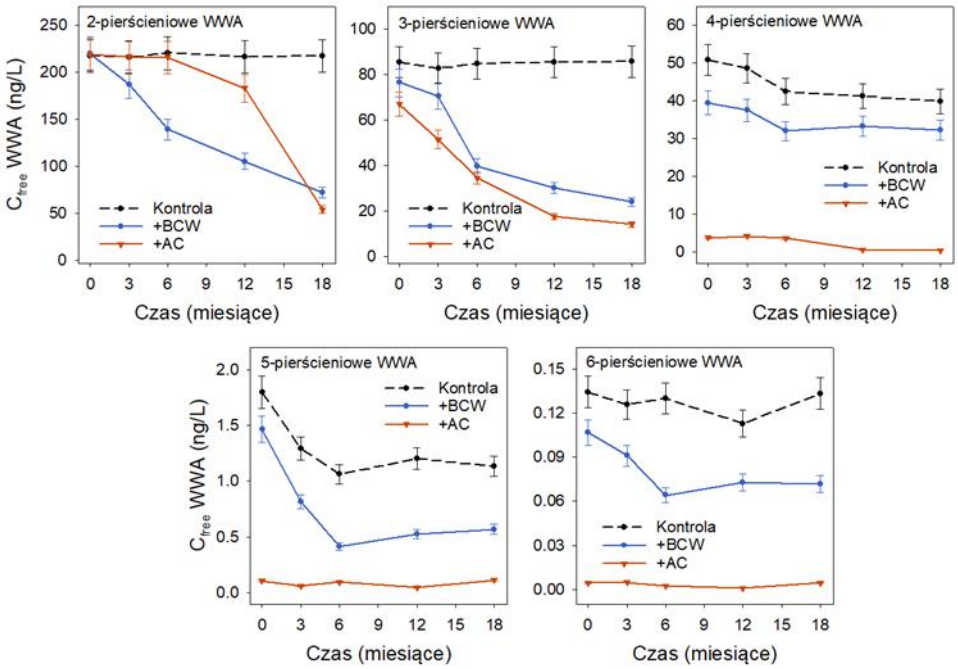
Rysunek 7. Obniżenie zawartości WWA w glebie z dodatkiem AC lub biowęgli

W miarę upływu czasu, zawartość C_{acc} WWA ulegała dalszemu zmniejszeniu **(Rys. 8)**. Wpływ roślin na zawartość C_{acc} uwidocznił się w przypadku gleby z dodatkiem AC (Σ16 WWA, naftalen), biowęgla 1 (5-pierścieniowe WWA) oraz w glebie nie zawierającej AC lub biowęgla (3- i 4-pierścieniowe WWA) **(Rys. 9)**. Wiklina uprawiana na glebie zawierającej AC i biowęgle, charakteryzowała się mniejszą zawartością fenantrenu niż uprawiana na glebie kontrolnej. Stwierdzono jednak,



Rysunek 8. Zmiana zawartości WWA w glebie w trakcie eksperymentu

że obecność AC w glebie negatywnie wpływała na plon wikliny i długość pędów. AC obniżał również zawartość rozpuszczalnego węgla organicznego w glebie, czego nie obserwowano w przypadku wariantu z biowęglami. Takie znaczne zmniejszenie rozpuszczalnego węgla organicznego (DOC) może mieć szkodliwy wpływ na organizmy glebowe i rośliny [55].



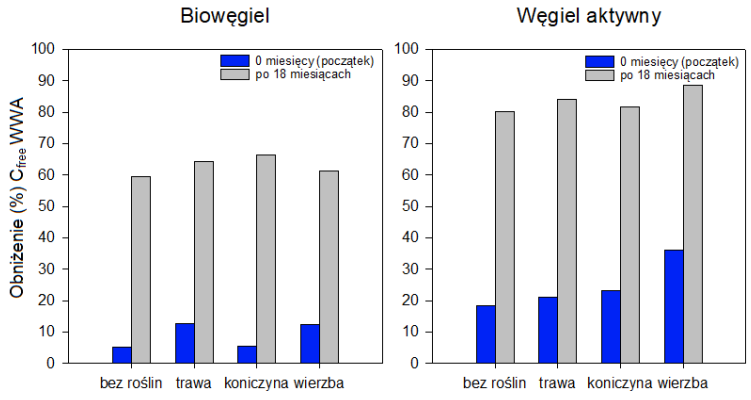
Rysunek 9. Zmiana zawartości WWA w glebie w trakcie trwania eksperymentu z uwzględnieniem liczby pierścieni

Podsumowując, AC okazał się bardzo szybki w wiązaniu C_{acc} WWA, co było powodowane jego dużo większym polem powierzchni w porównaniu do zastosowanych biowęgli. Biowęgle adsorbowały C_{acc} WWA wolniej w porównaniu do AC. Po kilku miesiącach jednak zaobserwowano istotne zmniejszenie C_{acc} WWA przez biowęgle. Uzyskane wyniki wskazują więc, że biowęgle również mogą być skuteczne w wiązaniu C_{acc} WWA, potrzebny jest jednak dłuższy czas do osiągnięcia pożądanego efektu.

5.2. Wpływ roślin na biodostępność WWA w glebie z dodatkiem węgla aktywnego lub biowęgla (publikacja D2)

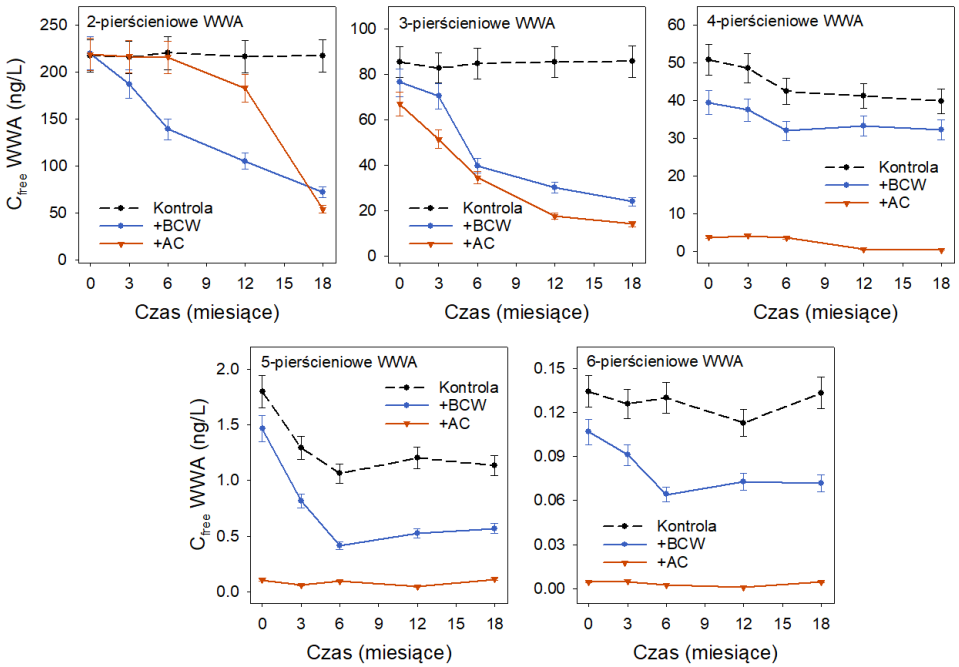
Celem badań było określenie wpływu dodatku AC lub biowęgla na zawartość biodostępnych (C_{free}) WWA w kontekście uprawy różnych gatunków roślin (koniczyna, trawa, wierzba). Dodatkowo oceniano wpływ dodatku AC lub biowęgla na zawartość azotu ogólnego oraz przyswajalnych form fosforu, potasu i magnezu w glebie.

Dodanie AC lub biowęgla do gleby na początku badań nie spowodowało w przypadku biowęgla lub spowodowało w przypadku AC tylko nieznaczne obniżenie się zawartości Σ16 C_{free} WWA **(Rys. 10)**. W obrębie poszczególnych grup WWA zaobserwowano jednak już od samego początku badań istotne obniżenie się C_{free} w przypadku 4-, 5- i 6-pierścieniowych WWA. Efekt ten był szczególnie widoczny po zastosowaniu AC **(Rys. 11)**. Z czasem, skuteczność wiązania C_{free} WWA przez AC i biowęgiel ulegała znacznemu zwiększeniu również w odniesieniu do pozostałych grup WWA **(Rys. 11)**. Efekt zmniejszenia zawartości C_{free} WWA był od 53 do 79 % wyższy dla AC niż biowęgla.



Rysunek 10. Obniżenie biodostępnych WWA w glebie z dodatkiem biowęgla i węgla aktywnego

Zarówno w doświadczeniu z biowęglem i AC, do ostatniego terminu badań obserwowano stopniowe obniżanie się zawartości C_{free} WWA w przypadku związków 2- i 3-pierścieniowych. Ostatecznie, bez względu na grupę WWA, lepszy w wiązaniu C_{free} WWA okazał się AC niż biowęgiel, chociaż w przypadku 2- i 3-pierścieniowych WWA różnice między AC a biowęglem nie były tak znaczące, jak dla związków o większej liczbie pierścieni (4-, 5- i 6-pierścieniowych) **(Rys. 11)**. Obecność AC i biowęgla zwiększały również dostępność składników odżywczych w porównaniu do gleb bez dodatków.



Rysunek 11. Zmiana indywidualnych grup C_{free} WWA w glebiebez uprawy roślin

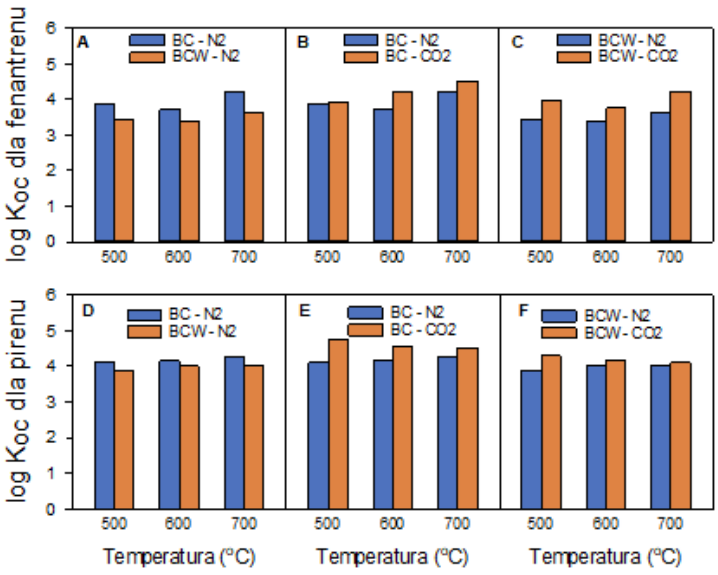
Podsumowując uzyskane wyniki, stwierdzono, że AC był nie tylko bardziej efektywny niż biowęgiel, ale również i szybszy w wiązaniu C_{free} WWA w zanieczyszczonej glebie. Sytuacja ta nie zmieniła się przez cały okres badań. Największe różnice w efektywności i szybkości wiązania między AC i biowęglem obserwowano dla WWA o ≥ 4 pierścieniach w cząsteczce. Wraz z upływem czasu efektywność biowęgla ulegała zwiększeniu, jednak nie osiągnięto poziomu efektywności obserwowanej dla AC. Należy podkreślić, że w przypadku „lekkich" WWA (2-3 pierścienie) różnice między biowęglem a AC były znacznie mniejsze niż w przypadku „ciężkich" WWA (≥ 4 pierścienie). Wpływ roślin zaznaczał się przez cały okres badań, jednak wykazywał on różne tendencje. Po 18 miesiącach zaobserwowano istotnie mniejszą zawartość C_{free} WWA w wariancie doświadczenia z roślinami niż bez roślin. Z jednej strony mogło to być efektem degradacji zanieczyszczeń, z drugiej natomiast unieruchomieniem zanieczyszczeń w wyniku ich adsorpcji przez materię organiczną „wspomaganą" obecnością AC lub biowęgla i enzymów roślinnych. AC i biowęgiel nie obniżały dostępności składników odżywczych, co w kontekście uprawianych roślin jest szczególnie istotne. Obserwowano nawet zwiększenie dostępności (szczególnie po zastosowaniu biowęgla), co może potencjalnie stymulować wzrost roślin i aktywność biologiczną gleb, zwiększając skuteczność remediacji.

5.3. Adsorpcja fenantrenu i pirenu przez biowęgle otrzymane z mieszanych surowców i w atmosferze N_2 lub CO_2 (publikacja D3)

Celem badań było określenie wpływu dodatku biomasy do osadu ściekowego oraz zastosowania CO_2 (zamiast N_2) podczas pirolizy, na właściwości adsorpcyjne biowęgli w stosunku do fenantrenu (PHE) (3-pierścieniowy) i pirenu (PYR) (4-pierścieniowy).

Badanie adsorpcji przeprowadzono metodą statyczną, wykorzystując próbniki pasywne z POM. Cztery nieliniowe modele izoterm adsorpcji (Freundlicha, Langmuira, Temkina i Dubinina- Raduszkiewicza) zostały przetestowane w celu dopasowania uzyskanych wyników do danych eksperymentalnych.

Adsorpcję PHE i PYR najlepiej opisywał model Freundlicha (Publikacja D3, Tabela 2). We wcześniejszych badaniach [18,51–56] dotyczących adsorpcji WWA przez biowęgle otrzymane w różnych warunkach i z różnych surowców model Freundlicha był również najlepszy do opisu danych eksperymentalnych. W modelu tym zakłada się, że adsorpcja związków na adsorbencie jest wielowarstwowa. W sorpcji uczestniczą różnego rodzaju miejsca sorpcyjne, różniące się między sobą ilością i swobodną entalpią [51]. Wartości znormalizowanego współczynnika *log* K_{OC} (C_w = 0,01 S_w) wahały się od 3,72 do 4,21 dla PHE i od 4,11 do 4,28 dla PYR **(Rys. 12)**. Uzyskane wartości *log* K_{OC} były zbliżone do wcześniejszych badań dotyczących biowęgli otrzymanych z SL [51], jak również biowęgli otrzymanych z innych materiałów pochodzenia organicznego [54,57]. Zarówno w przypadku PHE i PYR, najwyższą pojemność adsorpcyjną (*log* K_{OC}) uzyskano dla biowęgli otrzymanych w temperaturze 700°C. Biowęgle te charakteryzowały się jednocześnie



Rysunek 12. Współczynnik log Koc w badaniach adsorpcji fenantrenu i pirenu przez biowęgle

najwyższym polem powierzchni właściwej, co można tłumaczyć obserwowaną wysoką wydajność w adsorpcji zarówno PHE, jak i PYR [52,55,57].

Dodatek biomasy do osadu ściekowego przed pirolizą wpłynął na obniżenie powinowactwa (*log* K_{OC}) PHE i PYR do otrzymanych biowęgli. Stwierdzono (na podstawie wartości *log* K_{OC}) od 9 do 14% (PHE) oraz od 4 do 6% (PYR) niższą adsorpcję na biowęglach z dodatkiem biomasy niż na biowęglach otrzymanych z samych osadów ściekowych (N_2 jako gaz nośny) **(Rys. 12)**. Podobnie jak w przypadku biowęgli otrzymanych tylko z SL największe powinowactwo do PHE i PYR wykazywały biowęgle otrzymane w temperaturze 700^{o}C.

Istotny wpływ na zwiększenie pojemności adsorpcyjnej biowęgli miała zmiana atmosfery z N_2 na CO_2 podczas pirolizy (od 7% do 12% i od 6 do 16% wyższy *log* K_{OC} niż biowęgle otrzymane w N_2). Biowęgiel otrzymany w temperaturze 700^{o}C najlepiej sorbował PHE, natomiast PYR był najefektywniej sorbowany przez biowęgiel otrzymany w temperaturze 500^{o}C (w oparciu o *log* K_{OC}).

Zamiana gazu nośnego z N_2 na CO_2 zwiększyła również pojemność adsorpcyjną biowęgli otrzymanych z SL z dodatkiem wikliny w taki sposób, że biowęgle otrzymane w CO_2 charakteryzowały się wyższymi od 11 do 16% (PHE) i od 2 do 11% (PYR) wartościami *log* K_{OC} niż te same biowęgle otrzymane w atmosferze N_2. PHE był najlepiej sorbowany przez biowęgiel otrzymany w 700^{o}C, natomiast PYR był ponownie efektywniej sorbowany przez biowęgle otrzymane w 500^{o}C (na podstawie *log* K_{OC}).

Zmiana warunków pirolizy miała zróżnicowany wpływ na właściwości adsorpcyjne biowęgli w stosunku do PHE i PYR. Struktura i właściwości biowęgli uległy zmianom, co w znacznej mierze determinowało mechanizm wiązania badanych WWA przez biowęgiel. Dodatek wikliny do SL przed pirolizą wpłynął na zmniejszenie adsorpcji PHE i PYR przez biowęgle, natomiast gaz nośny powodował zwiększenie jej skuteczności. Obniżenie adsorpcji związane było z obniżeniem średnicy porów, przy niewielkich zmianach chemii powierzchni. Obserwowane zwiększenie adsorpcji wiązało się natomiast ze zwiększeniem głównie aromatycznego charakteru powierzchni biowęgla.

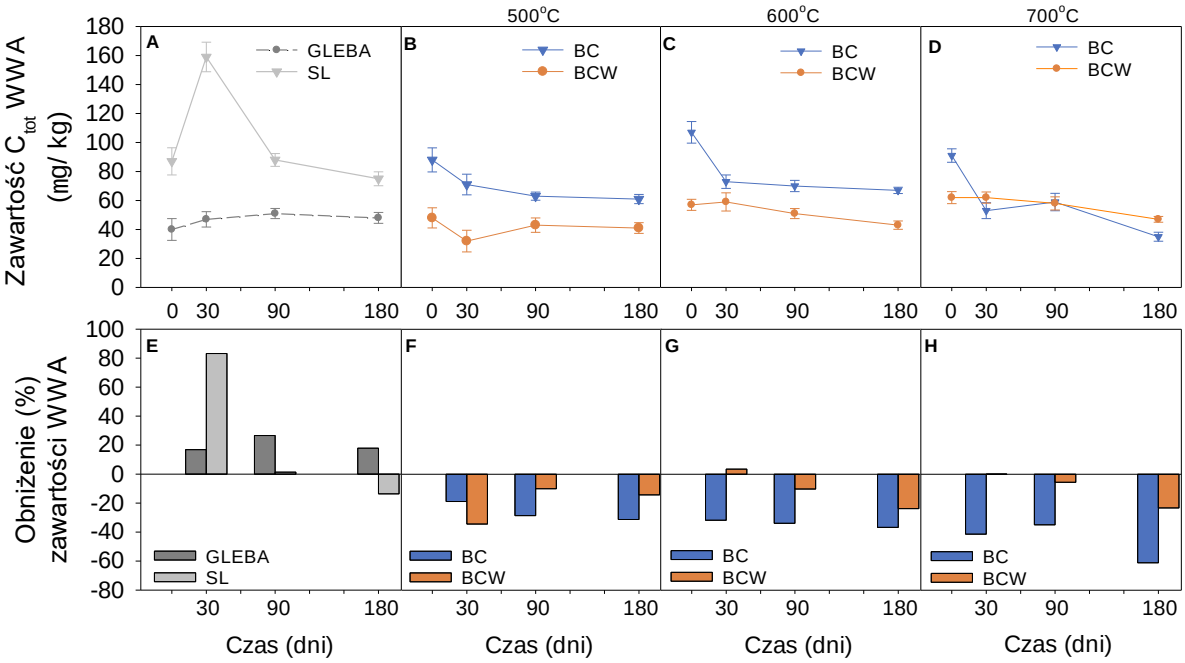
Stosowanie ditlenku węgla zamiast azotu jako gazu nośnego podczas pirolizy wpływało korzystnie na właściwości adsorpcyjne biowęgli otrzymanych zarówno z osadów ściekowych, jak i ich mieszaniny z wikliną w stosunku do PHE i PYR.

5.4. Trwałość i biodostępność WWA w glebie z dodatkiem biowęgla otrzymanego z mieszaniny osadu ściekowego i biomasy (publikacja D5)

Celem przeprowadzonych badań było określenie całkowitej (C_{tot}) i biodostępnej (C_{free}) zawartości WWA w glebie użyźnionej biowęglem otrzymanym z SL lub SL z dodatkiem wikliny. Dodatkowo badano, jaki wpływ na trwałość (na podstawie C_{tot}) i biodostępność (na podstawie C_{free}) WWA będzie miała temperatura (500, 600 i 700^{o}C) pirolizy w kontekście surowca stosowanego do otrzymania biowęgla.

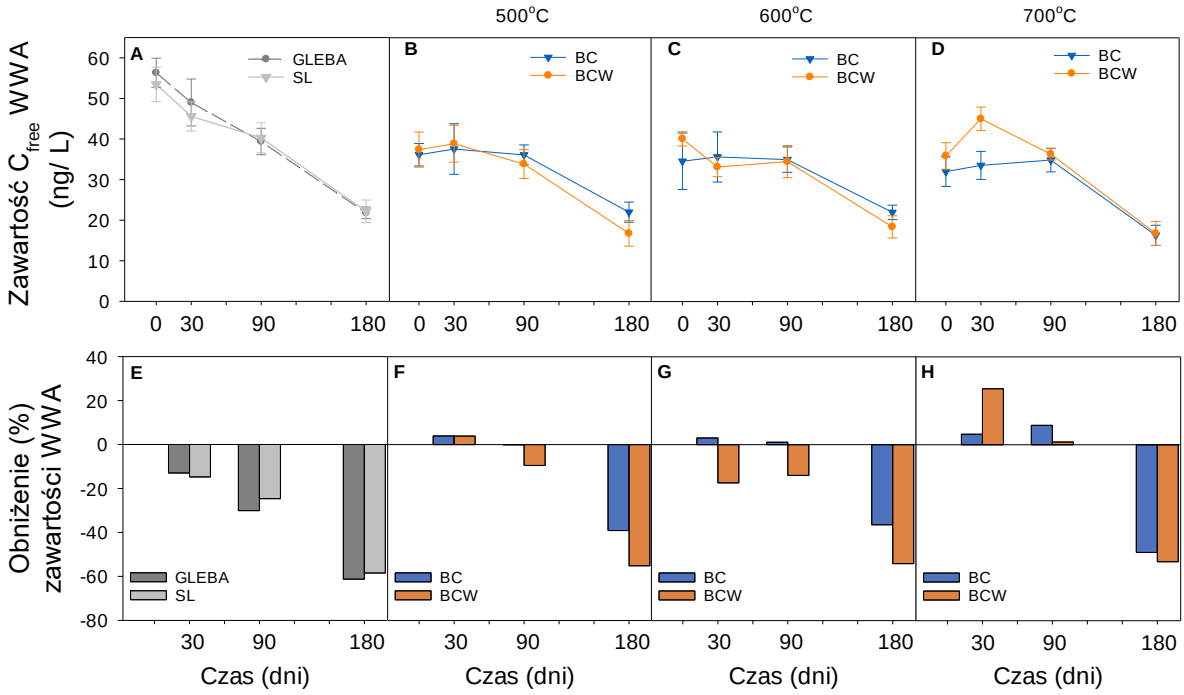
W pierwszym etapie badań przeprowadzono porównanie trwałości (C_{tot}) i biodostępności (C_{free}) WWA między glebą użyźnioną osadem ściekowym (SL) oraz biowęglem otrzymanym z osadu ściekowego w różnych temperaturach (BC500 – 500^{o}C, BC600 – 600^{o}C i BC700 – 700^{o}C). Dodanie do gleby biowęgli w zależności od temperatury ich otrzymania miało zróżnicowany wpływ na zawartość C_{tot} WWA **(Rys. 13)**. Bezpośrednio po dodaniu biowęgla otrzymanego w temperaturze 500 lub 700^{o}C zawartość Σ16 C_{tot} WWA nie różniła się istotnie statystycznie (P>0,05) w stosunku do gleby użyźnionej SL oraz była od 117 do 124% wyższa niż w glebie kontrolnej. W glebie z biowęglem otrzymanym w temperaturze 600^{o}C zawartość Σ16 C_{tot} WWA była o 23% wyższa niż w glebie z SL, co związane było głównie ze zwiększeniem zawartości w tej glebie 4-, 5- i 6-pierścieniowych WWA po dodaniu tego biowęgla.

Po 180 dniach zawartość Σ16 C_{tot} WWA w glebie z biowęglem otrzymanym z SL (BC) była od 31 do 61% niższa niż na początku badań w zależności od temperatury otrzymywania biowęgla **(Rys. 13)**. W odniesieniu do gleby kontrolnej w tym samym okresie zawartość Σ16 C_{tot} WWA była natomiast od 27 do 41% wyższa w glebie z BC500 i BC600 i o 26% niższa w glebie z BC700. Stwierdzono, że trwałość WWA w glebie z biowęglem była niższa niż w glebie z dodatkiem SL. Zawartość Σ16 C_{tot} WWA po 180 dniach uległa zmniejszeniu w większym stopniu (od 18 do 48%) w glebie z biowęglem niż SL.



Rysunek 13. Zmiana C_{tot} w glebie z dodatkiem biowęgli w trakcie 180 dni eksperymentu

W przypadku Σ16 C_{free} WWA bezpośrednio po dodaniu biowęgla (BC) do gleby ich zawartość nie różniła się istotnie między biowęglami otrzymanymi w różnych temperaturach. Gleba z dodatkiem biowęgli charakteryzowała się natomiast od 36 do 43% oraz od 32 do 40% niższą zawartością Σ16 C_{free} WWA w porównaniu odpowiednio z glebą kontrolną oraz glebą z dodatkiem SL **(Rys. 14)**. W trakcie pierwszych 90 dni ilość Σ16 C_{free} WWA utrzymywała się na stałym poziomie. Po 90 dniach zaobserwowano istotny spadek zawartości Σ16 C_{free} WWA w zakresie od 36 do 49%. Największe obniżenie Σ16 C_{free} WWA odnotowano dla gleby z BC700, a najmniejsze w glebie z dodatkiem BC600.



Rysunek 14. Zmiana C_{free} w glebie z dodatkiem biowęgli w trakcie 180 dni eksperymentu

Dodanie do gleby biowęgla otrzymanego z SL i wikliny (BCW) zwiększyło zawartość Σ16 C_{tot} WWA w mniejszym stopniu niż obserwowano to w glebie z biowęglem otrzymanym tylko z SL (BC). Związane to było z niższą od 7 do 52% ilością WWA w BCW niż BC (Publikacja D5, Tabela 1). Bezpośrednio po dodaniu BCW, Σ16 C_{tot} WWA była od 32 do 47% niższa niż w glebie z BC. Zaobserwowano wyraźny trend wskazujący, że stężenie Σ16 C_{tot} WWA w glebie użyźnionej BC ulegało zwiększeniu wraz ze wzrostem temperatury pirolizy, co jednocześnie korelowało z zawartością WWA w biowęglach. Dodatek biomasy do pirolizowanego osadu ściekowego wpłynął również na zmianę ilości poszczególnych związków z grupy WWA w glebie z BCW w porównaniu dla gleby z BC, jak i gleby kontrolnej (Publikacja D5, Rys. 2).

W zależności od temperatury otrzymania BCW, zmiany stężenia Σ16 C_{tot} WWA były różne w poszczególnych terminach i charakteryzowały się różną dynamiką **(Rys. 13)**. Ostatecznie, po 180 dniach zawartość Σ16 C_{tot} WWA obniżyła się w stosunku do początku badań od 14 do 24% (w zależności od temperatury otrzymania biowęgla). Najwyższe straty WWA notowano w glebie użyźnionej biowęglem BCW otrzymanym w temperaturze 500°C, natomiast najniższe w przypadku BCW otrzymanego w 600°C.

Porównując trwałość Σ16 C_{tot} WWA po 180 dniach między poszczególnymi wariantami eksperymentalnymi z BC i BCW, stwierdzono niższe straty, a przez to większą trwałość Σ16

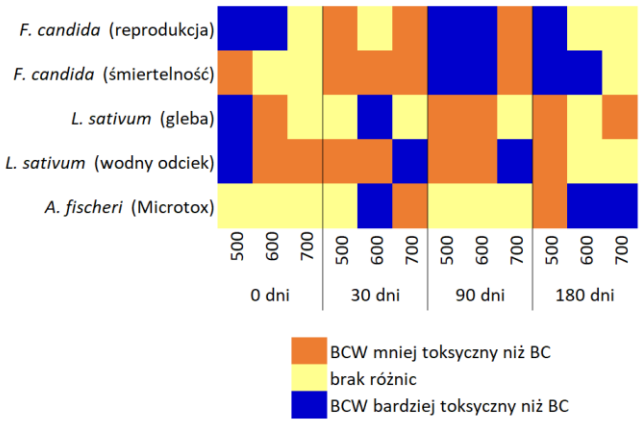
C_{tot} WWA w glebie z BCW. W zależności od temperatury pirolizy straty Σ16 C_{tot} WWA były od 13 do 38% mniejsze w glebie z BCW w porównaniu z glebą z BC.

Zawartość Σ16 C_{free} PAH w glebie z BCW w zależności od temperatury otrzymania biowęgla była od 4 do 16% wyższa niż w glebie z dodatkiem BC. Było to związane ze słabszą pojemnością adsorpcyjną BCW w stosunku do lekkich WWA (3-pierścieniowe) w porównaniu do BC [58]. We frakcji C_{free} WWA dominowały lekkie WWA, stąd też efekt ten był bardziej widoczny. Zmiany zawartości poszczególnych grup C_{free} WWA w glebie z dodatkiem BCW po 180 dniach różniły się istotnie od zmian tych związków w glebie z dodatkiem BC. Biowęgle BC charakteryzowały się mniejszą aromatycznością i hydrofobowością niż biowęgle BCW (Publikacja D5, Tabela S1), co wpłynęło na większe straty C_{free} WWA w przypadku BCW (lepsze wiązanie C_{free} WWA przez biowęgiel o powierzchni o charakterze bardziej aromatycznym).

Trwałość WWA (określona na podstawie C_{tot}) w glebie z dodatkiem biowęgla otrzymanego z osadu ściekowego z dodatkiem biomasy (BCW) była większa w porównaniu z w glebą z dodatkiem biowęgla otrzymanego z samego osadu ściekowego (BC). Oznacza to, że wprowadzenie współpirolizowanego (biomasa i SL) biowęgla przyczyni się do większej trwałości WWA w użyźnionej glebie w porównaniu do zastosowania biowęgla otrzymanego tylko z osadu ściekowego. Jednocześnie jednak, frakcja biodostępna (określona na podstawie C_{free}) WWA w glebie z dodatkiem BCW ulegała większym stratom, niż w glebie z BC. Na podstawie uzyskanych wyników można wnioskować, że bezpośrednie ryzyko środowiskowe związane z obecnością WWA będzie niższe po zastosowaniu BCW w porównaniu z BC. Większe straty C_{free} WWA w glebie z BCW w stosunku do gleby z BC przy jednoczesnym mniejszym powinowactwie WWA do BCW niż BC mogą wskazywać, że w glebie z BCW straty WWA były w większym stopniu związane z procesami biodegradacji w porównaniu z glebą z BC, w której z kolei dominowały procesy sekwestracji lub tworzenia pozostałości związanej [40,56]**. Z drugiej jednak strony C_{free} WWA w glebie z BCW mogły wzbogacać pulę C_{tot} WWA wpływając na mniejszy spadek zawartości tych związków w trakcie doświadczenia. Różnice w mechanizmie wiązania WWA przez biowęgle determinowały ich straty, na co wskazują zależności między stratami tych związków a właściwościami biowęgli. W przypadku BC mechanizm adsorpcji był zdominowany przez wypełnianie porów, natomiast w przypadku BCW mechanizm związany był z adsorpcją opartą na wiązaniach hydrofobowych i π-π.**

5.5. Ekotoksyczność gleby z dodatkiem biowęgla otrzymanego z osadów ściekowych lub osadów ściekowych i wikliny (publikacja D6)

Celem badań było porównanie toksyczności gleby z dodatkiem biowęgla otrzymanego z SL oraz SL i biomasy. Wpływ oceniano w stosunku do różnych grup organizmów reprezentujących różne ogniwa łańcucha troficznego (rośliny, bakterie i bezkręgowce). Ocenie poddano zarówno fazę stałą, jak również odcieki uzyskane z badanych gleb. Dodatkowo określano wpływ temperatury otrzymania biowęgla na obserwowany efekt.



Rysunek 15. Diagram obrazujący różnice w toksyczności między glebą z dodatkiem biowęgla z osadów i wikliny a glebą z biowęglem z osadów

Efekt toksyczny biowęgla w kontekście zastosowanego dodatku determinowany był głównie rodzajem organizmu, a w mniejszym stopniu terminem badań i temperaturą pirolizy **(Rys. 15)**. W teście z bakteriami *A. fischeri* (Microtox®) w większości terminów nie obserwowano istotnego wpływu dodatku biomasy do SL przed pirolizą na toksyczność biowęgla. Istotne różnice zanotowano jedynie w drugim i ostatnim terminie badań **(Rys. 15)**.

Ewidentny pozytywny wpływ dodatku wikliny dla większości biowęgli i terminów zaobserwowano natomiast w teście fazy stałej z *L. sativum* oraz w mniejszym stopniu dla fazy ciekłej, która była szczególnie widoczna w 90 i 180 dniu badań.

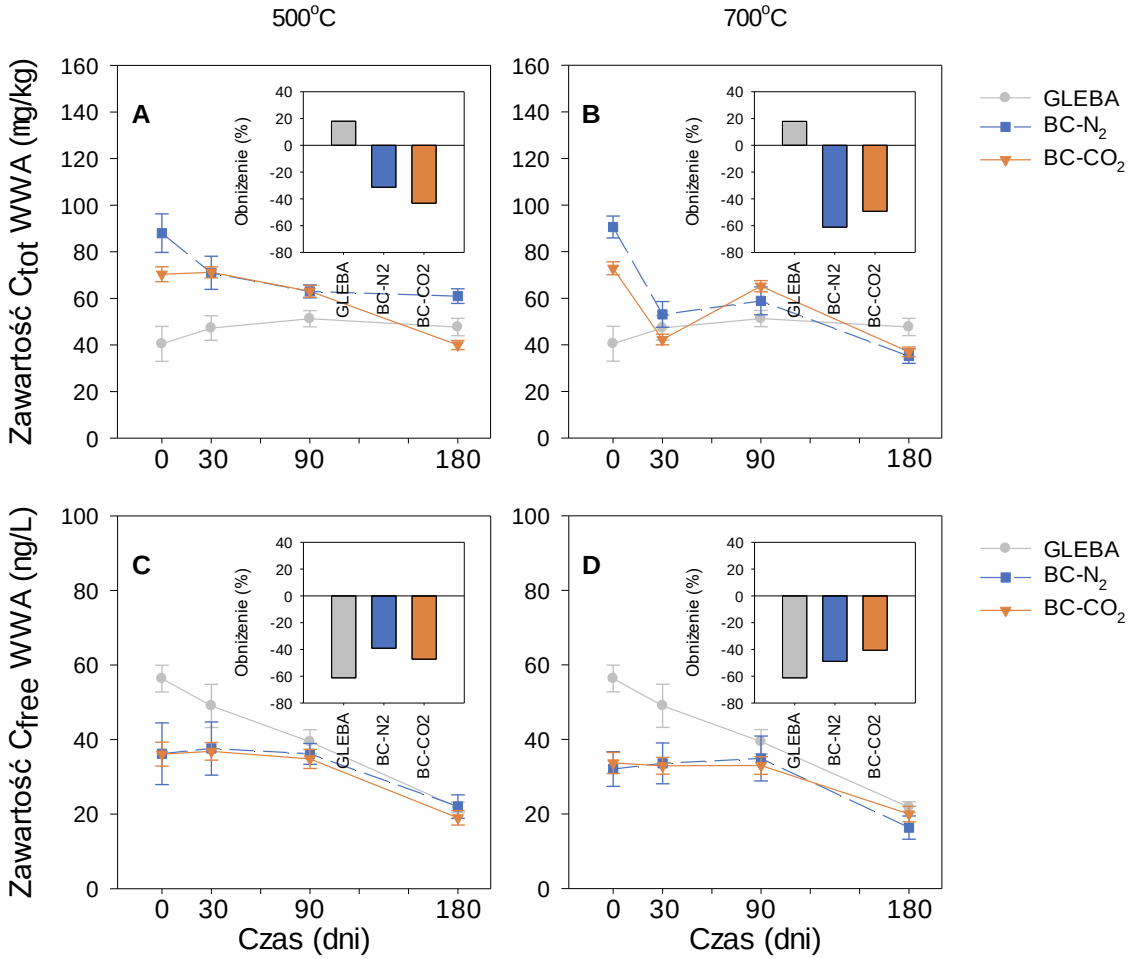
W testach z *F. candida* stwierdzono pozytywny wpływ dodatku biomasy w 30-tym dniu badań w przypadku śmiertelności, po czym po 90 i 180 dniach, zaznaczył się wyraźny negatywny wpływ dodatku biomasy w biowęglach otrzymanych w 500 i 600°C. Najbardziej zróżnicowany wpływ poszczególnych biowęgli obserwowano natomiast w odniesieniu do reprodukcji *F. candida*. Jedynie w trzech przypadkach (w zależności od terminu i rodzaju biowęgla) wpływ dodatku biomasy pozytywnie wpływał na reprodukcję, podczas gdy dla pozostałych biowęgli i terminów nie obserwowano istotnych różnic między BC i BCW lub dodatek biomasy powodował negatywy wpływ na badane organizmy **(Rys. 15)**.

W dłuższej perspektywie i w przypadku większości testów dodatek osadu ściekowego do gleby wywierał większy efekt toksyczny niż dodatek biowęgli. Efekty toksyczne gleby z dodatkiem BC lub BCW były zróżnicowane i zależne od rodzaju organizmu testowego, terminu badań i surowca. Zaobserwowano wpływ temperatury pirolizy na toksyczność biowęgli, jednak zróżnicowany w zależności od badanego organizmu. Należy również podkreślić, że efekt toksyczny uwidocznił się w różnych terminach badań, co może wskazywać na zróżnicowany wpływ różnych czynników odpowiedzialnych za toksyczność. W większości przypadków bardziej toksycznym dodatkiem okazał się biowęgiel otrzymany tylko z osadów ściekowych niż z osadów ściekowych i wikliny.

5.6. Wpływ gazu nośnego podczas pirolizy na trwałość i biodostępność WWA w glebie z dodatkiem biowęgla (publikacja D7)

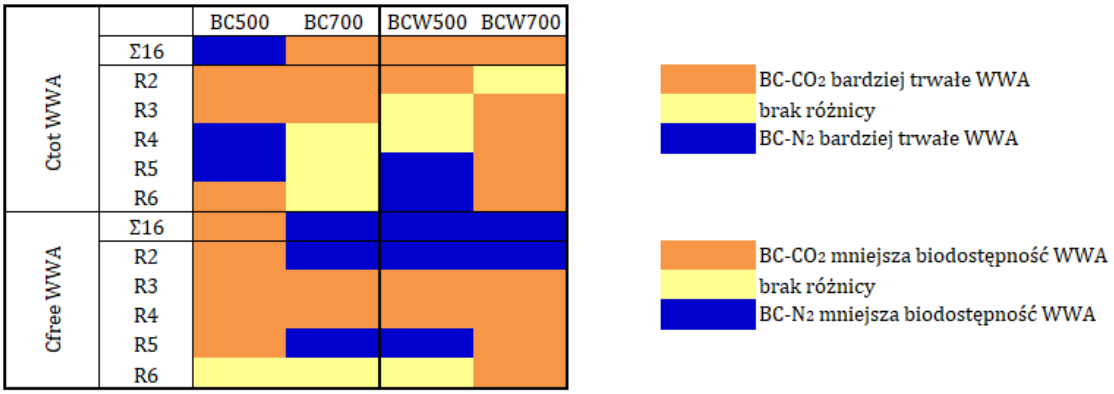
Celem pracy było określenie całkowitej (C_{tot}) i biodostępnej (C_{free}) zawartości WWA w glebie z dodatkiem biowęgla otrzymanego z SL lub mieszaniny SL z wikliną w atmosferze N_2 lub CO_2. Oceniano wpływ temperatury pirolizy (500 lub 700°C) na wspomniane parametry.

Warunki pirolizy determinowały trwałość i biodostępność WWA w glebie po zastosowaniu biowęgla. Zmiana gazu nośnego z N_2 na CO_2 spowodowała zwiększenie strat C_{tot} WWA (zmniejszenie trwałości) w glebie od 19 do 75% dla biowęgla z samego SL (BC) i od 49 do 206% w przypadku biowęgla otrzymanego z SL i wikliny (BCW) **(Rys. 16A, B)**. W przypadku C_{free} WWA zmiana N_2 na CO_2 zwiększyła straty C_{free} WWA jedynie w przypadku BC otrzymanego w temperaturze 500°C (o 21%). W glebie z pozostałymi biowęglami (BC otrzymanym w 700°C oraz BCW otrzymanymi w 500 i 700°C) zaobserwowano zwiększenie zawartości C_{free} WWA od 17 do 26% w porównaniu do tych samych biowęgli wytworzonych w atmosferze N_2 **(Rys. 17C, D)**.



Rysunek 16. Zmiana zawartości C_{tot} (A i B) oraz C_{free} (C i D) WWA w trakcie 180 dni

Na **rys. 17** przedstawiono wykres obrazujący różnice w trwałości i zmianie biodostępności sumy (Σ16) oraz poszczególnych grup WWA w ciągu 180 dni badań w zależności od zastosowanego gazu nośnego. W przypadku biowęgli otrzymanych z SL trwałość Σ16 C_{tot} WWA była większa, gdy do gleby dodano BC500-N_2 i BC700-CO_2. Różnice między dwoma wariantami temperaturowymi były związane z trwałością 3- i 4-pierścieniowych WWA. W przypadku 2- i 3-pierścieniowych WWA trwałość była większa, gdy do gleby dodano biowęgle otrzymane w CO_2. Podobnie w przypadku 6-pierścieniowych WWA i BC500-CO_2. Σ16 C_{tot} WWA w glebie z współpirolizowanymi biowęglami była trwalsza, gdy gazem nośnym był CO_2 w porównaniu do N_2. Najbardziej widoczne było to dla BCW700-CO_2 **(Rys. 17)**, gdzie trwałość większości grup (za wyjątkiem jedynie 2-pierścieniowych WWA) była większa, gdy gazem nośnym był CO_2. Natomiast w przypadku BCW500 zmiana gazu na CO_2 była korzystna tylko dla 2-pierścieniowych WWA **(Rys. 17)**.



Rysunek 17. Porównanie trwałości (na podstawie C_{tot}) i biodostępności (na podstawie C_{free}) WWA w glebie z dodatkiem biowęgli otrzymanych w azocie lub ditlenku węgla w trakcie 180 dni eksperymentu. BC- biowęgle otrzymane z SL, BCW- biowęgle otrzymane z mieszanki SL z wikliną

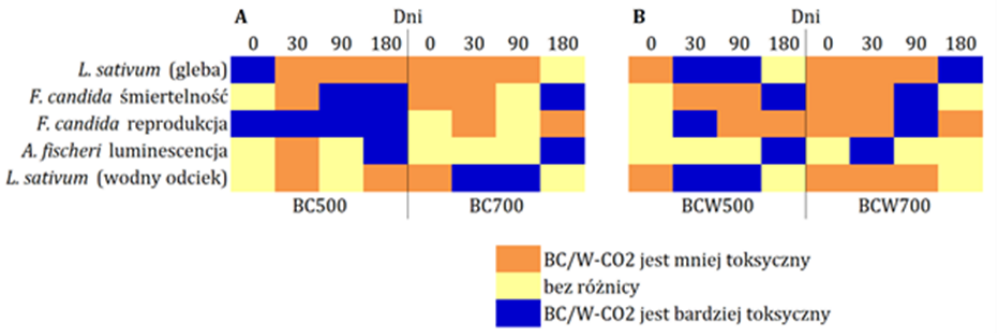
Na podstawie przeprowadzonych badań stwierdzono, że za różnice w trwałości WWA odpowiadały właściwości fizyko-chemiczne biowęgli, takie jak: wielkość powierzchni właściwej (S_{BET}) oraz porowatość (d, V_{tot}, V_{macro}, V_{micro}), które determinowały powinowactwo WWA do biowęgla (obniżenie adsorpcji), co zwiększało podatność WWA na biodegradację (po zastosowaniu biowęgli otrzymanych w CO_2).

Biodostępność WWA zmniejszyła się na skutek zmiany gazu z N_2 na CO_2 jedynie w przypadku BC otrzymanego w 500°C. W pozostałych wariantach nastąpiło zwiększenie biodostępności WWA. Obniżenie się biodostępności mogło być związane ze zwiększonym dostępem cząsteczek WWA do węgla organicznego, w wyniku czego następowała adsorpcja i zmniejszenie biodostępności szczególnie 3- i 4-pierścieniowych WWA.

5.7. Wpływ gazu nośnego podczas pirolizy na ekotoksyczność gleby z dodatkiem biowęgla (publikacja D8)

Celem pracy było określenie toksyczności biowęgli otrzymanych w różnych warunkach (temperatura: 500 lub 700°C, gaz nośny: N_2 lub CO_2, surowce: osad ściekowy lub osad ściekowy/biomasa) po dodaniu ich do gleby w długoterminowym eksperymencie wazonowym (180 dni). Testy przeprowadzono w oparciu o następujące organizmy testowe: *Aliivibrio fischeri*, *Lepidium sativum* i *Folsomia candida*.

Dla większości testów obserwowano obniżenie się toksyczności biowęgli (szczególnie po 180 dniach eksperymentu) po zamianie gazu nośnego z N_2 na CO_2 podczas pirolizy osadu ściekowego lub mieszaniny osadu i wikliny. Szczególnie korzystny wpływ zmiany gazu z N_2 na CO_2 zaznaczył się dla *L. sativum*. Najbardziej widoczny efekt zaobserwowano w przypadku biowęgla otrzymanego z mieszanki osadów i wikliny w 700°C (Rys. 18). Zmiana gazu w najmniejszym stopniu wpłynęła natomiast na *A. fischeri*. Wpływ zmiany gazu na *F. candida* był zróżnicowany w zależności od terminu badań **(Rys. 18)** oraz rodzaju biowęgla. Pozytywny wpływ zmiany gazu z N_2 na CO_2 był widoczny dla biowęgli otrzymanych z mieszkanki osadu i wikliny. Efekt ten zaznaczył się szczególnie w przypadku reprodukcji *F. candida*, gdy biowęgiel otrzymany z osadu i wikliny w 700°C w CO_2 stymulował (na poziomie 60%) reprodukcję przez cały czas trwania doświadczenia wazonowego.



Rysunek 18. Porównanie toksyczności gleby z dodatkiem biowęgli otrzymanych w azocie lub ditlenku węgla. A. biowęgle z osadów ściekowych, B. biowęgle ze współpirolizowanych osadów ściekowych i biomasy

W przypadku większości organizmów testowych zmiana gazu nośnego z N_2 na CO_2 podczas pirolizy powodowała obniżenie toksyczności biowęgla w ujęciu długoterminowym. Wykorzystanie CO_2 podczas pirolizy może być więc interesującym

kierunkiem poprawiającym (zmniejszającym) ekotoksykologiczny charakter biowęgla otrzymanego z osadu ściekowego. Korzystne działanie zmiany gazu szczególnie widoczne było w przypadku biowęgli otrzymanych z osadu ściekowego i wikliny. Wpływ zmiany gazu nośnego determinowany był jednak głównie rodzajem organizmu testowego. Czynnikami odpowiedzialnymi w największym stopniu za toksyczność gleby z dodatkiem biowęgli - wyznaczonymi na podstawie współczynników korelacji - była zawartość WWA, zawartość dostępnych form Mg, K i P oraz zawartość węgla organicznego w biowęglach.

6. Wnioski

1. Zarówno węgiel aktywny, jak i biowęgiel powodowały obniżenie frakcji potencjalnie biodostępnej (C_{acc}) WWA w glebie, przy czym lepszy efekt osiągano dla węgla aktywnego. Największe zmniejszenie C_{acc} obserwowano dla 5- i 6-pierścieniowych WWA (od 54 do 100%), podczas gdy najsłabsze dla związków 2-pierścieniowych (od 8 do 25%).

2. Wiklina uprawiana na glebie zawierającej węgiel aktywny i biowęgle, charakteryzowała się mniejszą zawartością fenantrenu niż na glebie kontrolnej. Jednakże obecność węgla aktywnego w glebie negatywnie wpływała na plon wikliny i długość pędów. Węgiel aktywny zmniejszał również zawartość DOC w glebie w zakresie od 54 do 67%. Nie stwierdzono natomiast wpływu biowęgli oraz roślin na zawartość DOC w glebie.

3. Dodanie adsorbentów do gleby nie spowodowało, w przypadku biowęgla, lub spowodowało tylko nieznaczne, w przypadku węgla aktywnego, zmniejszenie sumy biodostępnych (C_{free}) WWA. Wraz z upływem czasu skuteczność wiązania C_{free} WWA ulegała jednak zwiększeniu zarówno w doświadczeniu z biowęglem, jak i węglem aktywnym, przy czym lepszy efekt uzyskiwano dla węgla aktywnego.

4. Zmiana warunków pirolizy miała zróżnicowany wpływ na właściwości adsorpcyjne biowęgli w stosunku do fenantrenu i pirenu. Struktura i właściwości biowęgli uległy zmianom, co w znacznej mierze determinowało mechanizm wiązania badanych WWA przez biowęgiel.

5. Stosowanie ditlenku węgla zamiast azotu jako gazu nośnego podczas pirolizy wpływał korzystnie na właściwości adsorpcyjne biowęgli otrzymanych zarówno z osadu ściekowego, jak i ich mieszaniny z wikliną w stosunku do fenantrenu i pirenu.

6. Trwałość WWA (określona na podstawie C_{tot}) w glebie z dodatkiem biowęgla otrzymanego z osadu ściekowego z dodatkiem biomasy (BCW) była większa niż w glebie z dodatkiem biowęgla otrzymanego z samego osadu ściekowego (BC).

7. Frakcja biodostępna (określona na podstawie C_{free}) WWA w glebie z dodatkiem BCW ulegała wyższym stratom niż miało to miejsce w glebie z BC. Sugeruje to, że bezpośrednie ryzyko środowiskowe związane z obecnością WWA w biowęglach może być mniejsze w przypadku BCW niż BC.

8. Zamiana gazu nośnego podczas pirolizy z N_2 na CO_2 miała znaczący wpływ na właściwości biowęgli (przede wszystkim S_{BET}, średnica i objętość porów), które następnie, determinowały trwałość i biodostępność WWA w glebie z biowęglem.

9. Konwersja osadu ściekowego do biowęgla, dodatek wikliny podczas współpirolizy z osadem ściekowym oraz zamiana gazu nośnego z N_2 na CO_2 obniżały toksyczność w stosunku do większości badanych organizmów. Zaobserwowano wpływ temperatury

pirolizy na toksyczność biowęgli, jednak był on zróżnicowany w zależności od badanego organizmu.

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Streszczenie

Rozprawa doktorska pt.: „Ocena ryzyka w zastosowaniu biowęgla do gleb w kontekście trwałości i biodostępności WWA w glebach" składa się z cyklu powiązanych ze sobą tematycznie ośmiu artykułów opublikowanych w recenzowanych czasopismach naukowych, posiadających wskaźnik wpływu (ang. *impact factor*).

Badania przeprowadzone w ramach pracy doktorskiej składały się z dwóch głównych etapów. W pierwszym analizowano potencjał biowęgla do remediacji zanieczyszczonej gleby. Wyniki badań otrzymanych w ramach doświadczenia polowego (Publikacja D1 i D2) pozwoliły na analizę zmian zawartości biodostępnej frakcji WWA w zanieczyszczonej glebie z dodatkiem biowęgli lub węgla aktywnego. Zarówno węgiel aktywny, jak i biowęgiel powodowały obniżenie frakcji potencjalnie biodostępnej (C_{acc}) WWA w glebie, przy czym lepszy efekt osiągano dla węgla aktywnego. Jeśli chodzi o frakcję biodostępną (C_{free}) bezpośrednie dodanie adsorbentów do gleby nie spowodowało (w przypadku biowęgla) lub spowodowało tylko nieznaczne (w przypadku węgla aktywnego) zmniejszenie sumy C_{free} WWA. Oprócz tego zbadano wpływ zmiany warunków pirolizy na właściwości adsorpcyjne biowęgli otrzymanych z osadów ściekowych lub osadów ściekowych z dodatkiem wikliny w stosunku do dwóch związków zaliczanych do grupy WWA, fenantrenu i pirenu (Publikacja D3). Struktura i właściwości biowęgli uległy zmianom, co w znacznej mierze determinowało mechanizm wiązania badanych WWA przez biowęgiel.

W drugim etapie ocenie podlegało ryzyko dodawania biowęgla z osadów ściekowych do gleby niezanieczyszczonej. Przegląd literatury w zakresie tego tematu został przedstawiony w pracy przeglądowej (Publikacja D4). W ramach doświadczenia wazonowego przeprowadzone zostały badania gleby z dodatkiem biowęgli z osadów ściekowych otrzymanych w zróżnicowany sposób. Różnice obejmowały temperaturę otrzymywania (500, 600 i 700^{o}C), surowiec (dodatek wikliny do osadu przed pirolizą) oraz gaz nośny (azot lub ditlenek węgla). Badania dotyczyły zmian zawartości całkowitej i biodostępnej frakcji WWA w glebie (Publikacja D5 i D7) oraz właściwości ekotoksykologicznych gleb (Publikacja D6 i D8) w stosunku do organizmów pochodzących z różnych poziomów troficznych. Badania wykazały, że dodatek wikliny do osadu ściekowego zwiększa trwałość (na podstawie C_{tot}) i zmniejsza biodostępność (na podstawie C_{free}) WWA w glebie z dodatkiem biowęgla. Jest to korzystne dla środowiska i zwiększa bezpieczeństwo stosowania takiego materiału jako

dodatku do gleb. Ponadto zarówno obecność wikliny w osadzie, jak i zmiana gazu nośnego z N_2 na CO_2 zmniejszała wielokrotnie toksyczność biowęgli.

Abstract

Doctoral thesis entitled: „Risk assessment of biochar application to soil in terms of PAHs persistence and bioavailability in soil" comprises eight articles, thematically related to each other, and published in peer-reviewed scientific journals with an impact factor.

Research carried out as a part of the doctoral thesis consisted of two main stages. In the first one, the potential of biochar for the remediation of contaminated soil was analyzed. The results of the research obtained as part of the field experiment (Publications D1 and D2) allowed for the analysis of changes in the bioavailable fraction of PAHs in contaminated soil with the addition of biochars or activated carbon. Both activated carbon and biochar caused a decrease in the bioaccessible fraction (C_{acc}) of PAHs in the soil, with a better effect achieved for activated carbon. As for the bioavailable fraction (C_{free}), the direct addition of adsorbents to the soil did not (in the case of biochar) or caused only a slight (in the case of activated carbon) reduction in the sum of C_{free} PAHs. On top of it, biochars obtained from sewage sludge or sewage sludge with the addition of willow were tested for adsorption properties concerning two PAH compounds, phenanthrene and pyrene (Publication D3). Changing the pyrolysis conditions had a varied effect on the adsorption properties of biochars concerning phenanthrene and pyrene. The structure and properties of biochars changed, which largely determined the binding mechanism of the tested PAHs by biochar.

In the second stage, the risk of adding biochar from sewage sludge to uncontaminated soil was assessed. The literature review concerning the topic was published in one of the articles (Publication D4) As a part of the pot experiment, soil tests with the addition of biochars from sewage sludge obtained in various ways were carried out. The differences include the production temperature (500, 600, and 700°C), the feedstock (willow addition to the sludge before pyrolysis), and the carrier gas (nitrogen or carbon dioxide). The conducted research concerned changes in the content of total and bioavailable fractions of PAHs in the soil (Publications D5 and D7) and ecotoxicological properties of soil (Publications D6 and D8) in relation to organisms from different trophic levels. Research has shown that the addition of willow to sewage sludge increases the persistence (based on C_{tot}) and reduces the bioavailability (based on C_{free}) of PAHs in soil with the addition of biochar. This is beneficial for the environment and increases the safety of using such material as an additive

to soils. In addition, both the mixing of willow with sludge and the change of the carrier gas from N_2 to CO_2 reduced the toxicity of biochars.

Życiorys naukowy

W 2012 roku ukończyłam III Liceum Ogólnokształcące im. Unii Lubelskiej w Lublinie. W październiku 2012 r. rozpoczęłam studia I stopnia na kierunku Ochrona Środowiska na Wydziale Chemii Uniwersytetu Marii Curie- Skłodowskiej w Lublinie. W 2015 roku z wynikiem bardzo dobrym obroniłam pracę licencjacką pt. „Zastosowania metod termicznych w ochronie środowiska" napisaną pod kierunkiem Pani dr hab. Renaty Łyszczek, prof. UMCS. W październiku 2015 roku rozpoczęłam studia II stopnia na Wydziale Chemii UMCS w Lublinie, na kierunku Ochrona Środowiska. W 2017 roku uzyskałam tytuł magistra ochrony środowiska na podstawie pracy pt. „Wpływ modyfikacji powierzchni biowęgla związkami żelaza na adsorpcję jonów Se(VI)" (promotor: Pan prof. dr hab. Ryszard Dobrowolski/ Pan prof. dr hab. Patryk Oleszczuk) z wynikiem bardzo dobrym. W trakcie studiów I stopnia otrzymywałam stypendium z projektu „Od studenta do eksperta- ochrona środowiska w praktyce" (z Europejskiego Funduszu Społecznego), a w trakcie studiów II stopnia przez dwa lata otrzymywałam stypendium Rektora dla najlepszych studentów UMCS. W roku akademickim 2016/17 otrzymałam stypendium Ministra Nauki i Szkolnictwa Wyższego dla najlepszych studentów.

W październiku 2017 r. rozpoczęłam studia doktoranckie na Wydziale Chemii UMCS w Lublinie. Badania do rozprawy doktorskiej pt. „Ocena ryzyka w zastosowaniu biowęgla do gleb w kontekście trwałości i biodostępności WWA w glebach" realizowałam w Katedrze Radiochemii i Chemii Środowiskowej (wcześniej Zakładzie Chemii Środowiskowej) pod kierunkiem prof. dr hab. Patryka Oleszczuka. Mój dorobek naukowy składa się z 13 publikacji w czasopismach z listy JCR, 2 publikacji w języku polskim, 3 komunikatów na konferencjach międzynarodowych oraz 2 komunikatów na studenckich konferencjach krajowych. Uczestniczyłam w aplikowaniu oraz realizacji grantu z Narodowego Centrum Nauki - Preludium 16 jako kierownik i główny wykonawca w projekcie pt. „Trwałość i biodostępność macierzystych wielopierścieniowych węglowodorów aromatycznych z biowegla w glebach użyźnionych biowęglem otrzymanym w zróżnicowanych warunkach" (lipiec 2019 - obecnie).

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Projekty badawcze:

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- 2017 - Nauka i przemysł- lubelskie spotkania studenckie, temat posteru: Biowęgiel jako adsorbent zanieczyszczeń nieorganicznych
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- 10-14.11.2019 - IBI Biochar World Congress 2019, Seul, Korea Południowa; temat posteru: „Effect of mixture of sewage sludge and biomass and carrying gases (CO_2 and N_2) during pyrolysis on the toxicity of biochar-amended soil",
- 3-7.05.2020 - SETAC SciCon The SETAC Europe 30th Annual Meeting; referat na temat: „The effect of biomass addition to sewage sludge prior to pyrolysis on the polycyclic aromatic hydrocarbons persistence and bioavailability in biochar-amended soil",
- 3-6.05.2021 - SETAC Europe 31st Annual Meeting (virtual conference); referat na temat: : "The Effect of Carrier Gas Change During Pyrolysis on the Polycyclic Aromatic Hydrocarbons Persistence and Bioavailability in Biochar-Amended Soil".

Artykuły wchodzące w skład pracy doktorskiej

D1

P. Oleszczuk, P. Godlewska, D. D. Reible, P. Kraska,

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Bioaccessibility of polycyclic aromatic hydrocarbons in activated carbon or biochar amended vegetated (*Salix viminalis*) soil☆

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ABSTRACT

The aim of the present study was to determine the effect of activated carbon (AC) or biochars on the bioaccessibility (C_{bioacc}) of polycyclic aromatic hydrocarbons (PAHs) in soils vegetated with willow (*Salix viminalis*). The study determined the effect of willow on the C_{bioacc} PAHs and the effect of the investigated amendments on changes in dissolved organic carbon (DOC), crop yield and the content of PAHs in plants. PAH-contaminated soil was amended with 2.5 wt% AC or biochar. Samples from individual plots with and without plants were collected at the beginning of the experiment and after 3, 6, 12 and 18 months. The C_{bioacc} PAHs were determined using sorptive bioaccessibility extraction (SBE) (silicon rods and hydroxypropyl-β-cyclodextrin). Both AC and biochar caused a decrease in the C_{bioacc} PAHs. Immediately after adding AC, straw-derived biochar or willow-derived biochar to the soil, the reduction in the sum of 16 (Σ16) C_{bioacc} PAHs was 70.3, 38.0, and 29.3%, respectively. The highest reduction of C_{bioacc} was observed for 5- and 6-ring PAHs (from 54.4 to 100%), whereas 2-ring PAHs were reduced only 8.0–25.4%. The reduction of C_{bioacc} PAHs increased over time. Plants reduced C_{bioacc} in all soils although effects varied by soil treatment and PAH. Willow grown in AC- and biochar-amended soil accumulated less phenanthrene than in the control soil. The presence of AC in the soil also affected willow yield and shoot length and DOC was reduced from 53.5 to 66.9% relative to unamended soils. In the biochars-amended soil, no changes in soil DOC content were noted nor effects on willow shoot length.

1. Introduction

Soil contamination is a serious problem because it can lead to the accumulation of contaminants in plants and subsequently in the human food chain. Various methods of soil remediation have been used(de Boer and Wagelmans, 2016). However, conventional methods are costly and significantly interfere with the natural environment. The use of activated carbon (AC) has attracted great interest in recent years (Ghosh et al., 2011; Kupryianchyk et al., 2015) due to the strong affinity of contaminants for AC (Ghosh et al., 2011; Rakowska et al., 2014) and the resulting binding of the bioavailable/bioaccessible fraction of contaminants (Ghosh et al., 2011; Millward et al., 2005; Reible, 2014; Stringer et al., 2014). A significant reduction of bioaccessible contaminants in sediments contaminated with PAHs, PCB, mercury, methylmercury, PCDD/F and DDT was obtained by applying AC (Ghosh et al., 2011; Gomez-Eyles et al., 2013; Patmont et al., 2015; Samuelsson et al., 2015; Tomaszewski et al., 2008; Werner et al., 2005; Zimmerman et al., 2004). The binding of the bioaccessible fraction was often associated with a simultaneous reduction in the accumulation of these contaminants by various aquatic organisms (McLeod et al., 2007; Millward et al., 2005; Samuelsson et al., 2015; Tomaszewski et al., 2008). To date, research has been mainly focused on sediments and few studies of this type relate to soils (Brändli et al., 2008; Jakob et al., 2012; Kupryianchyk et al., 2016b; Oen et al., 2012) which have a different specificity and different properties than sediments. Studies on soil at field scale are relatively few (Hale et al., 2012a) and are typically of short duration.

Biochar is another adsorbent that could be an alternative to AC (Ahmad et al., 2014). Biochar has a smaller surface area than AC and its efficiency in binding organic contaminants (polycyclic aromatic hydrocarbons, polychlorinated biphenyls, etc.) is also smaller

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(Kupryianchyk et al., 2016b). However, biochar has many advantages. Biochar is cheaper comparing to AC. Conversion of biomass to biochar and its use for soil amendment have been proposed as one of the best methods of climate change mitigation through soil carbon sequestration (Lehmann, 2007). Moreover, biochar can be obtained from waste biomass or other waste products. Biochar contains many nutrients, which contributes to an improvement in soil properties and crop yields (Hussain et al., 2016). These advantages are particularly important when it is planned to grow crops in a rehabilitated area. In this case, the fertilizing advantages of biochar can be of great importance. The cultivation of plants (e.g. energy crops) can also aid remediation. The effect of plants on PAH degradation (phytoremediation) has been well described in the literature (de Boer and Wagelmans, 2016). It is known that root exudates can be a factor stimulating the growth of organisms capable of degrading PAHs.

The aim of the present study was to evaluate the efficiency of binding of the bioaccessible (C_{bioacc}) fraction of PAHs in soil contaminated with these compounds under natural conditions. The study also evaluated the effect of AC and biochar on changes in the content of soil dissolved organic matter (DOC), crop yield and PAH accumulation in willow as a secondary effect of amendment. The effect of willow on the content of C_{bioacc} PAHs in AC- or biochar-remediated soil was also evaluated.

2. Materials and methods

2.1. Adsorbents

The biochar used in the experiment was a commercial biochar provided by Fluid SA company (Poland) obtained from dried willow (*Salix viminalis*) (BCW) through a slow pyrolysis process in temperature range 600–700 °C. The second biochar was produced from wheat straw in temperature range 600–700 °C. This biochar was provided by MOSTOSTAL company (Poland). Activated carbon (AC) was purchased from POCH company (CAS: 7440-44-0, Poland). The physico-chemical properties of AC and biochars are presented in Table 1 and the methods of its determination in supporting information (SI).

2.2. Field experiment

The field experiment was performed nearby Chełm, Lubelskie, Poland (51°11′49.7″N 23°15′01.2″E). The experiment consisted of 7 plots (all plots were prepared in duplicates). The contaminated soil (Table 2) was transported from Dąbrowa Górnicza, Silesia, Poland and was associated with a Coking facility. The soil was homogenized and put in 2 m (w) x 2 m (l) x 0.2 m (d) plots. The study employed four mesocosms: (1) control (without any amendments), (2) treatment with biochar – BCW, (3) treatment with biochar – BCS and (4) treatment with activated carbon (AC). Particular amendments were added to the soil once in 2014 with the quantity of amendment corresponding to 50 t/ha (2.5 dry wt % of soil). The biochar dose was chosen as a most effective one based on previous study referred to the PAHs reduction in soils by biochars (Kołtowski et al., 2016b). Willow was planted on such prepared plots. Additionally, to evaluate the effect of plants on the content of C_{bioacc} PAHs, an experiment without plants (three additional mesocosms) was carried out concurrently to the experiment with willow. The unplanted experiment concerned only control and AC and BCW amendments. Soil samples were sampled five times from 2014 to 2015 (April 2014, July 2014, October 2014, April 2015, October 2015). Control soil (non-amended) and AC or biochar-amended soil samples were collected from the level of 0–20 cm with a (5–60 cm i.d.) stainless steel corer. Six independent samples (pseudo-replicates) were taken from each plot. The samples were transported to the laboratory, air dried in air- conditioned storage rooms (about 25 °C) for several weeks (in darkness), manually crushed, and sieved (<2 mm) prior to chemical analyses. After harvesting in October 2015 the willow shoots were thoroughly rinsed with water to remove soil and AC or biochar particles. Plant samples were air-dried at 25 °C and were ground to one sample and stored at −18 °C prior to analysis.

2.3. Bioaccessible (C_{bioacc}) PAH content

Bioaccessible concentration (C_{bioacc}) of PAHs was determined using silicon rods according to Gouliarmou and Mayer (2012). The silicon rods were cleaned before use by Soxhlet extraction with ethyl acetate for 100 h. Hydroxylpropyl-β-cyclodextrin (HPCD) solution was prepared by adding 75 g of HPCD and 200 mg of NaN_3 in 1 L of mili-Q water. Clean and dry silicone rods (length = 3 m) were placed in empty 100 mL Pyrex bottles. Then 100 mg of sample (soil or soil-biochar mixtures) and 50 mL of HPCD-solution were added to each bottle. Next, the samples were shaken in horizontal, orbital shaker at >200 rpm at room temperature for 30 d. HPCD were used a diffusive carrier to enhance desorption of PAHs from the matrix. The silicon rod continuously absorbs contaminants from the HPCD solution, effectively measuring both freely dissolved and reversibly bound PAHs, which together are defined as the bioaccessible fraction. Recovery standards were spiked and extraction was carried out using 2 × 100 mL of acetone without shaking once for 6 h and once overnight. The acetone extracts were combined and concentrated to 1 mL.

2.4. Gas chromatography – mass spectroscopy (GC-MS)

A qualitative and quantitative analysis of PAHs was carried out using gas chromatograph (Trace 1300) mass spectrometry (ISQ LT) (GC-MS, Thermo Scientific). The GC-MS was equipped with a single quadrupole and used under the select ion monitoring mode. A Rxi®-5 ms crossbond® 5% diphenyl and 95% dimethyl polysiloxane fused capillary column (30 m × 0.25 mm ID x 0.25 μm film thickness) from Restek (USA) was used with helium as the carrier gas at a constant flow rate of 1 mL min^{-1}. The detection was performed with a Thermo Scientific ISQ LT mass spectrometer in the electron impact mode with a −70 eV ionisation energy and a dwelling time of 22 milli-seconds.

Table 1
Properties of adsorbents used in the experiment.

Biochar	pH	C	H	N	O	Ash	H/C	O/C	(O+N)/C	S_{BET}	S_{mic}	V_p	V_{mic}	Σ16 PAHs
AC	6.0	85.50	0.27	0.62	3.45	10.16	0.038	0.03	0.036	617.6	306.7	0.374	0.148	0.9
BCW	9.1	52.20	2.23	1.13	25.15	19.07	0.043	0.273	0.380	5.3	4.1	0.009	0.002	4.6
BCS	9.9	53.87	1.76	0.91	2.32	41.15	0.039	0.032	0.046	26.3	10.8	0.026	0.005	19.9

pH: reactivity in KCl; C, H, N, O: elemental composition [%]; Ash: ash content [%]; H/C, O/C and (O+N)/C – molar ratios; S_{BET}: surface area [m^2/g]; S_{mic}: micropore area [m^2/g]; V_p: total pore volume [cm^3/g]; V_{mic}: micropores volume [cm^3/g]; R: average pore radious [nm]; Σ16 PAHs: sum of total content of 16 PAHs [mg/kg]; Σ16 C_{free}: sum of freely dissolved 16 PAHs [ng/L].

Table 2
Properties of the soils used in the experiment.

Soil	Sand (%)	Silt (%)	Clay (%)	pH	CEC (mmol/100 g)	TOC (%)	DOC (mg/L)	BC (%)	Nt (%)	P_2O_5 (mg/100 g)	K_2O (mg/100 g)	Mg (mg/100 g)	Σ16 PAHs (mg/kg)
DG	77	18	5	7.49	99.2	8.18	28.57	3.0	0.155	77.6	13.6	18.4	16.9

pH: reactivity in KCl; CEC - the cation exchange capacity, expressed as sum of the bases Ca, Mg, K, Na [mmol/100 g]; TOC – the organic carbon content [%], DOC- dissolved organic carbon [mg/L], BC- black carbon content [%], P_2O_5, K_2O and Mg – available forms of phosphorous, potassium and magnesium [mg/100 g], Σ16 PAHs – the sum of 16 PAHs according to US EPA.

The linearity (R2 > 0.99) was given for a calibration from 10 to 2500 ng/mL and for each PAH-compound. The limits of quantification (LOQ) ranged from 0.0002 (IPY) - 0.3110 (NAP) ng/L for POM and 0.1 (CHR) – 0.7 (DBA) μg/kgdry weight (dw) for total PAH concentrations and was obtained from three times the limit of detection (LOD). Blanks were run for total PAH concentrations (thimble filled with Na_2SO_4) and for the C_{bioacc} (silicon rods but no sample). The recoveries were quantified by the deuterated internal standards (added before extraction) to the recovery standard (TTB) over the same ratio in the calibration. The recoveries ranged from 77% to 108% for individual PAHs. The reported results have not been corrected for losses.

3. Results and discussion

3.1. PAHs content in control soil

The total content of the sum of 16 (Σ16) PAHs in the soil was 15,800 μg/kg. The 4- and 5-ring PAHs were mainly dominant in the soil, accounting for 46.2 and 25.7%, respectively. The 6-ring PAHs were also found to have a significant proportion in the soil (15.4%). Among the individual PAHs, fluoranthene (16.6%), pyrene (12.3%) and chrysene (11.1%) had the highest percentages (Table S1). The level of soil contamination is similar but lower than other sites with a similar use history (Liao et al., 2013; Liu et al., 2016). The values of the molecular diagnostic ratios for identification of PAH source (Fig. S1) for the soil fall within the range characteristic for contaminants of pyrogenic origin and diesel/petroleum, coal and/or wood combustion, and thus related with coking production.

The content of the Σ16 C_{bioacc} fraction in the control soil as measured by the method of (Kołtowski et al., 2016a) was 2880 μg/kg and accounted for 19.8% of the total PAH content in this soil. The fraction of bioaccessible PAH, C_{bioacc} PAHs/total PAH concentration, decreased with decreasing molecular mass of the investigated compounds and their affinity for lipids (log K_{ow}) (Fig. S2). This is understandable taking into account that light PAHs are more easily soluble in water and more mobile than heavier PAHs. Due to this, light PAHs will be transferred more easily from the soil to the soil solution, thus exhibiting higher bioaccessibility. The 2-ring (38.2%) and 4-ring (26.2%) PAHs were predominant in the C_{bioacc} fraction.

Fig. 1A shows the change in Σ16 C_{bioacc} PAHs in the control soil over the course of the experiment. A gradual reduction in the content of the studied compounds was noted throughout the entire study period. It was ultimately found that after 18 months of the experiment Σ16 C_{bioacc} PAHs had decreased by 51% compared to the beginning of the study. The highest reduction in the content was found in the case of 6-, 5- and 4-ring PAHs, respectively by 97, 75, and 74%. The observed changes are likely associated with sequestration rather than biodegradation due to the refractory nature of these PAHs. The preferential degradation of light PAHs would be expected since they are more easily accessible to microorganisms and are degraded more easily (Haritash and Kaushik, 2009). In this study, a much higher decrease in the control soil was observed in the 4-6-ring PAHs compared to 2-3-ring compounds. A reduction in C_{free} and C_{bioacc} PAHs over time has also been observed by other authors (Hale et al., 2012a).

3.2. Effect of AC or biochar application on C_{bioacc} PAHs

Fig. 1A shows the impact of the amendments on the soil content of Σ16 C_{bioacc} PAHs. Adding the BCW and BCS biochars to the soil reduced the C_{bioacc} content by 29.3 and 38.0%, respectively, in relation to the control soil (Fig. 1B). A slightly better effect achieved for BCS resulted from the larger surface area of this material compared to the BCW biochar (Table 2). The level of reduction observed after application of the biochars was similar to that found in studies of other authors (Beesley et al., 2010; Gomez-Eyles et al., 2013, 2011; Khan et al., 2015). Beesley et al. (2010) observed a more than 40% reduction of bioavailable PAHs after 60 days of soil incubation with hardwood-derived biochar. A lower reduction of freely dissolved PAHs (<34%) was observed by Gomez-Eyles et al. (2011) after 56 days of incubation of soil contaminated with these compounds, also using hardwood-derived biochar for PAH immobilization. The reduction of bioavailable PAHs observed by Khan et al. (2015) after application of biochars derived from sewage sludge, soybean straw, rice straw and peanut shells ranged from 27 to 80% depending on biochar dose and type. The addition of activated carbon (AC) to the soil reduced Σ16 C_{bioacc} PAHs to the greatest extent (by 70.3% relative to the control) among the materials tested (Fig. 1B).

Analyzing the changes in the particular PAH groups (Fig. 2), the effectiveness of the individual materials was determined by the material as well as the characteristics of the PAHs. Increasing reductions in C_{bioacc} were observed with increasing molecular mass (Fig. S3). This results from increasing affinity of PAHs for carbonaceous materials with higher molecular mass PAHs (Kupryianchyk et al., 2016a). The weakest effect of C_{bioacc} reduction was observed for 2-ring PAHs. The biochar amendment did not affect the reduction of C_{bioacc} naphthalene, whereas AC caused a 25% decrease in their content. The lack of change for the biochars or an insignificant increase could be attributable to the leaching of part of the PAHs from the biochars, which had also been observed in a previous study (Brennan et al., 2014a). The range of reduction of 3-, 4- and 5-ring PAHs was respectively from 24 to 94%, from 30 to 98%, and from 54 to 100%, depending on the adsorbent used. The 6-ring PAHs were the PAH group for which the best reduction was obtained, regardless of the material used. Depending on the material, the reduction ranged between 78 and 100%.

Previous studies, that focused on C_{free} as an indicator of bioavailability tended to show greater reductions (Brändli et al., 2008; Brennan et al., 2014a; Hale et al., 2012a; Reichenberg and Mayer, 2006; Zimmerman et al., 2004). This is understandable since the C_{bioacc} fraction includes a larger part of contaminants that the C_{free} fraction. Contaminants in C_{bioacc} fraction are less easily accessible and their transfer to AC is more restricted than for C_{free}. For example, Brändli et al. (2008) and Hale et al. (2012a) observed a greater reduction in C_{free} of light (2-4-ring) PAHs than in the case of heavy (>5-rings) PAHs, which they explained by the quicker transfer of the light PAHs to the biochar than that of the heavy PAHs. A similar relationship was also observed by Brennan et al. (2014a) after AC application to contaminated soil. However, the latter authors did not observe the biochar to affect the reduction of C_{free} PAHs. C_{free} defines the current concentration of compounds

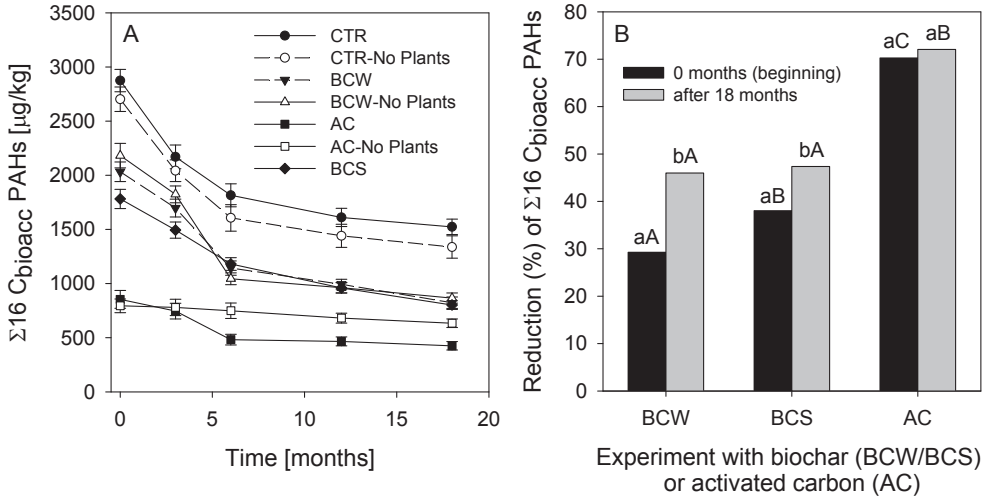

Fig. 1. Changes (A) and reduction (B) of/in Σ16 C_{bioacc} PAHs in AC or biochar (BCS, BCW)-amended soil in experiment with willow (solid line) or in unplanted soil (dotted line). Error bars represent standard deviation error (SD, n = 3 extractions). Different letters mean statistically significant differences (≤0.05) (small letters – between terms, capital letters – between amendments).

dissolved in the soil solution (pore water concentration), while C_{bioacc} additionally attempts to include contaminants that are adsorbed in a reversible manner, that after a certain time can undergo desorption (Reichenberg and Mayer, 2006). In determining C_{bioacc} PAHs using cyclodextrins, Gomez-Eyles et al. (2011) and Beesley et al. (2010) observed after biochar application a greater reduction of heavier PAHs than lighter PAHs. We observed a similar relationship in our previous study conducted under laboratory conditions (Kołtowski et al., 2016a). It should therefore be presumed that the observed differences are largely attributable to the different bioavailability methods used.

Regardless of the PAH group, the application of AC produced the greatest reduction in bioavailability, followed by BCS and BCW. The 6-ring PAHs, for which the differences between the BCW and BCS biochars were not statistically different, were an exception. The reduction of the individual C_{bioacc} PAH groups after AC application was each time from 15 to 75% higher than for the biochars.

3.3. Effect of time on C_{bioacc} immobilization by AC or biochar

As indicated earlier, unamended soil also showed decreasing C_{bioacc} PAHs over time. The Σ16 C_{bioacc} PAHs reductions with AC and biochar- amended soil, however, were significantly lower ($P \geq 0.05$) that in controls. The most rapid reductions relative to controls were noted in the 6 months after addition of the AC or biochar. In the experiment with AC, no significant change in Σ16 C_{bioacc} PAHs was found (Fig. 1A) in the 12th and 18th month samples relative to the 6th month.

In the case of 3-6-ring PAHs, adding AC was effective from the beginning of the experiment and the range of reduction did not vary significantly over time (Fig. 2). Only naphthalene decreased over the entire duration of the experiment with a more rapid reduction over the first 6 months followed by a continued slow reduction from the 6th to the 18th month, likely due to biodegradation, leaching or volatilization.

In the case of the biochars, substantial statistically significant differences ($P \geq 0.05$) between BCS and BCW amended soils were noted initially but these differences decreased over time. The initial differences in effectiveness of the BCW and BCS biochars is likely associated with the higher surface area of the BCS biochar. We observed a similar relationship in an earlier study where a high surface area biochar also bound more quickly but ultimately no significant differences between biochars were noted (Stefaniuk and Oleszczuk, 2016). Although the biochars continued to reduce C_{bioacc} over the entire 18 months, AC C_{bioacc} PAHs remained much lower (factor of 2). The different behaviors observed over the term of the experiment indicate the need for long-term experiments. For example, after 21 days of incubation Brennan et al. (2014a) observed in some cases even a several-fold difference in the reduction of C_{free} PAHs between biochar and AC. In our previous study (Kołtowski et al., 2016b) in which the aging lasted 31 days, the differences in the reduction of C_{free} PAHs after application of AC and biochars were also several-fold. Longer term these differences moderated significantly.

3.4. Effect of plants on C_{bioacc} PAHs

To evaluate the effect of plants on the content of C_{bioacc} PAHs, an experiment without plants was carried out concurrently to the experiment with willow. For Σ16 PAHs, plants were not found to have a significant effect on the C_{bioacc} content in the control soil and in BCW-amended soil throughout the entire study period (Fig. 1A). Nevertheless, significant differences ($P \geq 0.05$) were noted in AC-amended soil in the period from the 6th to the 18th month. In the soil with willow, the content of Σ16 C_{bioacc} PAHs in AC-amended soil was found to be lower from 46 to 55% (depending on the date) (Table S2) than in AC-amended soil without plants.

The effect of plants on the individual PAHs was dependent on the amendment used, experimental duration and the hydrophobicity of the PAH (Table S2, Fig. S4). In the case of 2-ring PAHs, lower values ($P \geq 0.05$) of C_{bioacc} PAHs (from 49 to 60%) were noted for AC amended soil with willow (Fig. S4) compared to AC-amended unplanted soil (between 6 and 18-months). In the case of 3-ring PAHs, significant differences ($P \geq 0.05$) between planted and unplanted soil were found at the beginning of the study for BCW-amended soil, 3rd month for AC-amended soil and from the 6th month until the end of the study for unamended soil (Table S2, Fig. S4). In this latter case, however, a higher content of C_{bioacc} PAHs (from 18 to 22%) was noted in soil with willow than in unplanted soil. A similar trend in unamended soil was also observed for 4-ring PAHs (the content of C_{bioacc} PAHs was higher from 16.3 to 47% in soil with willow compared to unplanted soil) and 5-ring PAHs (the

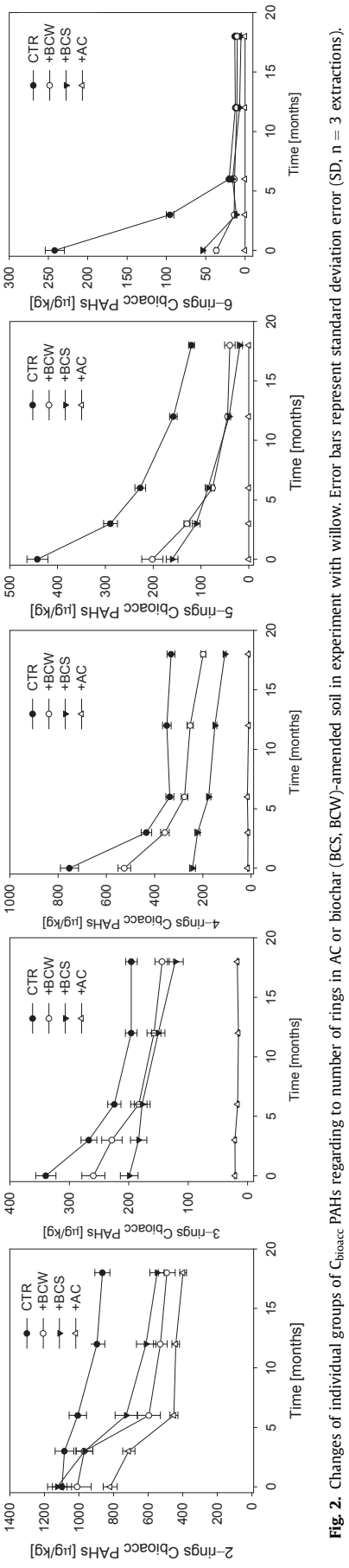

Fig. 2. Changes of individual groups of C_{bioacc} PAHs regarding to number of rings in AC or biochar (BCS, BCW)-amended soil in experiment with willow. Error bars represent standard deviation error (SD, n = 3 extractions).

content of C_{bioacc} PAHs was higher from 13 to 35% in soil with willow compared to unplanted soil).

In the case of BCW-amended soil (Fig. S4) the C_{bioacc} of 5 ring PAHs in soil with willow was lower (from 46 to 106%) compared to unplanted soil. Willow was not observed to have a significant ($P \geq 0.05$) effect on the content of 6-ring C_{bioacc} PAHs.

In general, in unamended soil the plants increased the accessibility of PAHs, whereas in amended soil they had no effect or decreased C_{bioacc}. The increase in C_{bioacc} content could have been due to the influence of root exudates (Gao et al., 2015; Reichenauer and Germida, 2008; Sun and Gao, 2013). For example Gao et al. (2015) stated that low-molecular-weight organic acids (components of root exudates) can promote the release of bound PAH residues and enhance the extractability and availability of PAHs in soil. Sun et al. (2013) found that the n-butanol extractable amounts of phenanthrene in soil increased with increasing the concentrations of root exudates. Our previous study also revealed (Oleszczuk and Baran, 2006) that rhizospheric soil contains more bioaccessible PAHs than bulk soil. It is also known that rhizospheric microbes actively produce rhamnolipids (bio-surfactants), which decrease the surface tension in such a way that contaminants can easily move into the liquid phase (Reichenauer and Germida, 2008). The lack of an observed effect of the plants on C_{bioacc} PAHs on amended soils may result from the adsorption of exudates by the amendment. Decreasing C_{bioacc} PAHs in presence of plants and amendments in some cases may be explained that biochar and/or AC can stimulate formation of strong bonds between contaminants and soil organic matter under the influence of plant enzymes. Moreover, biochar, can also enhance microbiological activity (Oleszczuk et al., 2014) and thus decrease the content of C_{bioacc} PAHs via biodegradation. However, this tendency was observed only for 5-rings (biochar) and 2-ring (AC) PAHs, which can be also the effect of random variations.

3.5. *Effect of AC or biochar amendment on PAHs content in willow*

Figs. 3A and S5 show the effect of biochar and AC addition, respectively, on selected willow parameters, including PAH content in the willow shoots. No significant differences were found in the case of the Σ16 PAH content in willow grown in the control soil compared to the treatments with the additions of BCW and BCS biochar. In AC-amended soil the content of Σ16 PAHs in the willow shoots was lower compared to unamended soil. The 5- and 6-ring PAHs were not found to be present in the plants, regardless of the experimental treatment. The absence of these PAHs is likely due to the limited transpiration associated with these highly hydrophobic compounds.

Willow grown on amended soils contained significantly less phenanthrene (by 9.8% - BCW, 13.7% - BCS, and 28% - AC) than willow grown in unamended soil (Fig. 3A). In this case, a significant correlation was observed (Fig. 3B) between the phenanthrene content in the plant and the C_{bioacc} phenanthrene content in the soil. Phenanthrene is a common PAH that can accumulate in plants (Chiapusio et al., 2011; Jakob et al., 2012). A significant reduction ($P \geq 0.05$) in accumulation was also found for acenapthene after application of BCW (by 36.6%) and AC (by 28.0%), chrysene after application of BCS (by 12.0%) and AC (by 23.8%) as well as for acenaphthylene after application of BCS (by 25.8%) (Fig. 3A, Table S3). However, there was no clear trend with the addition of sorbing amendments. To date, there have been few studies on the effect of AC or biochar on bioaccumulation of PAHs by plants. However, most studies demonstrate a significant reduction in PAH accumulation after application of AC or biochars (Brennan et al., 2014b; Jakob et al., 2012; Khan et al., 2015; Waqas et al., 2014). Jakob et al. (2012) observed a reduction in PAH accumulation by ryegrass,

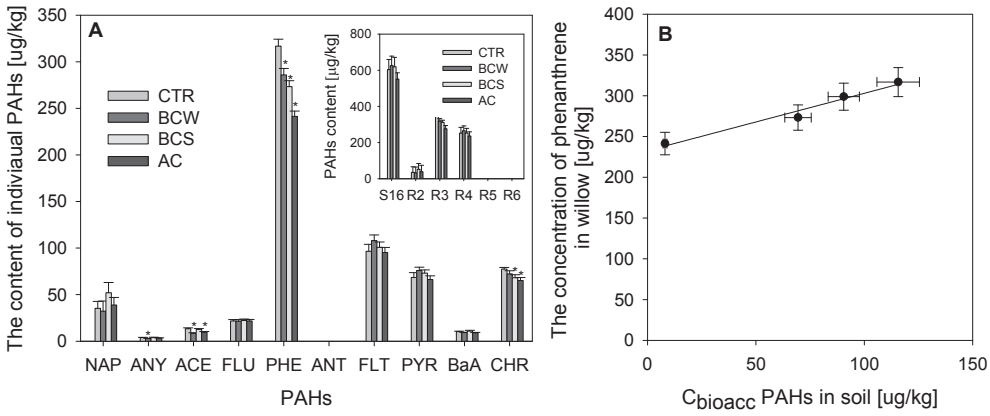

Fig. 3. The concentration of PAHs in willow (µg/kg) (A) and correlation between C_{bioacc} PAHs in soil (µg/kg) and in willow (µg/kg) (B). Error bars represent standard deviation error (SD, n = 3 extractions). * - mean statistically significant differences (≤0.05).

carrot and squash from 46 to 56% comparing to non-amended soil. A similar range of reduction in PAH accumulation (from 44 to 57%) was also observed in the fruit of *Cucumis sativa* L. by Waqas et al. (2014) and in *Brassica rapa* L. roots by Khan et al. (2015). The relatively higher values of bioaccumulation reduction observed in the above references are probably due to the different plant species and primarily the different part of plant investigated (roots, fruit vs shoots in the present experiment). Plants take up pollutants via roots and leaves. Roots have a direct contact with contaminated soil and hence the accumulation of contaminants in them is greater. The subsequent transport of contaminants to the shoots is smaller and its dynamics depends on many factors. Moreover, PAHs may be sorbed through the epidermis (Ogbonnaya et al., 2014) and remain in the roots. Jakob et al. (2012) also found a greater reduction of accumulation in roots than in stems after adding AC to the soil. Similarly as in the present study, Jakob et al. (2012) observed lower phenanthrene accumulation in shoots after AC application to soil, which explains a lower translocation of these compounds from roots to shoots. As in the present study, these authors did not observe a significant reduction in accumulation of heavier PAHs (≥4-rings) after AC application.

Thus far, the only comparative study concerning AC and biochars with respect to the accumulation of PAHs by plants has been carried out by Brennan et al. (2014b). AC and biochars reduced the PAH content in shoots but not in roots. These authors did not find a significant difference in accumulation between AC and biochar. In this study, except for phenanthrene no significant differences were found in plant accumulation between AC and the biochars. Frequently, the biochars reduced the bioaccumulation of the individual PAHs better than AC (e.g. acenaphtylene or acenaphtene). It may be explained by differences between particular biochars and biochars and AC properties creating differences in soil conditions, which affect PAHs accumulation by plant (Atkinson et al., 2010). As some suggest (Brennan et al., 2014b), the differences in the production of root exudates under the influence of biochar or AC can also affect the uptake of PAHs.

The amendments influenced only some willow parameters (Fig. S5). The greatest effect of all amendments could be seen for yield. The addition of BCW increased the yield more than twice, whereas the presence of BCS and AC reduced it. The addition of AC to the soils resulted in a decrease in the average shoot length, which was associated with an increased percentage of bark. The previous studies on AC have also shown that AC (in particular powdered AC) can reduce the growth and development of plants (Jakob et al., 2012; Kołtowski and Oleszczuk, 2016) and can negatively affect bacteria and invertebrates (Hale et al., 2013; Jonker et al., 2009). The observed negative influence of AC on the plants is primarily due to an indirect effect associated with the impact of AC on the soil physical and chemical properties, especially water and oxygen availability (Jośko et al., 2013) as well as DOC content (Hale et al., 2012b). In the case of biochars, an opposite effect can be observed as regards their impact on plants (Buss et al., 2016; Oleszczuk et al., 2013).

3.6. Dissolved organic carbon content

AC reduced the concentration of dissolved organic carbon (DOC) in the soil (Fig. 4). Depending on the date, the DOC content in AC-amended soil was 53.5–66.9% lower than in non-amended soil. The observed reduction in DOC was in the range that had been previously observed by Hale et al. (2012b) Such a substantial reduction in DOC may have a detrimental effect on soil organisms and plants (as was observed above). The biochars did not affect significantly the soil DOC content, which may be associated with the fact that biochars themselves can be a source of DOC (Tang et al., 2016). Despite that DOC continually fluctuated, irrespective of the type of biochar, no significant differences were found in its content in the period from the beginning to the 12th month of the study

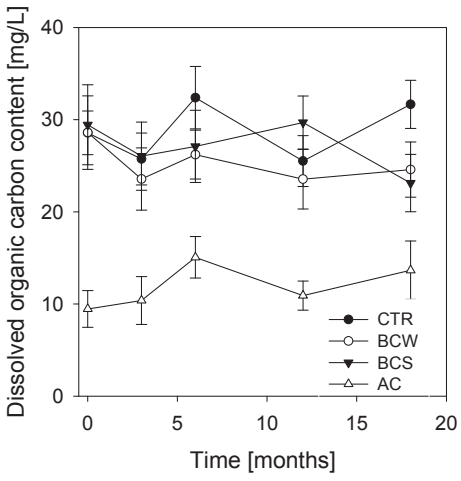

Fig. 4. Dissolved organic carbon (DOC) content (mg/L) in control soil and AC or biochar-amended soil. Error bars represent standard deviation error (SD, n = 3 determination).

(Fig. 4). Plants were also not found to significantly affect soil DOC content (Fig. S7).

4. Conclusion

This research is the first study which compared in a long-term experiment the efficiency of binding of C_{bioacc} PAHs by biochar and AC under in situ conditions and in the presence of plants. AC proved to be very quick in binding C_{bioacc} PAHs while the biochars bound C_{bioacc} PAHs more slowly but continuously reduced C_{bioacc} over the entire time period of experiment. Plants showed substantial reductions in accumulation with the amended soils relative to unamended soils. AC, however, also reduced plant growth and available DOC in soils while biochar enhanced plant growth and did not change DOC concentrations in the soil.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2017.04.064.

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Supporting information:

BIOACCESSIBLITY OF POLYCYCLIC AROMATIC HYDROCARBONS IN ACTIVATED CARBON OR BIOCHAR AMENDED VEGETATED (*SALIX VIMINALIS*) SOIL

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Analysis of physico-chemical properties of soil, biochars and activated carbon

The pH was measured potentiometrically in 1 M KCl after 24 h in the liquid/soil ratio of 10 (w/v). The cation exchange capacity (CEC, expressed as sum of the bases Ca, Mg, K, Na) was determined in the 0.1 M HCl extraction. The amounts of carbon, hydrogen and nitrogen were determined using a CHN equipment (Perkin–Elmer 2400, USA). The total organic carbon content (TOC) was determined by the dry combustion method using TOC-VCSH (SHIMADZU, Japan) with Solid Sample Module SSM-5000. The DOC content was determined according to Jones and Willett (2006). The textural characteristics of the biochars were recorded with a Micromeritics ASAP 2405 N_2 adsorption analyser (USA) by performing low-temperature (77.4 K) nitrogen adsorption–desorption isotherms. The specific surface area (SBET) of the AC and the biochars was determined according to the Brunauer – Emmett – Teller isotherm.

Table S1. The total polycyclic aromatatic hydrocarbons content (μg/kg) and contribution of individual PAHs in total PAHs content in soil used in the experiment

PAHs	Total	Contribution of individual PAHs [%]
NA	216.3±20.8	1.4
ACE	70.9±5.8	0.4
AC	80.4±5.9	0.5
FL	109.5±4.9	0.7
PHEN	1244.2±73.5	7.9
ANT	278.2±25.3	1.8
FLUO	2608.7±134.8	16.6
PYR	1944.4±104.8	12.3
BaA	974.7±59.7	6.2
CHR	1752.2±83.8	11.1
BbF	1414.2±94.0	9.0
BkF	1202.0±90.8	7.6
BaP	1232.3±60.3	7.8
IcdPd	1030.5±85.7	6.5
DahA	199.3±13.2	1.3
BghiP	1398.2±89.2	8.9
Σ16 WWA	15755.8±287.2	100

Values are mean of three repetitions. NAP - naphthalene; ACE - acenaphthylene; AC – acenaphthene; FL - fluorene; PHEN – phenanthrene; ANT – anthracene; FLUO – fluoranthene; PYR – pyrene; BaA - benzo(a)anthracene; CHR – chrysene; BbF - benzo(b)fluoranthene; BkF - benzo(k)fluoranthene; BaP- benzo(a)pyrene; IcdP- indeno(1,2,3-cd)pyrene; DahA – dibenz(a,h)anthracene; BghiP – benzo(ghi)perylene

Table S2. The difference (in %) between C_{bioacc} PAHs content in unplanted and planted soil regarding to number of rings

Number of rings	Non-amended					BCW-amended					AC-amended				
	0[#]	3	6	12	18	0	3	6	12	18	0	3	6	12	18
2-rings PAHs	2.6	-9.5	4.3	-1.6	-3.5	-1.4	-9.2	17.1*	14.2*	0.3	7.1	-3.6	-59.6*	-49.3*	-53.0*
3-rings PAHs	12.2	2.1	17.8*	16.3*	22.1*	-47.4*	-5.6	8.0	-0.9	-4.4	7.3	-39.8*	2.2	9.6	9.9
4-rings PAHs	8.3	25.7*	28.0*	37.1*	46.8*	-2.6	-6.6	5.4	2.3	1.0	-4.1	0.6	9.8	5.9	3.8
5-rings PAHs	6.7	35.4*	14.2*	13.4*	14.5*	1.2	9.4	-45.9*	-105.5*	-102.8*	-	-	-	-	-
6-rings PAHs	4.3	10.7	-4.8	10.2	9.7	-2.0	-7.4	6.0	-0.1	10.2	-	-	-	-	-
Σ16 C_{bioacc} PAHs	6.0	5.9	11.5	10.5	12.3	-7.3	-6.7	8.6	3.3	-5.2	7.0	-4.6	-53.3*	-46.1*	-49.0*

[#]-sampling terms; * - statistically significant differences (≤0.05); negative values mean lower C_{bioacc} PAHs in planted than unplanted soil; positive values mean higher C_{bioacc} PAHs in planted than unplanted soil.

Table S3. The content (μg/kg) and reduction (%) of PAHs bioaccumulation in willow cultivated on biochar or AC-amendment soil

PAHs	Control soil	BCW-amended soil		BCS-amended soil		AC-amended soil	
	C	C	%	C	%	C	%
NA	35.3±9.3	32.3±7.4	8.4	52.1±10.8	-47.7	38.8±10.9	-10.0
ACE	4.0±0.2	2.9±0.3	25.8	4.0±0.2	-1.08	3.5±0.3	11.9
AC	13.5±0.8	8.6±0.9	36.6	12.7±0.6	6.2	9.7±0.9	28.0
FL	21.7±1.5	21.6±1.5	0.5	22.3±1.5	-2.5	21.8±1.6	-0.4
PHEN	316.8±6.2	285.9±7.4	9.8	273.3±7.0	13.7	241.3±6.4	23.8
ANT	n.d.	n.d.	-	n.d.	-	n.d.	-
FLUO	96.6±7.0	108.0±7.5	-11.8	100.8±6.0	-4.3	95.3±5.7	1.4
PYR	68.5±4.4	76.0±5.2	-10.9	73.2±3.5	-6.9	66.2±3.4	3.3
BaA	10.3±0.5	9.4±0.5	8.9	11.0±0.3	-7.1	9.1±0.6	11.8
CHR	77.5±3.9	72.4±1.7	6.6	68.2±3.4	12.0	65.2±3.2	15.8
BbF	n.d.	n.d.	-	n.d.	-	n.d.	-
BkF	n.d.	n.d.	-	n.d.	-	n.d.	-
BaP	n.d.	n.d.	-	n.d.	-	n.d.	-
IcdPd	n.d.	n.d.	-	n.d.	-	n.d.	-
DahA	n.d.	n.d.	-	n.d.	-	n.d.	-
BghiP	n.d.	n.d.	-	n.d.	-	n.d.	-
Σ16 WWA	644.2±14.5	617.1±14.1	4.20	617.6±15.1	4.13	551.0±14.8	14.5

± values are mean of three repetitions; C – concentration; % - compound change regarding to willow cultivated on non-amended soil. NAP - naphthalene; ACE - acenaphthylene; AC – acenaphthene; FL - fluorene; PHEN – phenanthrene; ANT – anthracene; FLUO – fluoranthene; PYR – pyrene; BaA - benzo(a)anthracene; CHR – chrysene; BbF - benzo(b)fluoranthene; BkF - benzo(k)fluoranthene; BaP- benzo(a)pyrene; IcdP- indeno(1,2,3-cd)pyrene; DahA – dibenz(a,h)anthracene; BghiP – benzo(ghi)perylene

Table S4. The C_{bioacc} PAHs (μg/kg) in planted (willow) and unplanted control soil

PAHs	Planted (willow)					Unplanted				
	0	3	6	12	18	0	3	6	12	18
NA	1098.3±90.1	1083.8±104.6	1006.6±102.5	895.6±81.4	864.2±68.2	1069.7±101.5	1186.7±97.5	963.3±120.2	909.6±105.3	894.5±102.3
ACE	11.6±1.2	3.7±0.4	3.2±0.3	3.1±0.3	1.4±0.1	8.8±0.7	3.3±0.3	1.3±0.1	0.9±0.1	0.7±0.1
AC	23.1±2.3	27.1±2.2	20.6±2.1	20.6±2.1	21.5±2.1	20.8±1.5	33.2±2.2	21.1±1.6	17.9±1.5	15.5±1.7
FL	36.2±3.5	37.9±3.7	33.1±3.5	30.8±2.9	31±3.1	31.4±3.3	44.1±2.9	29.6±2.6	27.0±2.3	26.7±2.5
PHEN	211.6±21.8	156.0±15.4	132.5±13.3	119.9±9.8	115.6±10.6	191.8±16.8	147.8±16.1	109.5±9.5	99.9±9.7	91.8±7.4
ANT	57.0±4.9	42.3±4.2	34.7±2.5	21.2±1.7	25.9±1.7	45.1±3.5	33.0±3.8	22.8±2.7	18.1±1.5	17.5±1.4
FLUO	228.7±16.5	116.7±11.0	90.9±7.4	104.6±10.8	96.3±8.7	227.2±22.6	85.9±9.3	51.7±4.8	48.3±4.3	36.2±3.9
PYR	191.4±17.1	104.1±11.0	81.5±8.1	79.6±8.4	81.1±6.9	173.3±17.7	77.6±7.7	63.0±6.5	53.7±3.7	43.1±4.6
BaA	115.8±10.8	71.9±5.8	56.2±4.7	56.5±5.5	57.0±5.5	99.9±6.5	54.9±3.6	46.0±5	39.8±3.7	31.6±2.2
CHR	217.1±21.4	141.3±13.0	108.3±10.0	108.8±8.0	97.4±10.7	190.1±18.8	104.2±9.1	81.9±7.5	77.9±6.7	65.5±5.0
BbF	169.3±18.3	97.6±9.4	62.2±5.2	46.2±3.8	44.4±3.8	149.1±13.7	94.1±9.1	92.6±9.6	79.6±6.2	67.0±7.5
BkF	122.8±12.7	54.7±4.2	42.2±4.4	32±3.6	20.6±1.7	115.8±11.9	51.9±4.4	67.9±7.6	29.9±2.1	9.8±0.9
BaP	150.1±13.4	137.2±13.6	122.7±12.2	79.5±5.7	54.7±4.4	147.5±15.4	40.8±4.5	34.3±3.3	27.1±3.0	25.5±2.5
IcdPd	121.8±9.0	49.0±4.6	4.7±0.4	4.8±0.5	4.7±0.5	117.4±11.0	39.5±3.7	4.2±0.4	4.8±0.5	4.4±0.5
DahA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BghiP	120.1±12.5	46.4±3.7	15.7±1.5	7.5±0.6	8.3±0.9	114.2±9.8	45.7±4.6	17.0±1.4	6.3±0.6	7.3±0.7
2-rings	1098.3±90.1	1083.8±104.6	1006.6±102.5	895.6±81.4	864.2±68.2	1069.7±101.5	1186.7±97.5	963.3±120.2	909.6±105.3	894.5±102.3
3-rings	339.4±22.8	266.9±16.5	224.2±14.2	195.7±10.6	195.5±11.4	298.0±17.5	261.3±17.0	184.3±10.3	163.8±10.2	152.3±8.2
4-rings	752.9±33.8	434.0±21.1	336.8±15.5	349.5±16.8	331.8±16.4	690.4±34.9	322.6±15.6	242.6±12.1	219.7±9.5	176.4±8.1
5-rings	442.1±26.0	289.5±17.0	227.0±14.0	157.7±7.7	119.7±6.0	412.4±23.8	186.9±11.1	194.9±12.6	136.6±7.2	102.3±7.9
6-rings	241.9±15.4	95.4±5.9	20.3±1.6	12.3±0.8	13.0±1.0	231.6±14.8	85.2±5.9	21.3±1.4	11.1±0.8	11.8±0.8
Σ16 PAHs	2874.7±103.4	2169.6±109.5	1814.9±105.6	1610.8±84.2	1524.3±71.4	2702.1±112.4	2042.6±100.9	1606.4±121.9	1440.8±106.5	1337.3±103.3

Values are mean of three repetitions. NA - naphthalene; ACE - acenaphthylene; AC – acenaphthene; FL - fluorene; PHEN – phenanthrene; ANT – anthracene; FLUO – fluoranthene; PYR – pyrene; BaA - benzo(a)anthracene; CHR – chrysene; BbF - benzo(b)fluoranthene; BkF - benzo(k)fluoranthene; BaP- benzo(a)pyrene; IcdP- indeno(1,2,3-cd)pyrene; DahA – dibenz(a,h)anthracene; BghiP – benzo(ghi)perylene.

Table S5. The C_{bioacc} PAHs (μg/kg) in planted (willow) and unplanted BCW-amended soil

PAHs	Planted (willow)					Unplanted				
	0	3	6	12	18	0	3	6	12	18
NA	1010.1±85.9	969.1±78.2	595.5±62.8	529.2±43.1	431.8±50.9	1023.8±107.5	1057.8±74.2	493.6±51.1	454.3±44.5	430.7±44.9
ACE	11.4±1	2.8±0.2	1.8±0.2	1.5±0.1	0.8±0.1	34.8±2.6	3.1±0.3	1.2±0.1	2.6±0.2	1.1±0.1
AC	18.8±1.7	24±2	18.1±1.7	16.8±1.8	15.3±1.5	20.8±1.5	31.5±3	20.2±2	19.8±1.9	16.6±1.7
FL	34±3.7	34.2±2.8	25.2±2.6	23.2±2.3	21±1.7	51.4±3.3	36.9±3.9	23.1±2.6	18±1.6	16.9±1.7
PHEN	148.4±12.4	136.8±13.9	115.2±12.9	100.3±10	90.5±9.5	218.5±23	139.1±14.4	103.2±10.9	99.8±9.6	98.1±9.6
ANT	46.7±4.2	30.6±3.1	22.8±2.1	15.5±1.5	16.3±1	56.6±4.6	30.5±3.2	20.7±2.2	18.6±1.5	17.6±1.6
FLUO	164.9±12.2	103.2±8.9	77.8±8.9	81.9±5.8	60±5	173.1±14.9	103.3±7.6	61±6.3	57.5±6.6	52.5±4.0
PYR	142.7±11.9	95±8.6	75.8±7.6	64.6±6.7	56.5±6.1	133.4±9.8	93.7±8.1	71.1±8.2	64.4±5.7	54.9±5.4
BaA	79.8±7.4	56.5±4.4	44.7±4.3	35.9±3.2	33.7±3.5	79.6±8.4	65.1±5.2	40.8±4	37.7±3.6	30.7±3.2
CHR	137.9±14	102.7±9.5	77.5±7.7	70±7	48.1±4.5	153.0±12	119±9.3	87.8±6.6	87.0±6.7	58.1±4.1
BbF	95.3±9.3	73.5±7.8	55.7±5.7	26.8±2.7	22.5±1.8	113.3±7.9	68.5±6.1	62.5±4.8	44.6±4.2	43.1±3.1
BkF	68.1±6.6	12.6±1.2	7.6±0.8	8.2±0.8	9.3±1	55.6±1.6	17.1±1.2	16.9±1.7	14.7±1.6	8.5±0.9
BaP	38.3±4	43.2±3.3	12.2±0.8	9.4±0.9	7.7±0.8	30.3±2.4	31.6±2.5	30.8±3.3	31.9±2.7	28.5±2.8
IcdPd	18±1.5	4.6±0.4	5.7±0.5	4.2±0.4	5.5±0.6	7.1±0.7	4.7±0.5	5.2±0.5	5.7±0.4	5.8±0.6
DahA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BghiP	18.4±1.9	9.1±0.8	7.7±0.7	6.2±0.6	4.5±0.4	30.1±2.6	10±1.2	7.3±0.4	4.7±0.5	3.2±0.3
2-rings	1010.1±85.9	969.1±78.2	595.5±62.8	529.2±43.1	431.8±50.9	1023.8±107.5	1057.8±74.2	493.6±51.1	454.3±44.5	430.7±44.9
3-rings	259.4±13.7	228.3±14.6	183±13.5	157.4±10.5	143.9±9.8	382.2±23.9	241.1±15.5	168.4±11.5	158.8±10.1	150.2±10.1
4-rings	525.4±23.3	357.4±16.2	275.7±14.7	252.5±11.8	198.3±9.7	539±23	381.1±15.4	260.7±12.9	246.7±11.6	196.3±8.5
5-rings	201.7±12.1	129.2±8.5	75.5±5.8	44.4±3	39.5±2.2	199.2±8.4	117.1±6.7	110.2±6.1	91.2±5.2	80.1±4.3
6-rings	36.4±2.4	13.6±0.9	13.4±0.8	10.4±0.8	9.9±0.7	37.2±2.7	14.7±1.3	12.4±0.6	10.4±0.7	8.9±0.6
Σ16 PAHs	2033±90.9	1697.6±81.6	1143.2±66.2	993.8±46	823.4±52.8	2141.4±112.9	1811.8±77.7	1045.4±54.3	961.3±47.3	866.3±47

Values are mean of three repetitions. NA - naphthalene; ACE - acenaphthylene; AC – acenaphthene; FL - fluorene; PHEN – phenanthrene; ANT – anthracene; FLUO – fluoranthene; PYR – pyrene; BaA - benzo(a)anthracene; CHR – chrysene; BbF - benzo(b)fluoranthene; BkF - benzo(k)fluoranthene; BaP- benzo(a)pyrene; IcdP- indeno(1,2,3-cd)pyrene; DahA – dibenz(a,h)anthracene; BghiP – benzo(ghi)perylene.

Table S6. The C_{bioacc} PAHs (μg/kg) in planted (willow) and unplanted AC-amended soil

PAHs	Planted (willow)					Unplanted				
	0	3	6	12	18	0	3	6	12	18
NA	819±82.3	711.6±72.7	450.7±49.6	440.2±40.9	397.6±39.1	760.6±62.9	737.2±75.4	719.4±71	657±45.9	608.2±39.3
ACE	4.1±0.4	2±0.2	1.5±0.1	1.8±0.1	1±0.1	3.1±0.3	2.1±0.2	1±0.1	2.3±0.2	1.1±0.1
AC	1±0.1	2±0.2	1.7±0.2	1.5±0.1	1.9±0.1	1.4±0.1	7.3±0.9	1.7±0.1	1.4±0.1	3.6±0.3
FL	3.3±0.3	3.4±0.4	2.9±0.2	2.1±0.2	3.2±0.3	2.7±0.3	8.7±0.8	2.7±0.2	1.6±0.2	2.0±0.2
PHEN	9.4±0.8	10.3±1.1	7.1±0.7	7.5±0.7	8.1±0.8	9.2±0.9	8.6±0.8	7.6±0.9	6.9±0.5	6.7±0.5
ANT	3.4±0.4	3.6±0.3	3.5±0.3	2.7±0.3	3.1±0.3	3.2±0.3	3.1±0.3	3.4±0.4	1.9±0.2	2.1±0.2
FLUO	4.5±0.4	3.7±0.3	4.9±0.5	2.4±0.2	3.5±0.3	4.4±0.4	3.5±0.3	4.3±0.2	2.9±0.3	3.3±0.2
PYR	8.4±0.9	8.3±0.7	9.1±0.9	7.3±0.7	6.6±0.7	9.1±0.9	8±0.6	8.4±0.7	5.9±0.5	6.1±0.5
BaA	0.4±0.03	0.4±0.03	0.7±0.04	0.4±0.04	0.3±0.03	0.5±0.04	0.6±0.1	0.4±0.04	0.5±0	0.6±0.1
CHR	0.8±0.1	0.6±0.1	0.7±0.1	0.3±0.02	0.4±0.05	0.7±0.1	0.8±0.1	0.6±0.1	0.4±0	0.3±0.03
BbF	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BkF	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BaP	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
IcdPd	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DahA	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BghiP	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2-rings	819±82.3	711.6±72.7	450.7±49.6	440.2±40.9	397.6±39.1	760.6±62.9	737.2±75.4	719.4±71	657±45.9	608.2±39.3
3-rings	21.1±1	21.3±1.2	16.8±0.8	15.5±0.8	17.3±1	19.6±1.1	29.8±1.5	16.4±1.0	14±0.6	15.6±0.7
4-rings	14±1	13±0.7	15.3±1	10.4±0.7	10.8±0.8	14.6±1	12.9±0.7	13.8±0.7	9.8±0.6	10.4±0.6
5-rings	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
6-rings	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Σ16 PAHs	854.2±82.3	745.9±72.7	482.7±49.6	466.1±40.9	425.7±39.1	794.8±63	779.9±75.4	749.5±71	680.9±46	634.2±39.3

Values are mean of three repetitions. NA - naphthalene; ACE - acenaphthylene; AC – acenaphthene; FL - fluorene; PHEN – phenanthrene; ANT – anthracene; FLUO – fluoranthene; PYR – pyrene; BaA - benzo(a)anthracene; CHR – chrysene; BbF - benzo(b)fluoranthene; BkF - benzo(k)fluoranthene; BaP- benzo(a)pyrene; IcdP- indeno(1,2,3-cd)pyrene; DahA – dibenz(a,h)anthracene; BghiP – benzo(ghi)perylene.

Table S7. The C_{bioacc} PAHs (μg/kg) in planted (willow) BCS-amended soil

PAHs	Planted (willow)				
	0	3	6	12	18
NA	1125.5±125.8	967.2±81.8	726.5±73.4	611.8±59.6	548±59.3
ACE	12.9±0.9	3.5±0.3	8.2±0.7	2.6±0.3	0.7±0.1
AC	26.1±2.2	27.2±2.5	23.8±2.4	23.2±2.3	19.8±1.7
FL	27.9±2.4	32.1±3.3	32.3±2.8	22.6±2.3	17.6±1.7
PHEN	106.9±11.2	97.6±10.2	90.1±7.3	85.7±6.9	69.4±6.2
ANT	25.4±2.3	23±2	22.9±2.1	16.1±1.8	14.1±1.3
FLUO	78.7±8.5	68±4.7	33±3.2	30.3±3	21.7±2.1
PYR	70.2±6.9	65.6±5.1	50.6±4.9	43.9±3.8	33.9±2.5
BaA	31.2±2.9	30.4±3.3	31.2±2.8	25.8±2.2	6.7±0.6
CHR	62.6±7.4	58.2±5.5	61.3±5.2	50.1±4.3	46.9±3.8
BbF	48.3±5.2	40.5±3.3	37.8±4.1	22±2.3	12.9±1.5
BkF	30.3±3.1	29.1±2.6	19.2±1.7	11±0.9	5.7±0.4
BaP	81.8±8	40.3±4.2	27.9±2.9	8.1±0.7	0±0
IcdPd	22.6±2.2	3.6±0.4	2.9±0.3	3±0.3	4.1±0.4
DahA	n.d.	n.d.	n.d.	n.d.	n.d.
BghiP	31.1±2.8	7.4±0.6	12.8±0.8	3.7±0.2	0.8±0.1
2-rings	1125.5±125.8	967.2±81.8	726.5±73.4	611.8±59.6	548±59.3
3-rings	199.2±11.9	183.4±11.2	177.3±8.5	150.3±7.8	121.6±6.8
4-rings	242.7±13.5	222.1±9.4	176.1±8.4	150±6.8	109.1±5.1
5-rings	160.4±10	109.9±6	84.9±5.3	41.1±2.6	18.6±1.5
6-rings	53.7±3.5	11±0.7	15.7±0.9	6.7±0.4	5±0.4
Σ16 PAHs	1781.4±127.6	1493.7±83.3	1180.5±74.6	959.9±60.5	802.3±59.9

Values are mean of three repetitions. NA - naphthalene; ACE - acenaphthylene; AC – acenaphthene; FL - fluorene; PHEN – phenanthrene; ANT – anthracene; FLUO – fluoranthene; PYR – pyrene; BaA - benzo(a)anthracene; CHR – chrysene; BbF - benzo(b)fluoranthene; BkF - benzo(k)fluoranthene; BaP- benzo(a)pyrene; IcdP- indeno(1,2,3-cd)pyrene; DahA – dibenz(a,h)anthracene; BghiP – benzo(ghi)perylene.

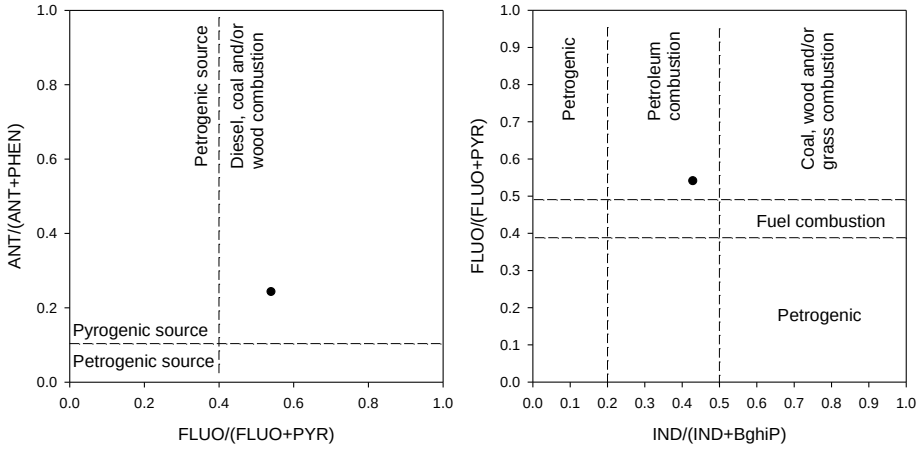

Figure S1. Molecular diagnostic ratios for identification of PAH source in area of contaminated soil sampling

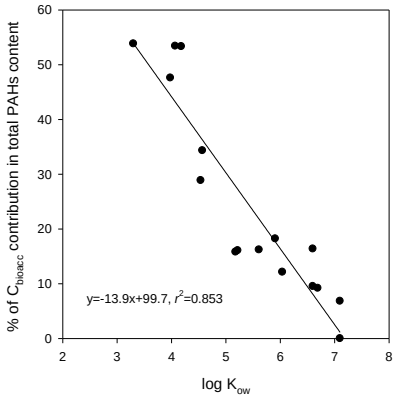

Figure S2. The relationship between contribution (%) of C_{bioacc} individual PAHs in non-amended soil to log K_{ow}

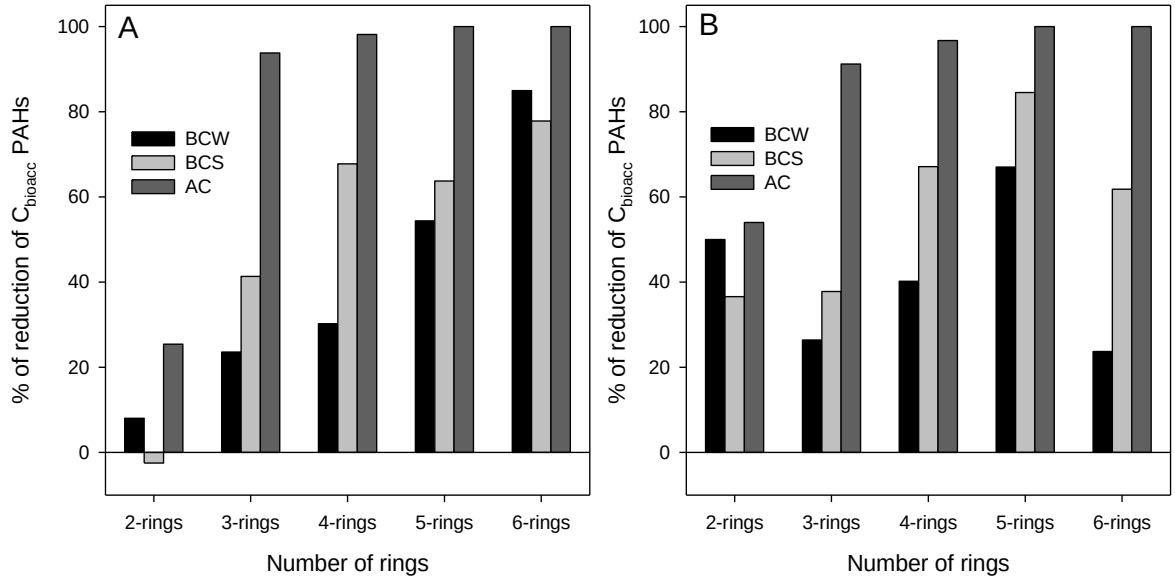

Figure S3. Reduction (%) of individual groups of PAHs in AC/biochar-amendment soil at the beginning (A) and end (B) of the experiment

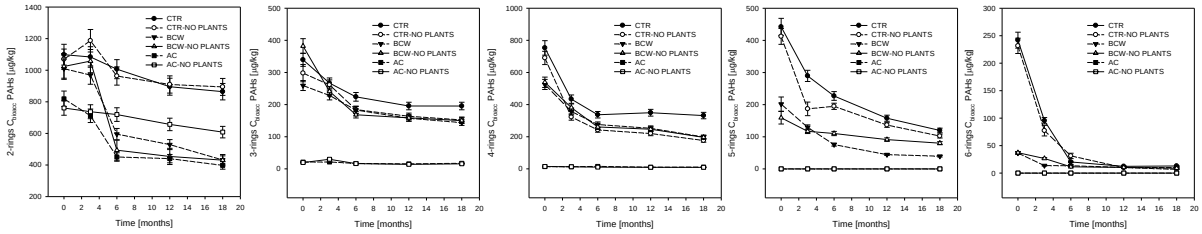

Figure S4. Effect of plants on individual groups of C_{bioacc} PAHs regarding to number of rings in AC or biochar (BCS, BCW)-amended soil

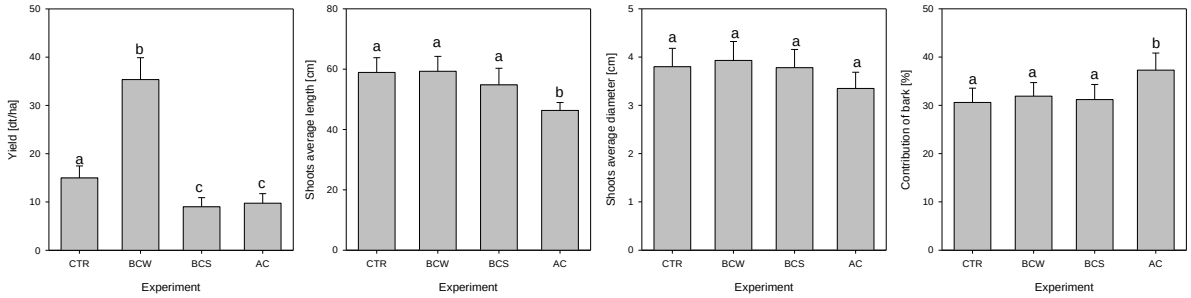

Figure S5. The yield, shoots length and diameter and contribution of bark in willow cultivated on non-amended and AC or biochar (BCW, BCS)-amended soil

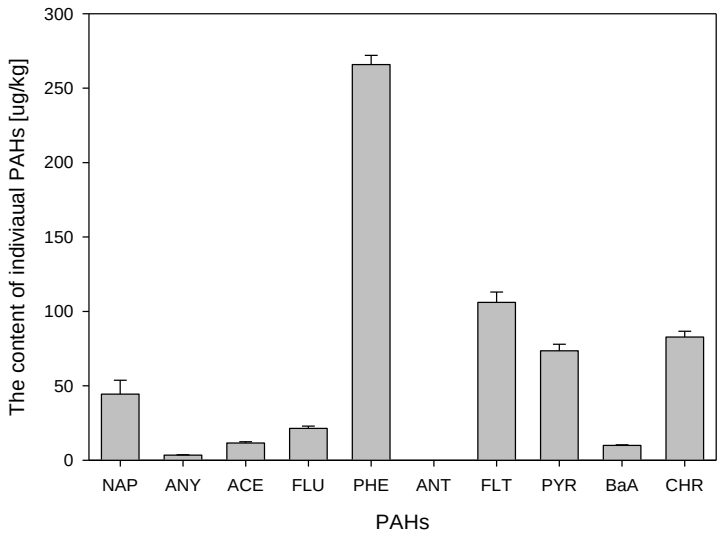

Figure S6. The concentration of individual PAHs (μg/kg) in willow growth on non-contaminated soil from area of field experiment

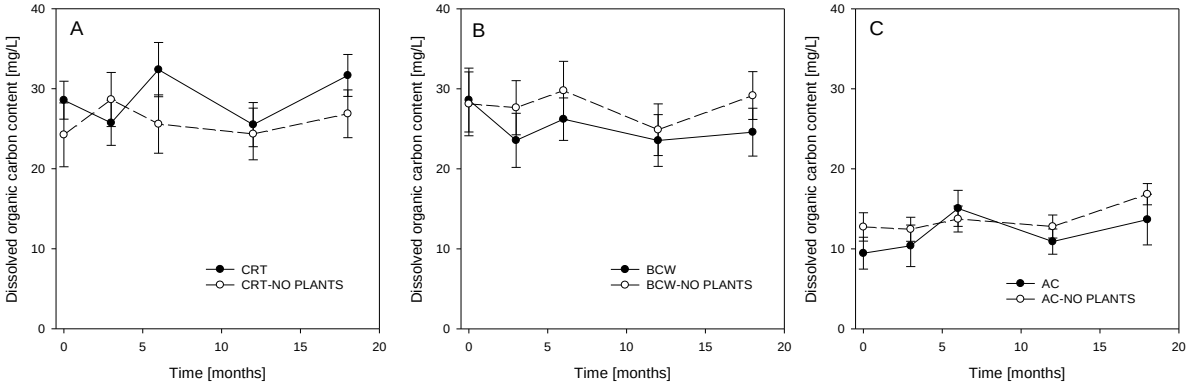

Figure S7. Dissolved organic carbon (DOC) content (mg/L) in unplanted (dotted line) and willow cultivated (solid line) non-amended and AC or biochar (BCW)-amended soil.

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Combined Effects of Plant Cultivation and Sorbing Carbon Amendments on Freely Dissolved PAHs in Contaminated Soil

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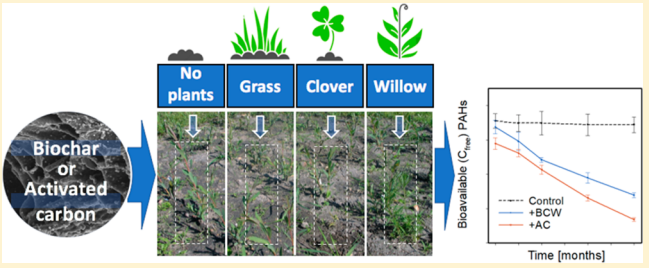
Supporting Information

ABSTRACT: We report freely dissolved concentrations (C_{free}) of PAHs in soils amended with 2.5% biochar and activated carbon (AC) during a long-term (18-months) field experiment. The study evaluates also the impact of different plants (clover, grass, willow) on C_{free} PAHs. The cumulative effect of treatments on nitrogen and available forms of phosphorus, potassium, and magnesium is also assessed. The direct addition of biochar to soil did not cause any immediate reduction of the sum of 16 C_{free} PAHs, while AC resulted in a slight reduction of 5- and 6 ring compounds. The efficiency of binding of C_{free} PAHs by biochar and AC increased with time. For biochar, the maximum reduction of 4−6-ring PAHs (18−67%) was achieved within 6 months. For 2- and 3-ring PAHs, a gradual decrease of C_{free} was observed which reached 60−66% at 18 months. AC proved to be better in reducing C_{free} PAHs than biochar, though for 2- and 3-ring PAHs, the differences in AC and biochar performances were smaller than those for 4−6-ring PAHs. After 18 months, a significantly lower content of C_{free} PAHs was observed in the soil with plants compared to the unplanted soil. Except for potassium, AC or biochar did not negatively impact nutrient availability.



INTRODUCTION

Remediation technologies for PAH-contaminated soils include solvent extraction, bioremediation, phytoremediation, chemical oxidation, photocatalytic degradation, electrokinetic remediation, and thermal treatment.[1] The greatest potential risk management benefit of in situ amendments with carbonaceous materials on large contaminated sites is due to containment and long-term exposure reduction of both organic and inorganic contaminants. The high affinity of contaminants for biochar or activated carbon (AC) provides a reduction of freely dissolved PAH concentrations (C_{free}) in sediment or soil porewater, which in turn is the most available fraction relevant for exposure, bioaccumulation, and effects.[2,3] Hence, the addition of biochar or AC reduces the mobility of HOCs and is expected to reduce adverse effects on organisms and the environment.[4−7] The use of carbon sorbents to immobilize contaminants has been demonstrated in freshwater and marine sediments.[6−11] It has been shown that a 2−5% addition of AC to sediment reduces the content of bioavailable PAHs up to 99.8%, depending on PAH hydrophobicity.[9,12−14] However, other reports have shown that AC may cause adverse effects in soil organisms.[15]

In recent years, the potential of contaminant immobilization has also been explored in soils amended with other carbonaceous sorbents, including biochar.[5,16−19] Although, the effectiveness of biochar in reducing contaminant availability in soils is lower compared to AC,[4,20] it may positively affect plant growth and development as well as soil enzymatic activity.[21,22]

To date, the performance of biochar or AC in soils has been studied mainly on a small scale (laboratory or greenhouse pot experiments), with exposure times of 1−15 weeks.[5,17,18,23] Long-term effects of carbonaceous amendment addition to soils under field conditions and for extended time (i.e., 18 months) have been studied less frequently.[17,20] The short-term studies demonstrated up to 80% reduction of bioavailable (freely dissolved or bioaccessible) PAHs after biochar addition to soils[5,18,23] and significantly reduced bioaccumulation in plants[5,23] and soil invertebrates.[18] Biochar was reported to reduce soil toxicity, although the effect strongly depended on the soil type and characteristics.[15] However, it is still uncertain to what extent the C_{free} values changed for individual PAHs throughout the experiment and how much time a system requires to reach steady-state under field conditions.

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In the literature, there is also a lack of long-term comparative studies between biochar and AC in the immobilization of PAHs under natural field conditions. Biochar requires more time than AC to bind individual PAHs,[16] which is an important factor in designing efficient remediation scenarios. Moreover, the sorption properties of biochar change with time,[24] which may also determine the (net) rate of adsorption and (possibly) desorption of contaminants.

Many sites have abundant vegetation, therefore from a practical point of view, understanding the effects of plants on the content of C_{free} PAHs in relation with biochar or AC application is advantageous. It has been shown that plants may increase bioavailable PAHs in soil.[25,26] Conversely, the plant rhizosphere may affect the degradation of PAHs and thus stimulate the growth of microorganisms.[27] However, biochar or AC addition to soil may also reduce the availability of nutrients necessary for plant growth and thus limit the applicability of this remediation technique. In both cases, an appropriate selection of plants can optimize the efficiency of elimination of bioavailable PAHs. Analysis of such secondary effects of biochar or AC amendment has been mainly limited to dissolved organic carbon (DOC) measurements,[17,19,20] whereas the accessibility of available forms of nutrients after biochar or AC application can play a key role in overall method effectiveness. To date, only one study has evaluated the influence of AC on nutrient concentration in leachates from AC-amended soils and did not observe AC to significantly affect nutrient content.[20] Nevertheless, biochars may have diverse capacities for nutrients, therefore different biochars and treatment scenarios need to be explored.

The primary aim of this study was to compare the efficiencies of AC and biochar in reducing C_{free} PAHs in soil in a long-term field experiment. A second aim was to determine the effect of different plants on the changes of PAH C_{free} in biochar- or AC-amended soil as well as the effect of AC and biochar on the content of available forms of nutrients. C_{free} PAHs were accurately determined at the beginning of the experiment and after 6, 12, and 18 months using 76 μm polyoxymethylene (POM) passive samplers. We believe this to be the first long-term study exploring the interplay of carbonaceous materials and plants crucial in the design of effective soil remediation scenarios.

MATERIALS AND METHODS

Adsorbents. Biochar (BCW) and AC were used in the field experiment. The BCW was provided by Fluid SA (Poland) and produced from dried willow (*Salix viminalis*). The slow pyrolysis temperature was set at 600−700 °C. The coal-based AC was purchased from POCH (CAS: 7440-44-0, Poland). BCW and AC were evaluated for pH, carbon, hydrogen and nitrogen content, the total organic carbon content (TOC), black carbon content (BC), N_2 adsorption, and specific surface area (SBET). Details of these methods are provided as Supporting Information (SI). The adsorption of representative 3-, 4-, and 5-ring PAHs (phenanthrene, pyrene, benzo[*a*]pyrene) to pure AC and BCW was also evaluated (detailed information about the sorption experiment is presented in SI).

Field Experiment. The field experiment was performed near Chełm, Poland (51°11′49.7″N, 23°15′01.2″E). Contaminated soil was transported from the location of a coking plant in Dąbrowa Górnicza, Silesia, Poland and homogenized using a mechanical (concrete) mixer before being placed in twelve 2 m (wide) × 2 m (long) × 0.2 m (deep) plots. The experiment was carried out outdoors in an agricultural station under the influence of environmental conditions (e.g., rainfall, sun exposure, etc.). No special treatment (no pesticides, no agricultural practices, no fertilization) was applied. The study employed three mesocosms (Figure S1): (1) control (without amendments), (2) treatment with biochar (BCW), and (3) treatment with AC. To explore the effects of diverse plants on C_{free} PAHs, parallel systems comprising willow (*Salix viminalis*), mix of grasses (*Lolium perenne* L., *Lolium multiflorum*, *Festuca arundinacea*, and *Dactylis glomerata* L.), white clover (*Trifolium repens* L.), and no plant addition were included in the field trial. Willows are used for short-rotation coppices because they grow rapidly (2−3 m per year), are easy to cultivate, and yield high biomass when planted at high densities. Willows can be used for different purposes, for example, as carbon dioxide neutral biofuel and are frequently applied in phytoremediation.[28,29] Perennial ryegrass is an important pasture and forage plant and is used in many pasture seed mixes. In fertile soil, it produces a high grass yield and is frequently sown for short-term ley grassland, often with red (*T. pratense*) or white clover (*T. repens*). The plants were sown at an approximate rate of seeds of 20 kg per 2.5 acres at the beginning of the field experiment in April 2014. Twelve plots were prepared, and eight were amended in early spring of 2014 with 2.5% of AC or BCW based on soil dry weight. The amounts of sorbents were selected based on our own experiences with earlier research[16] and typical numbers from the literature.[30] AC or biochar was added during spring tillage operations before sowing and was mixed with the soil by a rotatory tiller. Soil samples were collected during the field trial in 2014 and 2015, initially every 3 months and then every 6 months (i.e., April 2014, July 2014, October 2014, April 2015, October 2015). Nonamended and AC or biochar-amended soils were sampled at a 0−20 cm depth with a stainless-steel corer (5 cm i.d. and 60 cm long). Six independent subsamples were taken from each plot during individual sampling events. Subsamples (six from each plot) were mixed together to obtain a composite sample from each plot. The samples were transported to the laboratory, air-dried in an air-conditioned storage room (about 25 °C) for 9 weeks (in darkness), manually crushed, and sieved (<2 mm) prior to chemical analyses.

Freely Dissolved (C_{free}) PAH Content Determination. Freely dissolved concentrations in the porewater were quantified in triplicate with polyoxymethylene (POM) (thickness = 76 μm, CS Hyde, U.S.A.). The strips were cleaned according to Hale et al.[31] For each time point and treatment, two POM strips (about 0.35 g) were put into a conical flask with 1 g d.w. of composite soil or soil−biochar/AC mixture and 40 mL of Milli-Q water containing 0.2 g/L sodium azide (NaN_3) to inhibit microbial growth. The samples were mixed overhead at 10 rpm on a shaker (Rotax 6.8, VELP-Scientifica) for 30 days. Then, the strips were removed, cleaned in Milli-Q water, dried with lint free tissue (Kleenex), wrapped in aluminum foil, and kept in the fridge (4 °C) until extraction. Both POM strips were extracted together with 20 mL of acetone/heptane (20:80 v/v), and prior to the extraction, each sample received 20 μL of deuterated PAHs (PAH-mix 9 deuterated, Dr. Ehrenstorfer GmbH, Germany) at a concentration of 10 μg/mL for chemical quantitation. After 48 h of horizontal shaking, using an ELPIN 358A apparatus (Lubawa, Poland), the samples were concentrated to 1 mL using

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Table 1. Properties of Activated Carbon and Biochar Used in the Experiment[a]

Biochar	pH	C	H	N	O	Ash	H/C	O/C	(O+N)/C	S_{BET}	S_{mic}	V_p	V_{mic}	Σ16 PAHs
AC	6.0	85.50	0.27	0.62	3.45	10.16	0.038	0.03	0.036	617.6	306.7	0.374	0.148	0.9
BCW	9.1	52.20	2.23	1.13	25.15	19.07	0.043	0.273	0.380	5.3	4.1	0.009	0.002	4.6

[a]pH: reactivity in 0.1 M KCl; C, H, N, O: elemental composition [%]; Ash: ash content [%]; H/C, O/C, and (O+N)/C: molar ratios; S_{BET}: surface area [m^2/g]; S_{mic}: micropore area [m^2/g]; V_p: total pore volume [cm^3/g]; V_{mic}: micropore volume [cm^3/g]; R: average pore radius [nm]; Σ16 PAHs: sum of total content of 16 PAHs [mg/kg].

Table 2. Properties of Soils Used in the Experiment[a]

Soil	Sand (%)	Silt (%)	Clay (%)	pH	CEC (mmol/100g)	TOC (%)	DOC (mg/L)	BC (%)	Nt (%)	P_2O_5 (mg/100g)	K_2O (mg/100g)	Mg (mg/100g)	Σ16 PAHs (mg/kg)
DG	77	18	5	7.49	99.2	8.18	28.57	3.0	0.155	77.6	13.6	18.4	16.9

[a]pH: reactivity in KCl; CEC: cation exchange capacity expressed as sum of the bases Ca, Mg, K, Na [mmol/100g]; TOC: the organic carbon content [%]; DOC: dissolved organic carbon content [mg/L]; BC: black carbon content [%]; P_2O_5, K_2O, and Mg: available forms of phosphorus, potassium, and magnesium [mg/100 g]; Σ16 PAHs: sum of total content of 16 PAHs [mg/kg].

isooctane to avoid complete evaporation. 1,3,5-Tri-*tert*-butylbenzene (TTB) was used as a recovery standard, and each sample was spiked with 20 μL of TTB solution at 6.1 μg/mL.

Instrumental Analysis. The analysis of PAHs was carried out using a gas chromatograph (Trace 1300) coupled with a single quadrupole mass spectrometry detector (ISQ LT) (GC-MS, Thermo Scientific (Waltham, MA, U.S.A.). The GC-MS was operated under the selected ion monitoring mode. A Rxi-5 ms crossbond 5% diphenyl and 95% dimethyl polysiloxane fused capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) from Restek (Bellefonte, PA, U.S.A.) was used with helium as the carrier gas at a constant flow rate of 1 mL min^{-1}. Detailed information about the PAHs analysis by GC-MS is presented in the Supporting Information (SI).

Data Analysis. The concentration of PAH on POM passive samplers (C_{POM}) was calculated according to

$$C_{POM} = \frac{C_{extract} \cdot V_{extract}}{m_{POM}} \quad (1)$$

where $C_{extract}$ (ng/L) is the concentration of PAH determined via GC-MS, $V_{extract}$ is the volume of solvent (L), and m_{POM} (kg) is the mass of POM passive samplers.

Freely dissolved concentrations were calculated according to the equation

$$C_{free} = \frac{C_{POM}}{K_{POM}} \quad (2)$$

where C_{free} (ng L^{-1}) is the aqueous phase concentration of PAH, K_{POM} (L kg^{-1}) is the predetermined POM-water partitioning coefficient specific to each PAH compound obtained from Hawthorne et al.,[32] and C_{POM} (ng kg^{-1}) is the measured PAH concentration in POM strips.

The concentration of PAH determined in the bulk soil (details provided as SI) and the total organic carbon (TOC) content in the soil were used to calculate the TOC-water distribution coefficients (K_{TOC}) in the sorbent-free control soils using the following equation

$$K_{TOC} = C_s/(f_{TOC} \times C_{free}) \quad (3)$$

where C_S is the PAH concentration in the soil (ng kg^{-1}_{dw}), C_{free} is the PAH concentration determined with the POM passive samplers (ng L^{-1}), and f_{TOC} is the fraction of the TOC.[33]

Statistical comparisons between the PAHs content and experiments with different plants and between particular terms were evaluated using a one-way analysis of variance (ANOVA) and *t* tests at a 95% confidence level.

RESULTS AND DISCUSSION

Activated Carbon and Biochar Characteristics. The elemental carbon (C) content in AC was 86% and as such approximately 60% higher compared to BCW, whereas hydrogen (H), nitrogen (N), and oxygen (O) contents were, 2, 1, and 25%, respectively, more abundant in BCW (Table 1). This resulted in higher H/C, O/C, and (O+N)/C ratios for BCW, which is typical for pyrolysis temperatures of 400−700 °C and naturally derived biochars.[34,35] The surface area as determined by N_2 adsorption (S_{BET}) and total pore volume (V_p) were approximately 3 orders of magnitude higher for AC as compared to biochar (Table 1). The results agree with biochar and AC characteristics reported in the literature.[35] The adsorption isotherms and sorption affinity parameters for phenanthrene (PHE), pyrene (PYR), and benzo[*a*]pyrene (BaP) in pure water systems also varied between studied sorbents (Figure S2, Table S1). Adsorption affinity constants (log K_f, Freundlich adsorption coefficients) for AC in PAH-water systems ranged from 5.7 to 7.0 and were approximately a factor of 5 (PYR) to almost 1 order of magnitude (BaP) higher compared to that for BCW, which was probably due to its higher specific surface area and pore volume of adsorbents (Table 1). Both for biochar and AC, the determined sorption parameters were similar to values reported by other authors.[36]

Soil Properties. The concentration of 16 EPA PAH (PAH_{16}) in bulk soil was 15760 ± 290 μg/kg (Table 3), which is much lower than values typically reported for soils from areas with similar sources of contamination.[37,38] However, the study soil would still be classified as contaminated and require remediation according to Polish regulations.[39] Overall, 4-ring PAHs contributed 46% to the sum PAH_{16} concentration. The percentage of 5- and 6-ring PAHs was also substantial (26 and 15%, respectively), which indicates an anthropogenic source of contamination.[38] The TOC was 8.2%, whereas the black carbon (BC) and dissolved organic carbon (DOC) were 3.0% and 28.6 mg/L, respectively (Table 2). The BC/TOC ratio of 0.36 is comparable with values reported for sandy soils in highly industrialized regions.[40,41] Being a strong sorbent itself, the high BC content would be expected to appreciably affect biochar and, to a lesser extent, AC performance.

C_{free} of PAHs in Control-Unplanted Soil. The C_{free} of PAH_{16} in the control soil as initially determined by POM

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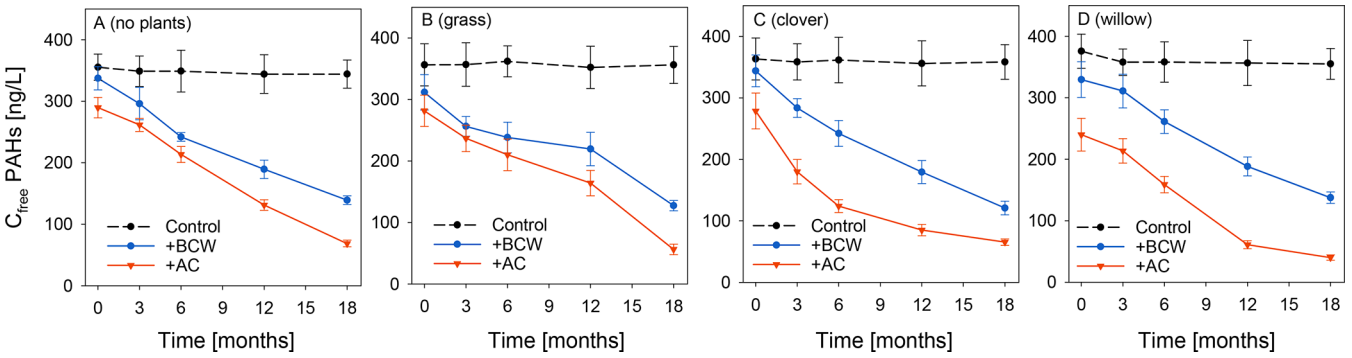

Figure 1. Changes of the sum of 16 freely dissolved (C_{free}) polycyclic aromatic hydrocarbons in control (nonamended) or activated carbon (AC)/biochar (BCW)-amended soil in experiments with unplanted soil (A), soil with grass (B), soil with clover (C), and soil with willow (D). Error bars represent standard deviation (SD, n = 3 extractions).

measurements was 356 ± 21 ng/L (Figure 1A, Table 3). It was similar to the previously observed values for soils subject to intensive anthropogenic pressure.[20,42] Naphthalene was observed in the highest concentration (217 ng/L) and accounted for 61% of the sum of identified PAH_{16} in the C_{free} fraction. Naphthalene has a high solubility and the lowest partition coefficient in soil which likely explains its prevalence in the C_{free} fraction.[16,42] The percentages of 3- and 4-ring PAHs were 24% and 14%, respectively. The 5- and 6-ring compounds accounted only for 0.5% and 0.04% in the dissolved PAH.

Table 3. Total and C_{free} PAHs Content (at Beginning of Study) and Log K_{TOC} for Polycyclic Aromatic Hydrocarbons in Soil Used in the Experiment[a]

PAHs	No. of rings	Total [μg/kg]	C_{free} [ng/L]	Log K_{TOC}
NAP	2	216.0 ± 21	217 ± 21	4.09
ACE	3	70.9 ± 5.8	10.19 ± 1.27	4.93
AC	3	80.4 ± 5.9	23.46 ± 1.99	4.62
FL	3	109.5 ± 4.9	13.88 ± 1.54	4.98
PHEN	3	1244.2 ± 73.5	26.91 ± 2.13	5.75
ANT	3	278.2 ± 25.3	11.10 ± 1.10	5.49
FLUO	4	2608.7 ± 134.8	27.77 ± 2.91	6.06
PYR	4	1944.4 ± 104.8	18.60 ± 1.13	6.11
BaA	4	974.7 ± 59.7	1.54 ± 0.08	6.89
CHR	4	1752.2 ± 83.8	2.89 ± 0.27	6.87
BbF	5	1414.2 ± 94.0	0.80 ± 0.05	7.33
BkF	5	1202.0 ± 90.8	0.41 ± 0.03	7.55
BaP	5	1232.3 ± 60.3	0.58 ± 0.04	7.42
IcdPd	6	1030.5 ± 85.7	0.07 ± 0.01	8.25
DahA	6	199.3 ± 13.2	0.01 ± 0.001	8.34
BghiP	6	1398.2 ± 89.2	0.06 ± 0.01	8.42
Σ16 PAH		15755.8 ± 287.2	355.45 ± 21.02	

[a]Values are the mean of three repetitions. NAP: naphthalene; ACE: acenaphthylene; AC: acenaphthene; FL: fluorene; PHEN: phenanthrene; ANT: anthracene; FLUO: fluoranthene; PYR: pyrene; BaA: benzo[*a*]anthracene; CHR: chrysene; BbF: benzo[*b*]fluoranthene; BkF: benzo[*k*]fluoranthene; BaP: benzo[*a*]pyrene; IcdP: indeno-[1,2,3-*cd*]pyrene; DahA: dibenz[*a,h*]anthracene; BghiP: benzo[*ghi*]-perylene.

The C_{free} values of 16 EPA PAHs in the control soil remained at a constant level over the course of the experiment (Figure 1A). No significant differences (ANOVA, $P \geq 0.05$) for the sum of PAH_{16} were found between particular sampling events. This is largely associated with the dominance of the 2- and 3-ring PAHs. Although, 2- and 3-ring PAHs are more prone to microbial degradation and more mobile in the environment,[16,43] no changes in C_{free} values were observed for these compounds (Figure 2). The kinetics of the release of these compounds from the soil was likely quicker than any of the loss processes. A decrease in the content of C_{free} of 4- and 5-ring PAHs (Figure 2) was observed. After 6 months, 4-ring PAHs declined (statistically significant, ANOVA, $P \geq 0.05$) by 16% and 5-ring PAHs declined by 45% compared to initial values. Over 18 months, the content of 4- and 5-ring PAHs in the control soil decreased by 22% and 65%, respectively. The observed changes are likely to be caused by biodegradation or sequestration of PAH by soil organic matter.[44] The BC might have contributed here since the soil was transported to the experimental facility and homogenized before placement. The BC originally present in the top soil layer may have been more evenly distributed in the soil thus causing the higher molecular weight PAH to sorb slowly to BC while the lower molecular

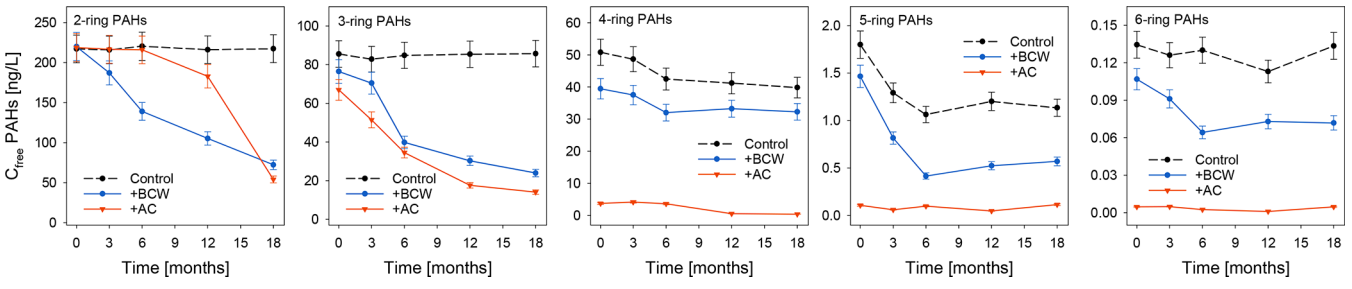

Figure 2. Changes of individual groups of freely dissolved (C_{free}) PAHs in the experiment without plants in control (nonamended) or activated carbon (AC)/biochar (BCW)-amended soil. Error bars represent standard deviation (SD, n = 3 extractions).

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weight PAHs may have equilibrated more rapidly with the BC during homogenization.

K_{TOC} values calculated for the individual PAHs in the studied soil are shown in Table 3. Ranging from 4.1 for NAP to 8.4 for B[*ghi*]P, they are 1−2 orders greater than those commonly found for OC normalized distribution coefficients in the literature[45,46] as a result of binding to the BC or dissipation as indicated above. The observed K_{TOC} values agree well with coal tar K_{TOC} (Figure S3), which was also previously observed by Arp et al.[42] The agreement with the coal tar model suggests that the molecules were probably introduced into the soil as a component of pyrogenic particles.[42]

Effect of Biochar and AC on C_{free} in Unplanted Soil. The effects of AC and BCW biochar on the content of Σ16 C_{free} PAHs in the investigated soil are shown in Figure 1A. Overall, AC was more effective compared to biochar. AC application resulted in an initial 18.5% decrease in C_{free} of Σ16 PAH, whereas porewater concentration in soil amended with biochar did not change. Interestingly, low molecular compounds (i.e., 2-ring PAHs) were not initially affected by biochar and AC addition. Three-ring PAHs declined only by 11% and 18.5%, respectively (Figure 2, Figure S4). The limited effects of BCW and AC on low molecular weight compounds were not expected given the fact that these species were dominant in porewater (i.e., contributed >60% to the Σ16 PAHs) (Table 3). The mass transfer from contaminated soil to AC particles can be faster for the smaller PAHs than for the larger ones because of higher mobility and water solubility of the smaller PAHs[41] suggesting that, as in the control soils, the release from the remaining carbon in the system was able to sustain C_{free} initially. It is likely that the relatively low dosage of BCW (2.5%) was insufficient in reducing available PAHs given the comparatively large amount of natural organic matter and black carbon in the soil as observed by an earlier study.[18] Larger effectiveness in reducing C_{free} has been reported in soils amended with biochar doses >5%.[5,16,23] It should also be emphasized that in the present study, for 2- and 3-ring PAHs, no significant differences were observed in the C_{free} contents between the AC- and biochar-amended soils (Figure 2), which likely indicates that the soil organic matter was able to sustain porewater concentrations of these species despite likely differences in sorption between AC and biochar.

Effective and immediate reduction of C_{free} (i.e., 92.6−96.6%) was observed in 4−6-ring PAHs in the AC application scenario. For the same compounds, the application of BCW resulted in 18.5% to 22.4% (Figure S4) decrease in C_{free} only, most likely due to the weaker affinity of PAHs to BCW as compared to AC (Figure S2). More effective reduction of heavy molecular weight (HMW) PAHs as compared to low molecular weight (LMW) compounds in AC systems was also observed in other studies.[16,47] This may be due to the fact that heavy molecular weight PAHs were present in the porewater at much lower concentrations compared to more labile 2−3 ring compounds as well as the relatively slow desorption of the heavier compounds. The desorption of heavy PAHs from native soil organic matter is relatively slow, whereas adsorption onto AC is relatively fast, leading to a depletion of these compounds in the aqueous phase (low C_{free}), which seems to be more prominent for heavy than for light PAHs. Typically, the nonlinear sorption onto carbonaceous materials leads to much greater effective partition coefficients at lower concentrations.

The porewater concentration results for Σ16 C_{free} PAHs in both biochar- and AC-amended soils showed a systematic decrease with time (Figure 1A). After 18 months, the content of C_{free} PAHs decreased in biochar and AC systems by 58.8 and 76.3%, respectively. When normalized to C_{free} in control soil, the reductions in AC and biochar systems were 59.5% (biochar) and 80.1% (AC). The largest reduction in 4−6 rings PAH was observed in AC during the initial stage of the study, and only minor further reductions were observed with time (Figure S4). However, over the 18 month period, significant changes were found for 2- and 3-ring PAHs, in both biochar and AC exposures (Figure S4) relative to unamended controls, as well as for 6-ring PAHs in the system with biochar.

This far, this has been the first comparative study between AC and biochar used to immobilize C_{free} PAHs that was conducted under natural conditions over a period of nearly two years. To date, comparative studies had been performed for periods not exceeding 60 days in laboratory experiments and revealed significant differences in C_{free} reduction between AC and biochar.[18] Our results show that for 2- and 3-ring PAHs, longer contact time is required between the contaminated soil and biochar. It is plausible that a decreasing trend could still be observed (even after 18 months) for these compounds. In case of the heavier compounds, steady state was reached after 6 months of biochar incorporation (4−6-ring PAHs) or immediately after AC incorporation (5- and 6-ring PAHs). The observed reduction of PAHs, after application of both biochar and AC, was comparable to that found in the previous studies concerning soils in the lab and the field scale.[5,16,18,20,23,48]

Effect of Plants on C_{free} in Sorbent Amended Systems. The changes in Σ16 C_{free} PAHs in the experiment involving the growth of different plants are shown in Figures 1B−D. Additional plots showing porewater changes for Σ16 PAHs are presented in Figure S5 (SI). Plots for individual PAH groups with different plants in control soil and in the biochar- or AC-amended soil are shown in Figure S6 (SI).

The effects of the plants on total C_{free} PAHs did not differ statistically (ANOVA, $P \geq 0.05$) between different sampling points in the control soil and for majority of time points in the biochar-amended soil (Figure 1A,C,D, Figure S5). However, significant differences were found in AC amended plots, where C_{free} reductions can be primarily explained by PAH sorption to AC. Statistically significant differences (ANOVA, $P \geq 0.05$) were noted for willow during all sampling events and for clover after 3, 6, and 12 months, whereas the differences for grass were noted only after 12 months. Depending on the sampling point, the porewater concentration decreased by 17−54% in the soil with willow and by 31−42% in the soil with clover in comparison to the unplanted soil (Figure 1A,C,D, Figure S5).

In the case of individual PAH groups (Figure S6−S9), significant differences (ANOVA, $P \leq 0.05$) in C_{free} content could be observed between the experiments with and without plants and between sampling events. We could not unambiguously demonstrate that the specific plants have a clear influence on the content of C_{free} PAHs in the soils. There were no clear trends with time despite the statistical differences between sampling periods. The ultimate changes were most clear by the comparison of the initial and the final sampling periods; for details, see Figure S4.

For most of the PAH groups, no significant differences were observed between the C_{free} PAHs at the last sampling date in the unamended soil with plants relative to the unamended and

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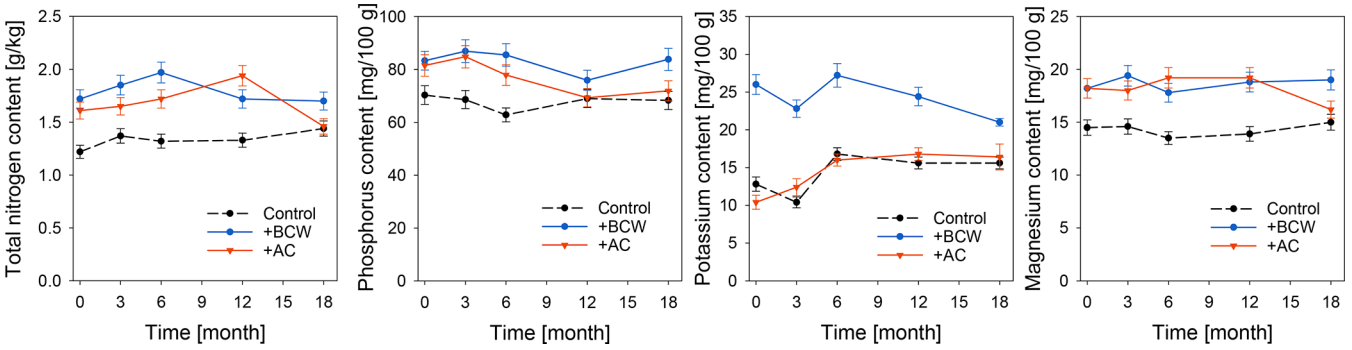

Figure 3. Effect of AC and biochar on the total organic nitrogen content and available forms of phosphorus, potassium, and magnesium in unplanted soil over time. Error bars represent standard deviation (SD, n = 3 extractions).

unplanted soil (Figure S6, first column). However, for 5-ring PAHs, the C_{free} values increased from 31 to 97% (grass < clover < willow), compared to systems without plants.

More pronounced effects of plants were found in the experiment with biochar (Figure S6, middle panel) and AC (Figure S6, last column). In the experiment with biochar, grass, and clover the content of 4-, 5-, and 6-ring PAHs decreased by 42 to 65% relative to unplanted controls. Moreover, a 30% reduction of 6-ring PAHs was also found in the case of willow. In the AC amended systems, a lower content of C_{free} PAHs in the soil with plants relative to unplanted controls was noted for 2-ring (31%, willow), 3-ring (72%, grass; 82%, willow), 4-ring (31%, willow), 5-ring (70%, grass; 100%, willow), and 6-ring (100%, grass) PAHs.

A positive influence of plants on the degradation of organic contaminants was shown in earlier studies[27,49,50] and was suggested as a remediation technique. The previous studies demonstrated that the positive influence is primarily associated with increased bioavailability of contaminants by plant exudates acting as surface-active compounds.[26,51] Sun and Gao[26] and Gao et al.[51] observed a higher content of bioavailable PAHs in the soil with plants compared to soil without plants, which would confirm these observations. A similar phenomenon was observed in our current study for the control soil and 5-ring PAHs (Figure S6). Nevertheless, in the experiment with biochar and AC, an opposite trend was observed; i.e., the C_{free} PAH content in the soil with plants was lower than that in the soil without plants. The inclusion of additional factors, which reduce bioavailability, i.e., biochar and AC, can stimulate the formation of strong bonds between contaminants and soil organic matter or humus under the influence of plant enzymes.[50,52] Moreover, biochar can also enhance microbiological activity[53] and thus decrease the content of C_{free} PAHs via biodegradation.[44] Two possible competitive mechanisms, providing a further decrease in PAH porewater concentrations, are plant detoxification and/or sorption onto the carbonaceous materials. The net effect is the reduced availability of the PAHs, although this is not a reduction in total bulk PAH levels as rapidly as in nonamended soils. The total bulk PAH concentrations may appear to be lower compared to the control soil, most likely due to reduced extractability of PAH in biochar and AC enriched soil caused by strong sorption.[17,40]

The differences in the reduction of C_{free} of PAHs between plants may originate from the different root systems of these plants[54] and the different compositions of their exudates.[26,54] In the biochar-amended soil, grass and clover outperformed other plants, while in the AC-amended soil, willow showed better effectiveness in reducing available PAHs (Figure S4).

Note that the results obtained are influenced by the large BC content and relatively low amendment levels of both carbonaceous materials. As a general rule, it is recommended that AC or biochar addition be performed at levels at least equal to the TOC content, whereas here, it is only about 30% of TOC, which is advantageous from a cost perspective (i.e., large effects in C_{free} reductions at relatively low amendment rates).

Secondary Effect of AC/Biochar-Soil Amendment. The effect of biochar or AC amendment on changes in total nitrogen content and available forms of phosphorus (P), potassium (K), and magnesium (Mg) in the unplanted soil are shown in Figure 3. The effect of the individual amendments was dependent on the type of nutrient. The addition of biochar or AC increased the availability of nutrients with the exception of K. In the case of K, only the application of biochar increased its availability. The increased availability of nutrients in the case of biochar and AC is not surprising since biochar and AC themselves can be sources of these nutrients.[55−57] It should however be noted that between the 12 and 18 month exposure time, both the total nitrogen content and available forms of magnesium and phosphorus significantly decreased in the experiment with AC. This can be associated on the one hand with the adsorption of these nutrients on the AC surface which reduces their availability and on the other hand with their degradation under the influence of microbial activity or assimilation by vegetation. Nevertheless, it should be emphasized that nitrogen and available forms of P and Mg in the AC-amended soil decreased to the levels observed in the control soil. Hence, AC behaved like a buffer for nutrients. Therefore, the obtained results demonstrate that the suggested negative effect of AC observed in the previous studies[15,19] is probably not associated with the reduced availability of nutrients, while in the case of biochar, this even results in their increased availability. It should also be stressed that in the biochar-amended soil, the content of all the elements was 10 to 120% higher than in the control soil throughout the entire study period (Figure 3).

AC was stronger and faster in binding C_{free} PAHs in the soils than biochar due to its much greater sorption capacity. The effect did not change even after a rather long time of contact of the investigated amendments with the soil. The greatest differences in binding effectiveness and speed between AC and biochar were observed for the heavy PAHs (>4-rings). Over time, the effectiveness of biochar increased, but it did not reach

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the level of AC effectiveness. Nevertheless, it should be noted that in the case of low molecular weight PAHs, the differences between biochar and AC were much smaller than in the case of heavy PAHs. The effect of the plants was noticeable throughout the entire study period and showed different trends. Earlier studies have shown that roots stimulate soil activity through the modification of microbial communities.[58] After 18 months, a significantly lower content of C_{free} PAHs was observed in the soil with plants compared to the unplanted soil which could have been effects of (1) facilitated biotic degradation, (2) plant uptake, and (3) enhanced sorption due to better incorporation of sorbents into soil by root growth. AC and biochar did not reduce the availability of nutrients, which is particularly important in the context of plant growth. Their increased availability was observed (in particular after biochar application), which may potentially stimulate the growth of plants and the biological activity of remediated soils, increasing remediation efficiency.

Soil treatment with carbonaceous materials resulted in a significant decrease of C_{free} PAHs with AC addition being the most efficient, followed by biochar. After 18 months, AC effectively reduced C_{free} PAHs with plants grown in conjunction with soil treatment. Furthermore, plants grew in all systems, which implies that the habitat quality was sufficient to support different plant species irrespective of treatment. The presence of biochar sustains nutrient availability, and we did not observe a shortage in nutrient supply, except that of potassium in AC amended soil. The addition of strongly binding carbonaceous materials with high surface areas has the potential to stimulate bacterial degradation of HOCs.[59] Soil treatment with AC or biochar can be coupled with other land management approaches. In conclusion, the application of 2−4% activated carbon to remediate contaminated soils can be considered beneficial and favorable for plant habitats.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.8b06265.

Scheme of the field experiment; detailed information about analysis of physicochemical properties of soil, biochars, and activated carbon; experimental procedure of adsorption experiment and adsorption isotherms and fitting parameters; experimental procedure of the total PAHs determination; detailed information about quality assurance and quality control; individual C_{free} PAHs content in control, AC, or biochar-amended soil (PDF)

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Notes

The authors declare no competing financial interest.

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Supporting information:

Combined effects of plant cultivation and sorbing carbon amendments on freely dissolved PAHs in contaminated soil

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Analysis of physico-chemical properties of soil, biochars and activated carbon

The pH was measured potentiometrically in 1 M KCl after 24 h in the liquid/soil ratio of 10 (w/v). The cation exchange capacity (CEC, expressed as sum of the bases Ca, Mg, K, Na) was determined in the 0.1 M HCl extraction. The amounts of carbon, hydrogen and nitrogen were determined using a CHN equipment (Perkin–Elmer 2400, USA). The total organic carbon content (TOC) was determined by the dry combustion method using a TOC-VCSH (SHIMADZU, Japan) with a Solid Sample Module SSM-5000. The DOC content was determined according to Jones and Willett.[1] Black carbon in soils was quantified using the chemo-thermal oxidation (CTO) method adapted for soil by Agarwal and Bucheli.[2] The textural characteristics of the biochars were recorded with a Micromeritics ASAP 2405 N_2 adsorption analyser (USA) by performing low-temperature (77.4 K) nitrogen adsorption–desorption isotherms. The specific surface area (S_{BET}) of the AC and the biochars was determined according to the Brunauer – Emmett – Teller isotherm.

Adsorption experiment

The adsorption experiment was carried out by the method proposed by Hale et al.[3] AC or biochar sample (50 mg) were added to 50 mL glass flasks with glass lids. The glass vials were tightly sealed and did not leak during the experiment. Millipore water (40 mL) with sodium azide (20 mg L^{-1}) to eliminate any possible effect of remaining microorganisms and strips of 55-µm thick polyoxymethylene (POM) passive samplers (0.3 g for all batches) were added to vials. Prior to use, POM samplers were cleaned by cold extraction overnight in methanol, then in heptane, rinsing thoroughly with Millipore water and finally drying. Batches were spiked with phenanthrene (PHE), pyrene (PYR) or benzo[a]pyrene (BaP) in methanol. The amount of spiked cosolvent (100 µl) did not exceed 0.25 % of the water volume and therefore the cosolvent effect was minimal in this system. To determine the initial concentration of PHE/PYR/BaP present in AC/biochar to glass flasks only AC/biochar + POM + water were added. Flasks were rolled end over end for 21 days at 1 RCF, after which POM samplers were removed, cleaned with Millipore water and wiped with a tissue to ensure they were dry and visibly clean. POM samplers were extracted in 20 mL of 20:80 acetone:heptane for 2 days. The solvent was reduced to about 1 mL using rotary vacuum concentrator RVC 2-25 CD plus (Martin Christ, Germany). A quantitative analysis of PAHs was carried out on Thermo Scientific Trace 1 300 Gas Chromatograph equipped with a Restek Rxi-5ms Column (length 30 m, 0.25 mm id and 0.25 µm film thickness). Detailed information about PAHs analysis is presented below.

The concentration of PHE/PYR/BaP on POM passive samplers was calculated according to the equation (1):

$$C_{POM} = \frac{C_{extract} \cdot V_{extract}}{m_{POM}} \quad (1)$$

where $C_{extract}$ (ng L^{-1}) is the concentration of PAH determined via GC-MS, $V_{extract}$ is the volume of solvent (L) and m_{POM} (kg) is the mass of POM passive samplers.

The concentration of PHE, PYR or BaP in water after 21 days of mixing was calculated on the basis of equation (2):

$$C_w = \frac{C_{POM}}{K_{POM}} \quad (2)$$

where C_w (ng L^{-1}) is the aqueous phase concentration of PHE, PYR or BaP, K_{POM} (L kg^{-1}) is the predetermined POM-water partitioning coefficient specific to PHE, PYR or BaP obtained from Hawthorne et al.[4] and C_{POM} (ng kg^{-1}) is the measured PAH concentration in POM strips.

The concentration of PHE, PYR or BaP on AC/biochar was calculated on the basis of equation:

$$C_s = \frac{C_{int} - C_w}{m_{sorbent}} V_r \qquad (3)$$

where C_s (μg kg^{-1}) is PHE, PYR or BaP on AC/biochar, C_{int} (μg L^{-1}) is the initial concentration of PHE, PYR or BaP in the water solution, C_w (ng L^{-1}) is the aqueous phase concentration of PHE, PYR or BaP, V_r (L) is the volume of solution, $m_{sorbent}$ (kg) is the mass of sorbent used for the experiment.

The sorption data were fitted with Freundlich sorption isotherm equation (logarithmic form):

$$log\ q_e = log\ K_F + n\ log\ C_e \qquad (4)$$

where q_e is the solid-phase concentration (μg/kg), C_e is the solution phase concentration (μg/L), K_F is the sorption affinity parameter ((μg/kg)/(μg/L)n), *n* is the nonlinear coefficient, The fitting was processed with Sigmaplot 10.0.

Determination of total PAHs content

The samples were extracted with hexane in Soxhlet for 36 h, 1 mL of isooctane was added as a keeper to concentrate the extracts to 1 mL using a rotary vacuum concentrator RVC 2–25 CD plus (Martin Christ, Germany). Then, the soil, biochar, activated carbon were subjected to the clean-up procedure (liquid-liquid partitioning) according to Brändli et al. (2006).[5] After liquid-liquid partitioning, the recollected phase was reduced and applied to an open micro glass column (150 mm × 7 mm i.d.) filled with (from bottom to top) glass wool, deactivated silica gel (10% milli-Q water, 3 cm), water free sodium sulphate, and prewashed with 5 mL heptane. The extract was eluted with 10 mL of heptane. After the clean-up the extracts were concentrated to 0.5 mL, transferred into the vials and each sample was spiked with 20 μL of a TTB (1,3,5-tri-tert-butylbenzene) solution of 6.1 μg/mL.

The final concentrated extracts analyzed using gas chromatograph (Trace 1300) mass spectrometry (ISQ LT) (GC – MS, Thermo Scientific). The GC – MS was equipped with a single quadrupole and used under the selected ion monitoring mode. A Rxi®-5ms crossbond® 5% diphenyl and 95% dimethyl polysiloxane fused capillary column (30 m x 0.25 mm ID x 0.25 μm film thickness) from Restek (USA) was used with helium as the carrier gas at a constant flow rate of 1.5 ml/min. The GC oven temperature was programmed to ramp from 75°C (hold time – 0.5 min) to 245°C at 25°C/min, then to 300°C at 4°C/min (hold time – 1.0 min). The injector temperatures were 310°C. The detection was performed with a Thermo Scientific ISQ LT mass spectrometer in the electron impact mode with a −70 eV ionization energy and a dwelling time of 22 ms. Sixteen PAHs from US EPA list were determined: NAP - naphthalene; ACE - acenaphthylene; AC – acenaphthene; FL - fluorene; PHEN – phenanthrene; ANT – anthracene; FLUO – fluoranthene; PYR – pyrene; BaA – benzo[*a*]anthracene; CHR – chrysene; BbF – benzo[*b*]fluoranthene; BkF – benzo[*k*]fluoranthene; BaP- benzo[*a*]pyrene; IcdP- indeno[1,2,3-*cd*]pyrene; DahA – dibenz[*a,h*]anthracene; BghiP – benzo[*ghi*]perylene.

The linearity ($R^2 > 0.99$) was given for a calibration from 10 to 2500 ng/mL and for each PAH compound. The limits of quantification (LOQ) ranged from 0.0002 (IcdP) to 0.3110 (NAP) ng/L for POM and 0.1 (CHR)–0.7 (DahA) µg/kg dry weight (dw) for total PAH concentrations and was obtained from three times the limit of detection (LOD). Blanks were run for total PAH concentrations (thimble filled with Na_2SO_4) and for the Cfree (POM strips but no sample). The recoveries were quantified by the deuterated internal standards (added before extraction) to the recovery standard (TTB) over the same ratio in the calibration. The recoveries ranged from 77 to 108 % for individual PAHs. The reported results have not been corrected for losses.

Quality assurance and quality control (QA/QC)

All samples were taken according to the PN-ISO 10381-2:2007P (ISO 10381-2:2002 - Soil quality -- Sampling -- Part 2: Guidance on sampling techniques, 2002). Chemical analyses were conducted at the University of Maria Skłodowska-Curie of Lublin (UMCS, Poland) in Department of Environmental Chemistry and Analytical Laboratory UMCS. The Analytical Laboratory UMCS is accredited by the Polish Centre for Accreditation (PCA). The procedures and methods of the chemical tests in lab were controlled according to existing standards or published papers. The QA/QC checks of the testing instruments (GC-MS, pH meter, TOC-VCSH etc.) in lab were conducted during and after installation by the supplier. To ensure quality assurance and quality control (QA/QC), we analyzed a blank sample, duplicate sample (n=3) and a standard reference material (PAHs - Loamy Sand, Sigma Aldrich) with each batch of samples. The all analytical instruments were also calibrated in the lab before the chemical analysis. Blank sample values were very low or below detection limits for the corresponding method. For each PAHs, the response factor percent relative standard deviations (% RSDs) typically were 4 to 15% and always less than 24%.

Table S1. Freundlich isotherm parameters for phenanthrene, pyrene and benzo[*a*]pyrene sorption by biochar and activated carbon

Compound	*log* K_F $(mg\ kg^{-1})(mg\ L^{-1})^{-n}$	*n*	N[a]	R^2
Biochar:				
Phenanthrene	5.13	0.684	8	0.950
Pyrene	5.53	0.630	8	0.949
Benzo[*a*]pyrene	6.34	0.631	8	0.948
AC:				
Phenanthrene	5.68	0.845	8	0.996
Pyrene	5.94	0.774	8	0.998
Benzo[*a*]pyrene	7.00	0.718	8	0.991

[a] Number of data points.

Table S2. The individual C_{free} PAHs content in no planted and under willow cultivation control soil

PAHs	No plants					Willow				
	0	3	6	12	18	0	3	6	12	18
NA	217±20	216±24	220±33	216±31	217±22	229±27	220±21	220±32	219±36	221±24
ACE	10.2±1.0	9.3±1.1	9.8±1.0	9.9±1.2	9.3±1.3	9.3±1.2	9.5±0.8	9.3±1.5	9.1±1.3	10.2±1.2
AC	23.5±3.1	22.1±3.1	21.6±3.0	22.8±3.4	23.4±3.4	24.2±2.4	24.1±2.8	24.6±2.2	24.7±2.0	21.9±3.1
FL	13.9±1.4	14.0±2.1	13.4±1.5	13.0±1.9	12.9±1.9	12.9±1.8	12.5±1.8	13.7±1.4	12.2±1.3	12.3±2.0
PHEN	26.9±3.64	28.5±3.3	30.9±4.0	28.7±3.2	29.0±2.6	35.6±5.2	33.5±3.5	33.2±5.6	34.0±3.2	34.9±4.4
ANT	11.1±1.3	9.0±1.1	9.2±1.3	11.0±1.3	11.2±1.5	10.1±1.4	9.2±1.5	8.1±1.0	6.5±0.8	9.5±1.2
FLUO	27.8±3.0	25.7±2.8	21.1±3.0	19.2±2.0	17.5±2.2	26.6±3.2	24.3±2.9	24.6±4.0	25.3±2.6	24.2±2.7
PYR	18.6±1.8	20.4±2.3	18.6±2.6	19.8±3.1	18.9±1.6	19.8±2.0	19.2±2.1	19.1±2.6	20.2±2.6	16.6±2.5
BaA	1.54±0.19	0.71±0.12	0.85±0.06	0.68±0.09	1.42±0.17	2.01±0.29	1.05±0.11	1.24±0.15	1.06±0.08	1.00±0.16
CHR	2.89±0.35	1.90±0.27	1.96±0.18	1.53±0.11	2.02±0.31	4.07±0.65	2.69±0.37	3.09±0.30	2.65±0.40	2.10±0.19
BbF	0.80±0.12	0.43±0.06	0.23±0.03	0.23±0.02	0.26±0.03	1.10±0.16	0.81±0.12	0.72±0.06	0.66±0.07	0.63±0.09
BkF	0.41±0.06	0.21±0.04	0.12±0.02	0.09±0.01	0.09±0.01	0.62±0.08	0.44±0.06	0.41±0.05	0.39±0.06	0.37±0.05
BaP	0.58±0.08	0.34±0.05	0.19±0.03	0.27±0.04	0.27±0.04	0.35±0.04	0.30±0.04	0.26±0.04	0.25±0.04	0.24±0.03
IcdPd	0.07±0.01	0.06±0.01	0.06±0.01	0.06±0.01	0.08±0.01	0.11±0.01	0.09±0.01	0.10±0.01	0.09±0.01	0.08±0.01
DahA	0.01±0.001	0.02±0.001	0.01±0.001	0.02±0.001	0.01±0.001	0.03±0.001	0.02±0.001	0.02±0.001	0.02±0.001	0.01±0.001
BghiP	0.06±0.01	0.04±0.01	0.04±0.01	0.03±0.001	0.02±0.001	0.05±0.01	0.06±0.01	0.06±0.01	0.04±0.001	0.04±0.01

Values are mean of three repetitions. NA - naphthalene; ACE - acenaphthylene; AC – acenaphthene; FL - fluorene; PHEN – phenanthrene; ANT – anthracene; FLUO – fluoranthene; PYR – pyrene; BaA – benzo[*a*]anthracene; CHR – chrysene; BbF – benzo[*b*]fluoranthene; BkF – benzo[*k*]fluoranthene; BaP- benzo[*a*]pyrene; IcdP- indeno[1,2,3-*cd*]pyrene; DahA – dibenz[*a,h*]anthracene; BghiP – benzo[*ghi*]perylene.

Table S3. The individual C_{free} PAHs content in under grass and clover cultivation control soil

PAHs	Grass					Clover				
	0	3	6	12	18	0	3	6	12	18
NA	219±34	212±35	221±24	221±34	221±30	225±34	225±29	227±36	226±36	228±27
ACE	9.02±1.30	8.56±1.05	9.96±1.30	5.87±0.51	7.09±0.92	6.61±0.68	8.69±1.21	8.26±1.23	8.49±1.18	7.10±0.53
AC	21.7±2.9	21.2±3.5	21.5±2.9	25.2±3.1	23.6±1.8	24.7±2.0	25.1±2.4	20.5±3.5	21.1±2.9	24.1±3.6
FL	11.1±0.8	13.4±1.5	12.5±1.2	13.4±2.0	14.9±1.9	20.1±2.3	16.7±2.4	14.2±1.6	16.8±2.7	16.8±2.0
PHEN	39.9±4.4	41.8±5.0	41.2±6.3	40.7±4.6	40.3±3.8	33.7±4.5	33.3±5.4	35.6±5.1	32.9±5.7	33.4±5.4
ANT	9.05±1.31	5.98±0.74	10.4±1.2	3.49±0.41	8.36±1.17	6.59±0.89	8.38±1.29	6.86±0.94	7.80±0.88	7.91±1.0
FLUO	25.2±3.7	23.9±3.6	22.7±2.4	23.2±2.6	19.0±2.2	19.6±1.6	18.2±2.7	25.0±2.4	23.0±2.3	20.3±1.9
PYR	17.0±2.5	18.1±2.9	17.2±2.4	15.6±1.8	18.4±2.6	19.5±1.2	17.2±2.3	18.7±2.9	16.1±1.6	17.0±2.1
BaA	1.28±0.11	1.04±0.16	1.33±0.15	0.81±0.12	0.72±0.11	1.75±0.22	1.08±0.10	1.08±0.16	0.81±0.10	0.86±0.11
CHR	2.66±0.35	2.79±0.28	3.04±0.32	1.88±0.26	1.75±0.21	3.42±0.45	2.68±0.43	2.60±0.38	2.01±0.31	2.07±0.27
BbF	0.51±0.08	0.49±0.06	0.45±0.06	0.47±0.08	0.42±0.05	1.00±0.12	0.85±0.13	0.83±0.09	0.72±0.11	0.65±0.10
BkF	0.27±0.03	0.21±0.01	0.18±0.01	0.15±0.02	0.15±0.02	0.40±0.07	0.31±0.03	0.25±0.03	0.21±0.02	0.11±0.02
BaP	0.27±0.03	0.23±0.04	0.22±0.03	0.20±0.02	0.24±0.02	0.68±0.10	0.44±0.07	0.42±0.06	0.39±0.06	0.38±0.06
IcdPd	0.08±0.01	0.08±0.01	0.09±0.01	0.07±0.01	0.06±0.01	0.07±0.01	0.07±0.01	0.07±0.01	0.07±0.01	0.06±0.001
DahA	0.02±0.001	0.02±0.001	0.03±0.001	0.02±0.001	0.02±0.001	0.01±0.001	0.01±0.001	0.01±0.001	0.01±0.001	0.01±0.001
BghiP	0.03±0.001	0.02±0.001	0.03±0.001	0.03±0.001	0.03±0.001	0.05±0.01	0.04±0.001	0.04±0.001	0.04±0.01	0.05±0.01

Values are mean of three repetitions. NA - naphthalene; ACE - acenaphthylene; AC – acenaphthene; FL - fluorene; PHEN – phenanthrene; ANT – anthracene; FLUO – fluoranthene; PYR – pyrene; BaA – benzo[*a*]anthracene; CHR – chrysene; BbF – benzo[*b*]fluoranthene; BkF – benzo[*k*]fluoranthene; BaP- benzo[*a*]pyrene; IcdP- indeno[1,2,3-*cd*]pyrene; DahA – dibenz[*a,h*]anthracene; BghiP – benzo[*ghi*]perylene.

Table S4. The individual C_{free} PAHs content in no plants and willow cultivation biochar-amended soil

PAHs	No plants					Willow				
	0	3	6	12	18	0	3	6	12	18
NA	220±18	187±28	170±6	125±15	82.3±6.6	217±29	213±27	169±19	107±15	76.2±7.5
ACE	11.2±1.3	8.87±1.44	4.42±0.62	6.02±0.85	5.51±0.79	10.3±0.9	6.93±1.00	6.58±0.77	3.47±0.35	1.44±0.36
AC	19.0±2.9	18.5±2.5	9.29±1.12	9.03±0.98	5.27±0.71	14.2±1.9	12.4±1.5	10.5±1.3	9.03±1.32	5.70±2.20
FL	10.2±1.6	14.2±2.0	5.79±0.94	5.33±0.68	3.57±0.29	11.9±1.7	8.95±0.76	7.87±1.12	6.73±1.05	5.69±0.86
PHEN	29.6±3.7	22.0±3.5	14.5±2.0	6.77±0.99	6.67±0.69	26.7±1.8	24.9±3.9	22.8±2.9	22.0±1.8	12.0±4.3
ANT	6.48±1.05	6.99±0.91	5.77±0.78	3.12±0.23	2.91±0.41	6.33±0.99	5.84±0.68	5.77±1.00	3.30±0.48	2.75±0.77
FLUO	21.0±2.3	18.9±2.5	17.2±2.7	17.6±2.2	17.0±2.2	22.0±2.8	18.6±2.5	20.4±2.9	19.5±1.4	19.6±2.0
PYR	15.1±2.0	14.9±1.6	13.0±1.2	12.6±1.8	12.6±1.2	18.0±2.8	17.5±2.3	15.2±1.8	14.6±1.4	11.9±1.3
BaA	1.07±0.14	1.03±0.15	0.42±0.06	0.81±0.12	0.74±0.12	0.94±0.12	0.88±0.07	0.91±0.12	0.62±0.08	0.50±0.04
CHR	2.25±0.21	2.69±0.26	1.32±0.20	2.32±0.32	1.89±0.27	1.96±0.29	1.65±0.24	1.27±0.21	1.67±0.22	1.29±0.13
BbF	0.65±0.08	0.38±0.06	0.23±0.03	0.214±0.02	0.25±0.12	0.60±0.10	0.34±0.03	0.28±0.04	0.21±0.03	0.29±0.04
BkF	0.42±0.04	0.09±0.01	0.034±0.02	0.031±0.001	0.03±0.02	0.31±0.05	0.21±0.03	0.07±0.01	0.06±0.01	0.07±0.01
BaP	0.38±0.06	0.32±0.04	0.24±0.03	0.188±0.03	0.14±0.01	0.23±0.03	0.11±0.01	0.12±0.02	0.10±0.02	0.08±0.01
IcdPd	0.07±0.01	0.08±0.01	0.042±0.01	0.047±0.01	0.054±0.001	0.05±0.01	0.04±0.01	0.05±0.001	0.03±0.01	0.02±0.001
DahA	0.01±0.001	0.02±0.001	0.01±0.001	0.02±0.001	0.02±0.001	0.01±0.001	0.01±0.001	0.01±0.001	0.01±0.001	0.01±0.001
BghiP	0.04±0.01	0.02±0.001	0.03±0.001	0.03±0.001	0.037±0.01	0.04±0.001	0.04±0.001	0.04±0.001	0.03±0.001	0.04±0.01

Values are mean of three repetitions. NA - naphthalene; ACE - acenaphthylene; AC – acenaphthene; FL - fluorene; PHEN – phenanthrene; ANT – anthracene; FLUO – fluoranthene; PYR – pyrene; BaA – benzo[*a*]anthracene; CHR – chrysene; BbF – benzo[*b*]fluoranthene; BkF – benzo[*k*]fluoranthene; BaP- benzo[*a*]pyrene; IcdP- indeno[1,2,3-*cd*]pyrene; DahA – dibenz[*a,h*]anthracene; BghiP – benzo[*ghi*]perylene.

Table S5. The individual C_{free} PAHs content in under grass and clover cultivation biochar-amended soil

PAHs	Grass					Clover				
	0	3	6	12	18	0	3	6	12	18
NA	195±28	166±16	163±25	173±27	81.4±7.7	226±25	193±15	176±21	129±19	86.3±11.0
ACE	10.2±1.4	9.07±1.07	6.23±0.79	3.36±0.33	2.78±0.46	9.41±1.51	9.49±1.48	7.69±1.21	6.14±0.55	4.28±0.57
AC	24.2±2.2	17.6±1.9	15.3±2.4	9.33±1.43	7.01±0.61	19.5±2.3	18.7±1.6	16.5±2.3	13.5±1.4	9.19±1.34
FL	10.7±1.7	7.18±0.84	6.92±0.62	2.96±0.31	2.00±0.25	18.1±2.0	8.23±1.17	5.04±0.77	2.63±0.41	2.24±0.28
PHEN	25.6±3.9	15.6±2.3	14.0±1.5	10.8±1.1	11.1±2.8	28.1±3.1	19.6±2.4	16.4±2.9	9.57±1.48	4.04±0.45
ANT	6.37±0.85	6.30±0.61	3.64±0.34	1.57±0.15	2.81±0.41	5.22±0.81	2.48±0.28	1.45±0.22	0.38±0.04	0.21±0.03
FLUO	20.2±2.3	18.5±2.9	15.0±2.1	9.86±1.30	8.93±1.33	19.3±3.3	16.0±2.0	12.0±1.8	11.4±1.4	7.33±0.76
PYR	15.3±2.1	13.7±1.5	11.7±1.1	7.57±1.18	6.73±1.04	13.9±2.1	12.7±1.6	5.57±0.61	5.55±0.72	5.92±0.78
BaA	1.00±0.16	0.59±0.09	0.56±0.08	0.24±0.04	0.19±0.02	1.03±0.09	0.78±0.10	0.84±0.13	0.61±0.08	0.31±0.04
CHR	2.05±0.16	1.69±0.24	1.44±0.14	0.78±0.11	0.67±0.07	2.17±0.26	1.90±0.23	0.94±0.13	0.90±0.14	0.82±0.10
BbF	0.45±0.07	0.29±0.04	0.20±0.03	0.17±0.01	0.10±0.01	0.51±0.07	0.33±0.05	0.24±0.02	0.21±0.01	0.19±0.02
BkF	0.21±0.03	0.17±0.02	0.09±0.01	0.04±0.001	0.02±0.001	0.35±0.04	0.12±0.001	0.04±0.001	0.04±0.001	0.03±0.001
BaP	0.23±0.02	0.17±0.02	0.07±0.01	0.04±0.01	0.03±0.001	0.29±0.03	0.14±0.02	0.03±0.001	0.02±0.001	0.03±0.001
IcdPd	0.06±0.001	0.06±0.01	0.05±0.01	0.04±0.001	0.02±0.001	0.06±0.01	0.05±0.001	0.03±0.001	0.03±0.001	0.02±0.001
DahA	0.02±0.001	0.01±0.001	0.01±0.001	0.01±0.001	0.01±0.001	0.00	0.02±0.001	0.00	0.01±0.001	0.01±0.001
BghiP	0.03±0.01	0.03±0.001	0.02±0.001	0.03±0.001	0.02±0.001	0.04±0.01	0.03±0.001	0.03±0.001	0.02±0.001	0.01±0.001

Values are mean of three repetitions. NA - naphthalene; ACE - acenaphthylene; AC – acenaphthene; FL - fluorene; PHEN – phenanthrene; ANT – anthracene; FLUO – fluoranthene; PYR – pyrene; BaA – benzo[*a*]anthracene; CHR – chrysene; BbF – benzo[*b*]fluoranthene; BkF – benzo[*k*]fluoranthene; BaP- benzo[*a*]pyrene; IcdP- indeno[1,2,3-*cd*]pyrene; DahA – dibenz[*a,h*]anthracene; BghiP – benzo[*ghi*]perylene.

Table S6. The individual C_{free} PAHs content in no plants and willow cultivation AC-amended soil

PAHs	No plants					Willow				
	0	3	6	12	18	0	3	6	12	18
NA	219±16	191±10	144±12	113±8	54±5	186±26	178±20	137±13	53.6±6.6	37.3±4.4
ACE	9.69±1.45	9.59±0.96	9.54±1.19	3.42±0.33	3.36±0.52	9.33±1.41	5.59±0.53	3.41±0.53	0.74±0.10	0.37±0.07
AC	20.9±3.0	19.3±2.5	19.3±2.3	8.48±0.96	7.76±0.89	9.16±1.42	5.05±0.74	3.37±0.49	1.81±0.15	0.58±0.08
FL	10.2±1.0	11.2±1.5	11.2±1.7	4.29±0.37	1.33±0.21	9.02±0.98	7.28±0.71	4.89±0.65	0.93±0.07	0.55±0.08
PHEN	25.6±3.0	25.8±2.6	25.7±4.0	1.19±0.15	1.18±0.12	17.7±2.5	11.7±1.5	6.35±0.65	1.93±0.27	0.59±0.05
ANT	0.63±0.10	0.56±0.05	0.48±0.06	0.22±0.03	0.44±0.06	4.54±0.69	3.83±0.47	3.49±0.45	1.37±0.17	0.39±0.05
FLUO	0.44±0.05	0.25±0.04	0.18±0.03	0.14±0.02	0.10±0.01	2.41±0.32	1.26±0.18	0.16±0.02	0.11±0.01	0.09±0.01
PYR	3.28±0.35	3.93±0.58	3.44±0.42	0.38±0.06	0.31±0.04	1.25±0.12	0.73±0.08	0.42±0.04	0.30±0.04	0.19±0.03
BaA	0.01±0.001	0.00	0.00	0.00	0.01±0.001	0.01±0.001	0.00	0.00	0.00	0.00
CHR	0.01±0.001	0.01±0.001	0.01±0.001	0.01±0.001	0.01±0.001	0.13±0.01	0.02±0.001	0.00	0.00	0.00
BbF	0.01±0.001	0.01±0.001	0.00	0.00	0.00	0.02±0.001	0.00	0.00	0.00	0.00
BkF	0.00	0.00	0.00	0.00	0.00	0.09±0.01	0. 00	0.00	0.00	0.00
BaP	0.10±0.01	0.06±0.01	0.05±0.01	0.048±0.01	0.01±0.001	0.16±0.03	0.03±0.001	0.03±0.001	0.12±0.02	0.00
IcdPd	0.001±0.0001	0.002±0.001	0.002±0.001	0.001±0.001	0.001±0.0001	0.022±0.001	0.006±0.001	0.005±0.001	0.004±0.001	0.002±0.001
DahA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
BghiP	0.004±0.001	0.003±0.001	0.00	0.00	0.00	0.008±0.001	0.00	0.00	0.00	0.00

Values are mean of three repetitions. NA - naphthalene; ACE - acenaphthylene; AC – acenaphthene; FL - fluorene; PHEN – phenanthrene; ANT – anthracene; FLUO – fluoranthene; PYR – pyrene; BaA – benzo[*a*]anthracene; CHR – chrysene; BbF – benzo[*b*]fluoranthene; BkF – benzo[*k*]fluoranthene; BaP- benzo[*a*]pyrene; IcdP- indeno[1,2,3-*cd*]pyrene; DahA – dibenz[*a,h*]anthracene; BghiP – benzo[*ghi*]perylene.

Table S7. The individual C_{free} PAHs content in under grass and clover cultivation AC-amended soil

PAHs	Grass					Clover				
	0	3	6	12	18	0	3	6	12	18
NA	201±25	170±21	168±26	159±21	52.0±8.5	205±29	125±20	93.6±10.4	61.2±9.2	51.1±5.3
ACE	9.55±1.24	9.52±1.47	6.50±1.10	0.87±0.09	0.25±0.03	8.02±1.01	5.51±0.86	3.01±0.39	2.31±0.33	1.75±0.18
AC	20.3±2.8	16.8±2.5	10.7±1.1	1.87±0.25	1.36±0.11	16.4±2.7	16.0±2.1	11.6±1.0	7.87±0.76	4.24±0.65
FL	10.4±1.5	9.11±0.79	7.95±1.06	0.93±0.10	0.99±0.07	7.69±1.12	6.80±0.73	6.40±0.77	4.78±0.49	2.35±0.24
PHEN	23.5±2.8	18.8±1.5	13.5±1.8	1.07±0.15	0.84±0.13	26.0±3.0	20.7±2.6	7.59±0.97	6.15±0.42	3.28±0.23
ANT	0.62±0.10	0.73±0.11	0.45±0.05	0.00	0.50±0.07	6.89±0.60	3.78±0.58	1.38±0.11	2.28±0.29	2.36±0.31
FLUO	9.25±1.60	9.07±1.40	3.16±0.52	0.22±0.03	0.09±0.01	5.19±0.41	1.19±0.15	0.19±0.03	0.13±0.02	0.10±0.01
PYR	6.32±0.91	2.62±0.23	0.41±0.04	0.32±0.03	0.25±0.03	2.25±0.18	0.92±0.12	0.21±0.03	0.22±0.03	0.21±0.03
BaA	0.01±0.001	0.00	0.00	0.00	0.00	0.30±0.05	0.09±0.01	0.01±0.001	0.00	0.00
CHR	0.01±0.001	0.01±0.001	0.00	0.00	0.00	0.90±0.07	0.10±0.01	0.01±0.001	0.00	0.00
BbF	0.43±0.06	0.29±0.04	0.14±0.02	0.00	0.00	0.01±0.001	0.00	0.00	0.00	0.00
BkF	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
BaP	0.12±0.01	0.06±0.01	0.03±0.001	0.03±0.001	0.00	0.08±0.01	0.13±0.02	0.04±0.001	0.04±0.02	0.013±0.001
IcdPd	0.00	0.00	0.00	0.00	0.00	0.010±0.001	0.009±0.001	0.007±0.001	0.006±0.001	0.002±0.0001
DahA	0.00	0.00	0.00	0.01±0.001	0.00	0.00	0.00	0.003±0.001	0.024±0.001	0.003±0.001
BghiP	0.00	0.00	0.00	0.00	0.00	0.009±0.001	0.00	0.00	0.00	0.00

Values are mean of three repetitions. NA - naphthalene; ACE - acenaphthylene; AC – acenaphthene; FL - fluorene; PHEN – phenanthrene; ANT – anthracene; FLUO – fluoranthene; PYR – pyrene; BaA – benzo[*a*]anthracene; CHR – chrysene; BbF – benzo[*b*]fluoranthene; BkF – benzo[*k*]fluoranthene; BaP- benzo[*a*]pyrene; IcdP- indeno[1,2,3-*cd*]pyrene; DahA – dibenz[*a,h*]anthracene; BghiP – benzo[*ghi*]perylene.

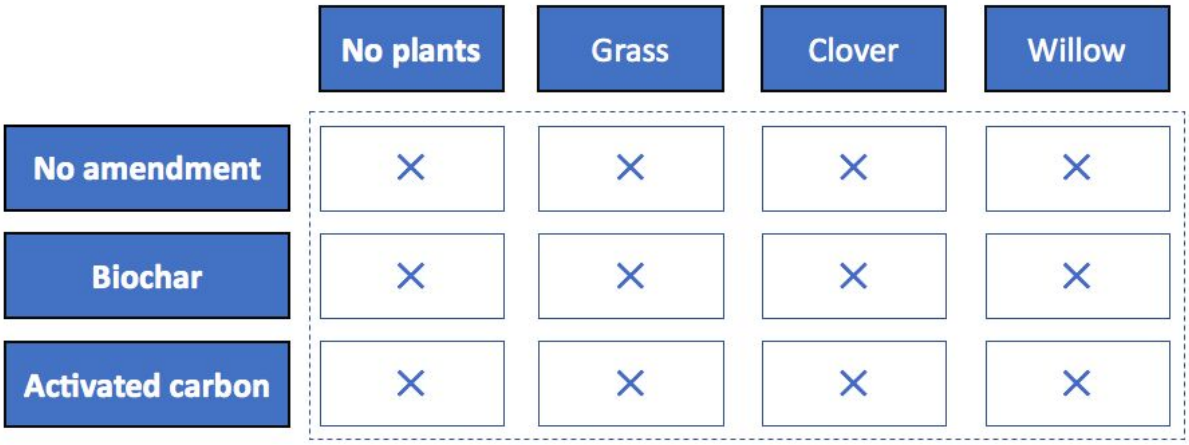

Figure S1. The general scheme of the field experiment

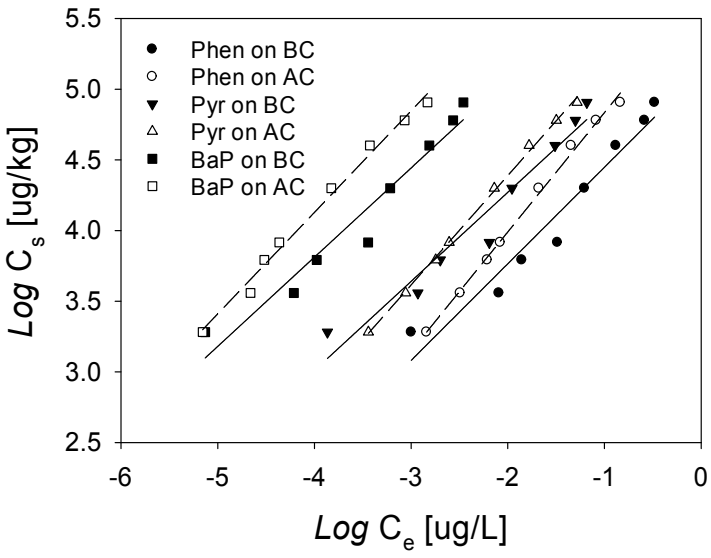

Figure S2. Adsorption isotherms of phenanthrene (Phen), pyrene (Pyr) and benzo[*a*]pyrene (BaP) on native biochar (BC) and activated carbon (AC) used in the experiment

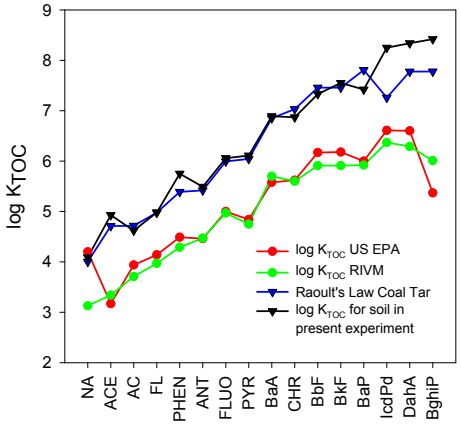

Figure S3. Log K_{TOC} values (L/Kg) for PAHs measured in present experiment comparing to recommended K_{TOC} values used by the US EPA for sediments [4], RIVM for soils and sediment [5] and Raoult's Law Coal Tar sorption model [6].

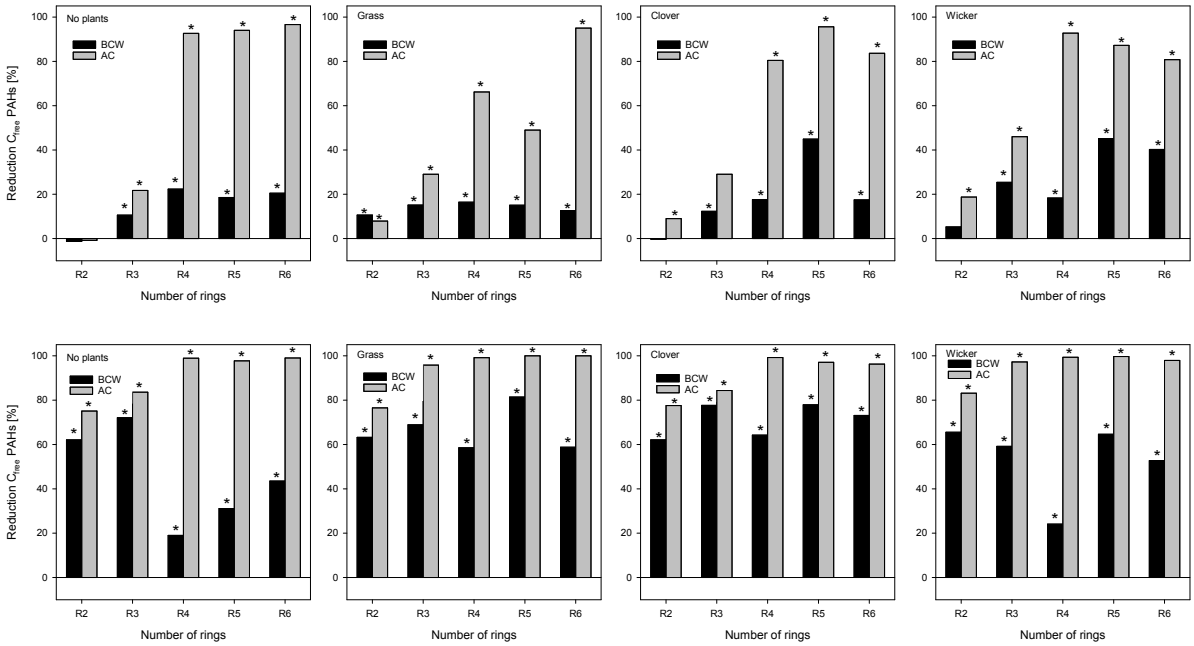

Figure S4. The reduction (%) of C_{free} PAHs after biochar or AC-soil amendment comparing to the control soil at the beginning of the experiment (top panel) and after 18-months (lower panel). * - means statistically significant differences compared to control soil (non-amended soil).

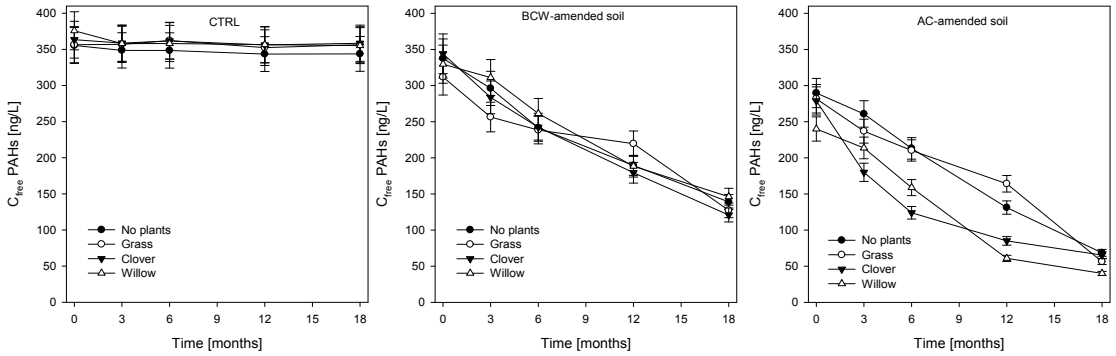

Figure S5. The content of Σ16 C_{free} PAHs in control (left panel), BCW- (middle panel) and AC-amended soil (right panel) depending on the plants cultivated. Error bars represent standard deviation error (SD, *n*=3 extractions).

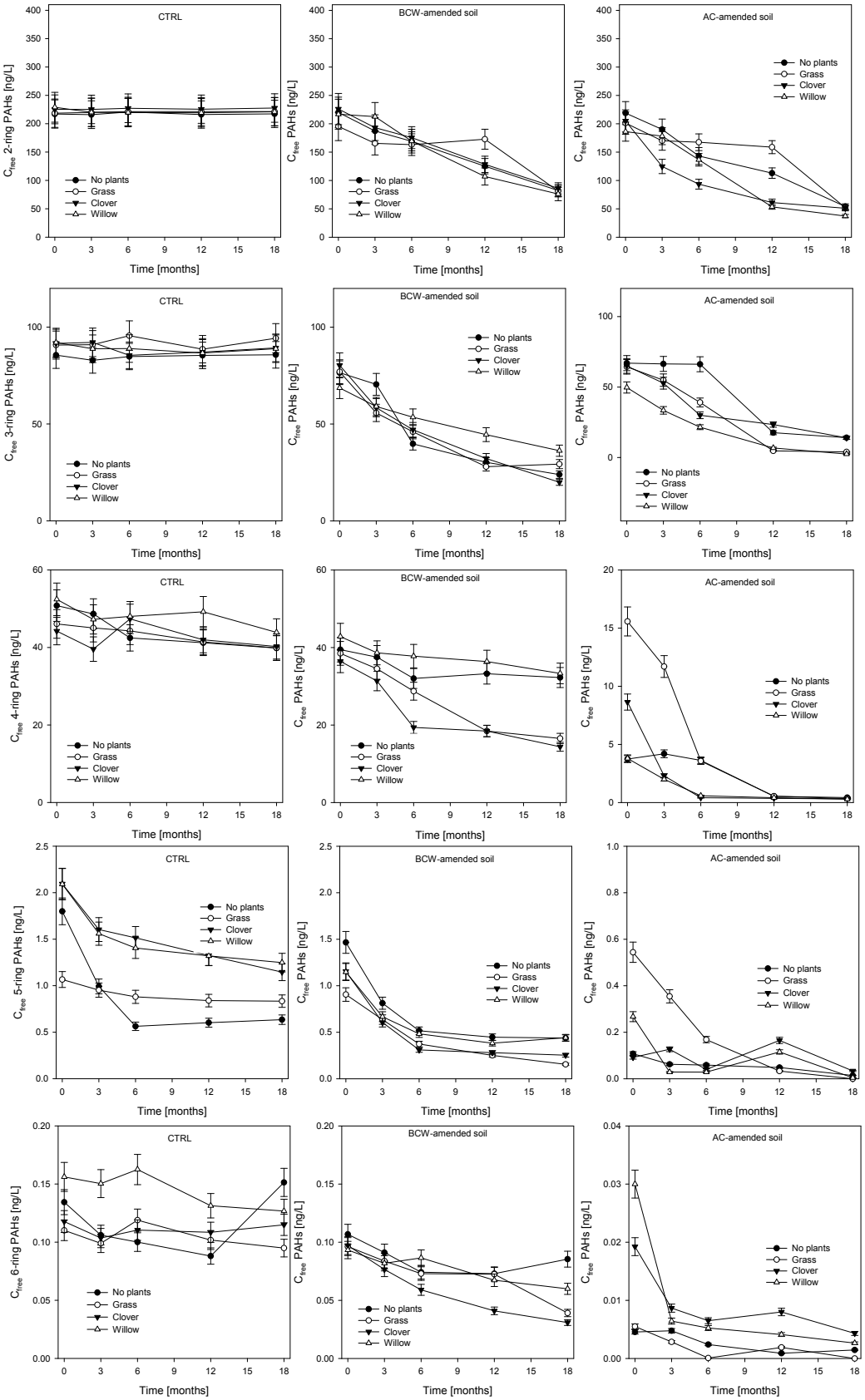

Figure S6. The content of individual groups of C_{free} PAHs in control (left panel), BCW- (middle panel) and AC-amended soil (right panel) depending on the plants cultivated. Error bars represent standard deviation error (SD, *n*=3 extractions).

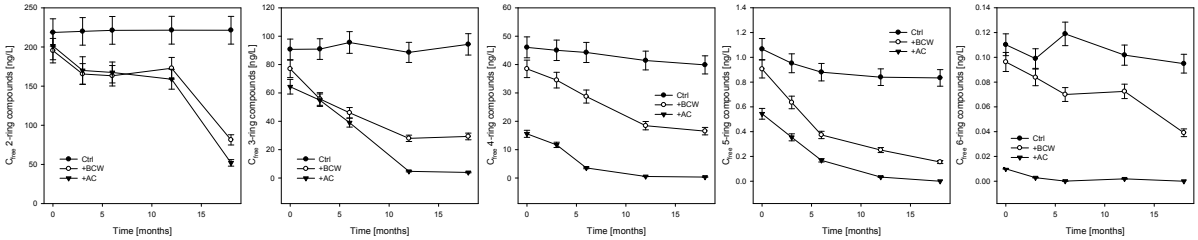

Figure S7. Changes of individual groups of C_{free} PAHs in experiment with grass in control (non- amended) or activated carbon (AC)/biochar (BCW)-amended soil. Error bars represent standard deviation error (SD, *n*=3 extractions).

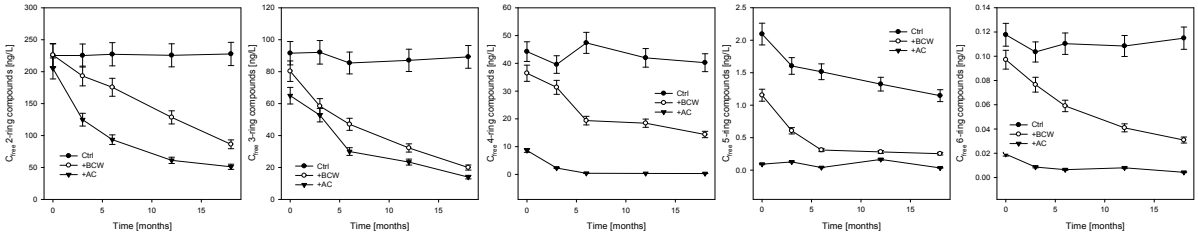

Figure S8. Changes of individual groups of C_{free} PAHs in experiment with clover in control (non-amended) or activated carbon (AC)/biochar (BCW)-amended soil. Error bars represent standard deviation error (SD, *n*=3 extractions).

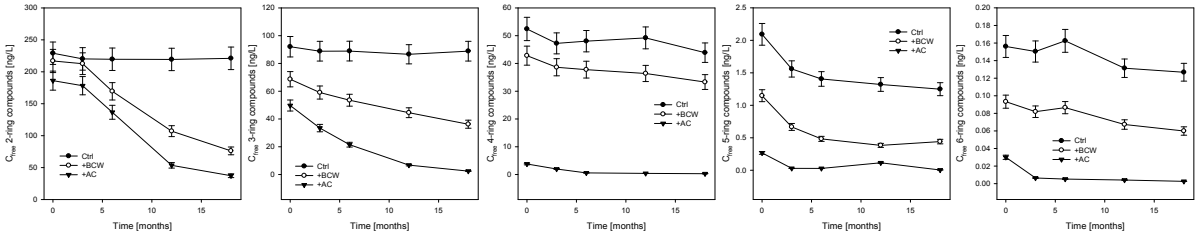

Figure S9. Changes of individual groups of C_{free} PAHs in experiment with willow in control (non-amended) or activated carbon (AC)/biochar (BCW)-amended soil. Error bars represent standard deviation error (SD, *n*=3 extractions).

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Adsorption capacity of phenanthrene and pyrene to engineered carbon-based adsorbents produced from sewage sludge or sewage sludge-biomass mixture in various gaseous conditions

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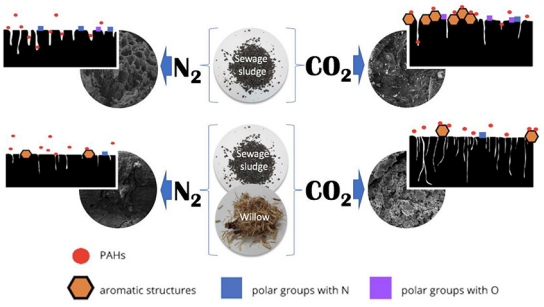
GRAPHICAL ABSTRACT



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ABSTRACT

Adsorption of phenanthrene (PHE) and pyrene (PYR) by engineered carbon-based adsorbents produced from sewage sludge in an atmosphere of nitrogen (N_2) or carbon dioxide (CO_2) at temperatures of 500, 600, and 700 °C was investigated. The addition of willow to the SSL decreased the biochar adsorption capacity. However, there was an increase in the adsorption capacity after changing N_2 to CO_2. The addition of willow to SSL and the type of carrier gas affected the mechanism of adsorption. The adsorption of PHE and PYR on the SSL-derived adsorbents produced in N_2 occurred through pore filling. The adsorption on the SSL-derived adsorbents with willow followed the mechanism of π-π electron-donor-acceptor (EDA) interactions and hydrophobic interactions. A similar mechanism was observed with regard to the biochars produced from SSL in atmosphere of CO_2. For the SSL-derived adsorbents with willow in CO_2, the adsorption mechanism was observed to vary between PHE and PYR.

1. Introduction

Carbon adsorbents, such as activated carbon (AC) (Fu and Wang, 2011; Mohan et al., 2014) are commonly used to clean up water and soil from various contaminants. The popularity of AC results from the high specific surface area and the presence of functional groups, which are efficient to adsorb different compounds. As a result of the continuous examination for cheaper and effective substitutes of AC, biochar (BC) is possessing an increasingly greater interest (Ahmad et al., 2014; Mohan et al., 2014; Zielińska and Oleszczuk, 2015a). Biochar is a

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carbon-rich product of thermo-chemical conversion of biomass (usually) in the absence of oxygen or with limited oxygen presence (< 1%) (pyrolysis). Feedstock for BC production can be widely understood biomass (Ok et al., 2016) as well as biowastes, e.g. sewage sludge (biosolid) or biogas residues (Inyang et al., 2016). The properties of biochar and thus its ability to adsorb different compounds depend on pyrolysis conditions (temperature, time) and the type of feedstock (Ahmad et al., 2014).

Sewage sludge (SSL) is a byproduct of sewage treatment and it may contain harmful organic and inorganic substances as well as biological hazardous materials (parasite eggs, weed seeds, etc.) (Zhang et al., 2017). The use of SSL in agriculture as well as its incineration and storage are the most common methods for disposal of SSL (Cieślik et al., 2015; Raheem et al., 2018). Pyrolysis of SSL to biochar (BC) could be an interesting method for converting this waste into a useful adsorbent (Zielińska and Oleszczuk, 2015a). During pyrolysis, the volume of SSL decreases and, at the same time, pathogens and the harmful substances contained in SSL are reduced (Waqas et al., 2014; Zielińska and Oleszczuk, 2015b). Nonetheless, the properties of SSL-derived BCs are far from the properties of an "perfect" adsorbent. Sewage sludge-derived BCs usually have a low specific surface area and a low carbon content, and biochar may contain concentrated toxic substances, e.g. heavy metals (Agrafioti et al., 2013; Callegari and Capodaglio, 2018; Figueiredo et al., 2018; Huang et al., 2017; Raheem et al., 2018). One of the directions for improving the above-mentioned properties of sewage sludge-derived biochar and reduce the risk is to enrich SSL before pyrolysis with a material that will contribute to an improvement in these parameters. Biomass-derived biochars are usually characterized by a higher specific surface area and a higher carbon content than biochars obtained from SSL (Huang et al., 2017, 2015). Mixing biomass with SSL results in an increase in the specific surface area and carbon content in biochar (Kończak et al., 2019) and, moreover, it has an effect on increasing the biochar's cation exchange capacity (CEC), decreasing the PAH content in it, and reducing the bioavailability of heavy metals (Huang et al., 2017; Jin et al., 2017a).

Apart from co-application of various feedstocks, the biochar properties can also be affected by the type of carrier gas used during pyrolysis (Azuara et al., 2017; Liu et al., 2018; Tan and Yuan, 2017). The use of carbon dioxide (CO_2) instead of N_2 as carrier gas during pyrolysis has attracted a special interest in recent years. The few existing data show (Azuara et al., 2017; Tan and Yuan, 2017) that using of CO_2 during pyrolysis instead of nitrogen (N_2) leads to better properties of the BC produced, e.g. an increased specific surface area (Liu et al., 2018). The specific surface area, in turn, has a significant impact on the adsorption of various organic contaminants (Ahmad et al., 2014). Furthermore, biochar produced under such conditions is characterized by a higher content of phosphorus (P) and potassium (K) than that obtained in a N_2 atmosphere (Tan and Yuan, 2017), which is of significant importance from the fertilization point of view. Nevertheless, in the literature there is a lack of data on how biomass addition or CO_2 application affects biochar properties in the context of contaminant adsorption. This issue is important for two reasons, i.e. for a practical reason and due to environmental risk. In the former case, it is important whether the adsorption capacity of BCs in relation to contaminants increases, which is especially important if a biochar is to be used for remediation of contaminated soils or for water treatment. In the latter case, attention should be paid to how biochars obtained under such conditions, with different properties than previously, will affect the cycling (bioavailability) of contaminants present in soils or their interactions with natural soil components.

The aim of this study was to determine the effect of biomass addition to sewage sludge and CO_2 application during pyrolysis on the adsorption properties of biochars obtained from SSL and from a SSL and biomass mixture in relation to selected representatives from the group of polycyclic aromatic hydrocarbons (PAHs), i.e. phenanthrene (PHE) (3-ring) and pyrene (PYR) (4-ring). The selected compounds belong to the group of organic pollutants commonly found in contaminated areas and their sorption is a frequent subject of research (Jin et al., 2017b, 2018; Kang et al., 2018; Wang et al., 2016; Zielińska and Oleszczuk, 2015a).

2. Materials and methods

2.1. Sorbent materials

SSL was obtained from municipal (mechanical-biological) wastewater treatment plant (WWTPs). Willow (*Salix viminalis*) was obtained from biomass-producing farm. Production of biochars and particular preparation steps of feedstock were described elsewhere (Kończak et al., 2019). Briefly, the SSL before pyrolysis was grinded and sieved through a 2 mm sieve. The willow was dried and then cut into small pieces and then sieved through 2 mm sieve. Mixtures of SSL and willow were obtained by mixing both materials in glass bottles (1000 mL) for 24 h in the dark at 10 rpm (Rotax 6.8. VELP, Italy). SSL was mixed with willow in the 6:4 (w/w) ratio. Feedstocks (SSL alone and SSL with willow) were pyrolyzed in 500, 600 and 700 °C, with the heating rate 10 °C/min. Temperature was held for 3 h (slow pyrolysis) During the pyrolysis the oxygen free atmosphere was maintained by constant flow of N_2 or CO_2 and the flow was monitored with mass flow controller (BETA-ERG, Poland).

2.2. Adsorption experiment

In the adsorption experiment the method proposed by Hale et al. (2011) was used. Biochars (50 mg) were added to 50 mL glass flasks with glass lids. Millipore water (40 mL) with sodium azide (200 mg L^{-1}) (to prevent microorganisms activity) and strips of 55 µm thick polyoxymethylene (POM) passive samplers (0.3 g for all batches) were added to the flasks. Before use, POM samplers were cleaned using overnight in methanol, next in heptane and then were rinsed in Millipore water and dried. Batches were spiked with PHE or PYR in methanol so that the end concentration was between 20 and 800 µg L^{-1}. The amount of spiked co-solvent (100 µL) was less than 0.25% of the water volume so the co-solvent effect was minimal in the system. The initial concentration of PHE or PYR in biochars was determined by batches without spiked PAHs (only BC, water with sodium azide and POM strips were added). Flasks were rolled end over end 28 days at 1 RCF. Next, POM strips were removed, cleaned with Millipore water and wiped with a tissue until they were dry and visibly clean. The extraction of POM samplers was carried out in 20 mL of 20:80 acetone:heptane for 48 h. The solvent was reduced to about 1 mL using rotary vacuum concentrator RVC 2-25 CD plus (Martin Christ, Germany) and spiked with d-10 phenanthrene as internal standard for quantification via GC/MS. A quantitative analysis of PAHs was carried out on Thermo Scientific Trace 1 300 Gas Chromatograph equipped with a Restek Rxi-5 ms Column (length 30 m, 0.25 mm id and 0.25 µm film thickness). Detailed information about PAHs analysis is presented elsewhere (Zielińska and Oleszczuk, 2015b).

The concentration of PHE/PYR on POM passive samplers was calculated according to the Eq. (1):

$$C_{POM}(\mu g\ kg^{-1}) = \frac{m_{PHE\ or\ PYR}\ (\mu g)}{m_{2POM}\ (kg)} \quad (1)$$

where $m_{Phe\ or\ Pyr}$ is the mass of PAH determined via GC/MS and m_{2POM} is the mass of two passive samplers used in the system.

The concentration of the initial PHE or PYR in biochars was determined by substracting $c_{POM\text{-}control}$ for control from c_{POM} for biochars.

The concentration of PHE or PYR in water after 28 days of mixing and days in the fridge was calculated according to the Eq. (2):

$$C_e(\mu g\ L^{-1}) = \frac{c_{POM\ (\mu g\ kg^{-1})} - c_{POM-control\ (\mu g\ kg^{-1})}}{K_{POM-w}\ (L\ kg^{-1})} \quad (2)$$

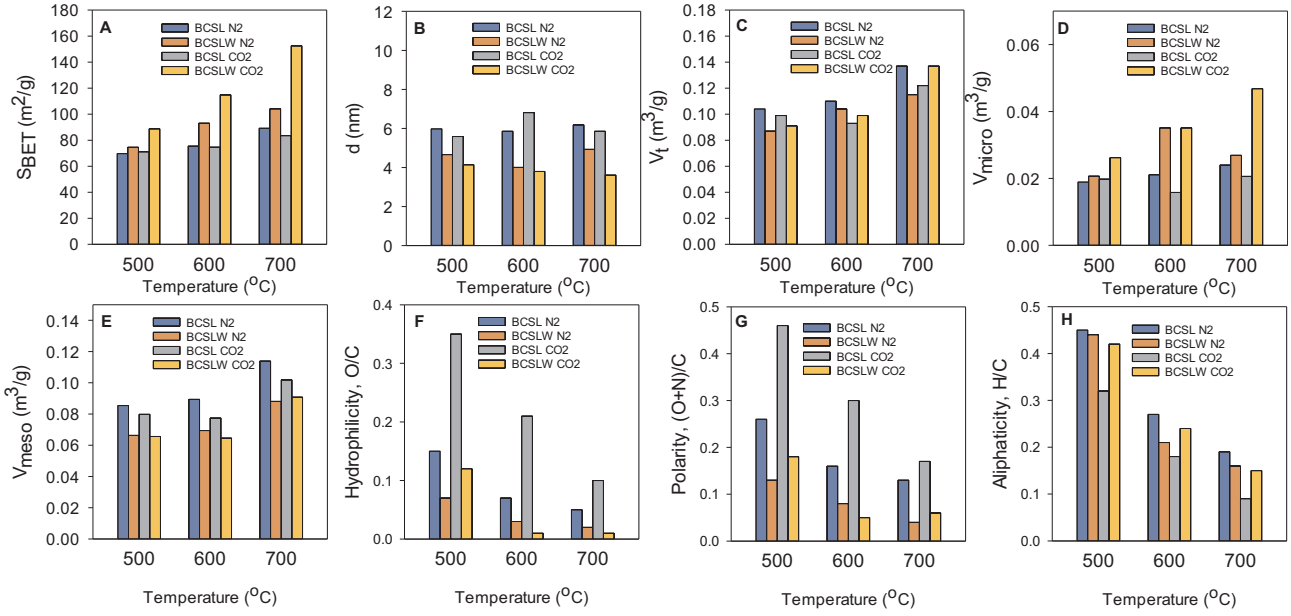

Fig. 1. Physical (A–E) and chemical (F–H) properties of studied biochars depending on the pyrolysis temperature. S_{BET} – surface area, d – width of pores, V_t – total pore volume, V_{micro} – volume of micropores, V_{meso} – volume of mesopores, hydrophilicity- oxygen to carbon content ratio, polarity- sum of oxygen and nitrogen to carbon content ratio, aliphaticity- hydrogen to carbon content ratio.

where K_{POM-W} is the sorbate POM- water partitioning coefficient ($logK_{POM-w}$ of 4.20 L kg^{-1} for PHE and 4.55 L kg^{-1} for PYR) obtained from Hawthorne et al. (2011).

The concentration of PHE or PYR on biochars was calculated according to Eq. (3):

$$C_S(\mu g\ kg^{-1}) = \frac{C_{PHE\ or\ PYR}\ (\mu g\ L^{-1}) - C_e(\mu g\ L^{-1})}{m_{sorbent}\ (kg)} V_r(L) \quad (3)$$

where $C_{PHE\ or\ PYR}$ is the initial concentration of PHE or PYR in the water solution of sodium azide, V_r is the volume of extractant (here acetone plus heptane, 0.02 L), $m_{sorbent}$ is the mass of sorbent used for the experiment (here 0.00005 kg of biochar).

2.3. Data analysis

Four different models were applied to fit the adsorption data:

$$\text{Freundlich (FM):}\ \log c_s = n\log c_w + logK_F \quad (4)$$

$$Langmuir\ (LM):\ \frac{1}{C_s} = \frac{1}{Q_L K_L}\frac{1}{C_e} + \frac{1}{Q_L} \quad (5)$$

$$Temkin\ (TM):\ \ln c_s = \frac{RT}{b}\ln c_w + \frac{RT}{b}\ln A \quad (6)$$

$$Dubinin - Radushkevich\ (DRM):\ logc_s = logQ_D - \frac{R^2T^2}{2E^2}log^2\left(1 + \frac{1}{C_e}\right) \quad (7)$$

where c_s (μg kg^{-1}) is the solid-phase concentration, C_w (μg L^{-1}) is the equilibrium solution-phase concentration, K_F ((μg kg^{-1}) (μg L^{-1})$^{-n}$) is the Freundlich constant or capacity factor, n (dimensionless) is the Freundlich exponent, Q_L (μg L^{-1}) is the maximum monolayer sorption capacity, K_L (L μg^{-1}) is the adsorption equilibrium constant corresponding to the inverse of the concentration that produces half maximal adsorption capacity, R (8.314 J mol^{-1} K^{-1}) is the universal gas constant, T (K) is the absolute temperature (here 298 K), b (J mol^{-1}) is the heat of adsorption, A (L μg^{-1}) is the binding constant, Q_D (μg kg^{-1}) is the micropores adsorption capacity and E (J mol^{-1}) is the bonding energy for the ion-exchange mechanism.

To assess the extent to which a compound is associated with solid phases in a given system at equilibrium, the ratio of the compound total equilibrium concentration in the solids and in the aqueous solution is need to know. The solid- water distribution coefficient can be denoted as (Jin et al., 2017a):

$$K_d(Lkg^{-1}) = \frac{c_s(\mu gkg^{-1})}{c_e(\mu gL^{-1})} \quad (8)$$

When dealing with non-linear isotherms (where n ≠ 1) the value of this ratio may apply only at given solute concentration (here at S_w and at C_w = 0.01 S_w and at C_w = 0.1 S_w, where S_w is aqueous solubility of PHE (1150 μg L^{-1}) or PYR (135 μg L^{-1}). In the case of Freundlich model the solid- water distribution coefficient K_d takes the following form:

$$K_d(Lkg^{-1}) = K_F((\mu gkg^{-1})(\mu gL^{-1})^{-n})c_e(\mu gL^{-1})^{n-1} \quad (9)$$

In order to evaluate the ability of natural organic materials to sorb organic pollutants, it is useful to define an organic carbon normalized sorption distribution coefficient (Jin et al., 2017b):

$$K_{OC}(Lkg^{-1}) = \frac{K_d(Lkg^{-1})}{f_{OC}} \quad (10)$$

where f_{OC} is the fraction of organic carbon

The organic carbon normalized sorption distribution coefficient log K_{OC} (C_w = 0.01 S_w) was related to the widely used octanol-water distribution coefficient log K_{OW} for PHE (4.57) or PYR (5.13) (Schwarzenbach et al., 2016).

3. Results and discussion

3.1. Characteristic of biochars

The properties that most influence the effectiveness of adsorption of non-ionizable organic contaminants on biochar include specific surface area (S_{BET}), porosity (V_t, V_{micro}, V_{meso}), and surface chemistry, i.e. polarity expressed as O/C and (O + N)/C ratios and aromaticity expressed as H/C ratio, which is determined by the presence of functional groups (Ahmad et al., 2014). Fig. 1 and Table 1 present the selected physicochemical properties of the biochars studied.

The biochar pyrolysis conditions had a significant effect on the

Table 1
Elemental composition, pH and ash content in biochars used in the experiment.

	pH[a]	Ash content[b] (%)	C[c] (%)	H[c] (%)	N[c] (%)	O[d] (%)
BCSL500N2	9.4	64.1	26.3	0.99	3.26	5.38
BCSL600N2	12.1	67.6	26.5	0.60	2.93	2.41
BCSL700N2	12.4	71.4	24.5	0.29	2.10	1.71
BCSLW500N2	10.8	46.4	44.6	1.66	3.33	3.93
BCSLW600N2	12.1	49.3	45.2	0.81	2.85	1.86
BCSLW700N2	12.5	50.9	46.2	0.62	2.09	0.21
BCSL500CO2	9.2	59.2	25.1	0.68	3.17	11.80
BCSL600CO2	9.5	64.4	25.5	0.39	2.82	6.99
BCSL700CO2	9.8	69.7	22.7	0.16	1.86	2.64
BCSLW500CO2	9.3	43.3	44.7	1.58	3.35	7.08
BCSLW600CO2	9.5	44.7	51.1	1.04	2.58	2.63
BCSLW700CO2	9.8	48.6	47.7	0.59	2.49	0.69

[a] pH measured after 24 h in water (1:10 w/v).
[b] ash content measured by weight loss after 6 h in 750 °C.
[c] C (carbon), H (hydrogen) and N (nitrogen) content measured using CHN analyser (Perkin-Elmer 2400).
[d] O (oxygen) content calculated by substracting ash, C, H and N content from total mass of the sample.

biochar properties. The biochars produced from SSL with willow addition in an atmosphere of N_2 were characterized by a greater specific surface area (S_{BET}) from 7 to 23% and higher microporosity (V_{micro}) from 9 to 66% (Fig. 1A and D) compared to the SSL-derived biochars. These biochars also showed a higher C content (from 44.6 to 46.2%) than the biochars obtained from SSL alone (Table 1).

The biochars produced from SSL, but in a CO_2 atmosphere, did not differ significantly in the porous structure (S_{BET} as well as V_t, V_{meso}, and V_{micro}) from the biochars produced from SSL in an atmosphere of N_2 (Fig. 1). However, CO_2 using increased the hydrophilicity (O/C) (from 2 to 3 times) and polarity ((O + N)/C) (from 30 to 88%) of biochars, but also increased the aromaticity (H/C) (a decrease in H/C from 29 to 36%) (Fig. 1). The increase in hydrophilicity was associated with an increase in the percentage of oxygen in the biochars, whereas the increase in aromaticity was related to a decrease in the percentage of H in the biochars, with relatively small changes in the carbon content.

In an atmosphere of CO_2, on the other hand, the addition of willow to SSL had a significant importance on surface properties (S_{BET}, V_t, V_{meso}, V_{micro}) (Fig. 1). These biochars were characterized by a greater specific surface area (S_{BET}) from 19 to 47% than the same biochars (the mixture of SSL and willow), but produced in an N_2 atmosphere. The total porosity (V_t) and microporosity (V_{micro}) of the biochars produced in CO_2 (for the biochars obtained at the temperatures of 500 and 700 °C) were also higher from 4.6 to 19% and from 26.6 to 74%, respectively, compared to V_t and V_{micro} of the biochars produced in N_2.

It can be noticed (Fig. 2) that the SSL-derived BCs with willow addition produced in an atmosphere of N_2 match (in terms of the relationship between the H/C and O/C) materials such as black carbon/soot and coal, similarly to the SSL-derived biochars. In comparison to the material obtained from SSL alone, the SSL-derived biochars with willow were slightly more carbonized. The higher the pyrolysis temperature, the more carbonized and dehydrated the biochar was. The biochars produced from SSL in CO_2 were more carboxylated relative to the biochars obtained from SSL in N_2 (Fig. 2). As far as the H/C and O/C ratios are concerned, these biochars were more similar to black carbon/soot and char than the biochars produced in N_2. Apart from that, as regards the biochars obtained from SSL in CO_2, pyrolysis temperature was shown to have a greater impact on their origin (Fig. 2) than for the biochars produced in N_2 and those with willow addition produced in CO_2. This is indicated by the larger differences between the SSL-derived biochars obtained at the different temperatures in CO_2 compared to the other biochars. In terms of the H/C and O/C ratios (Fig. 2), the addition of willow contributed to increased decarboxylation of the biochars in relation to the biochars produced from SSL in CO_2.

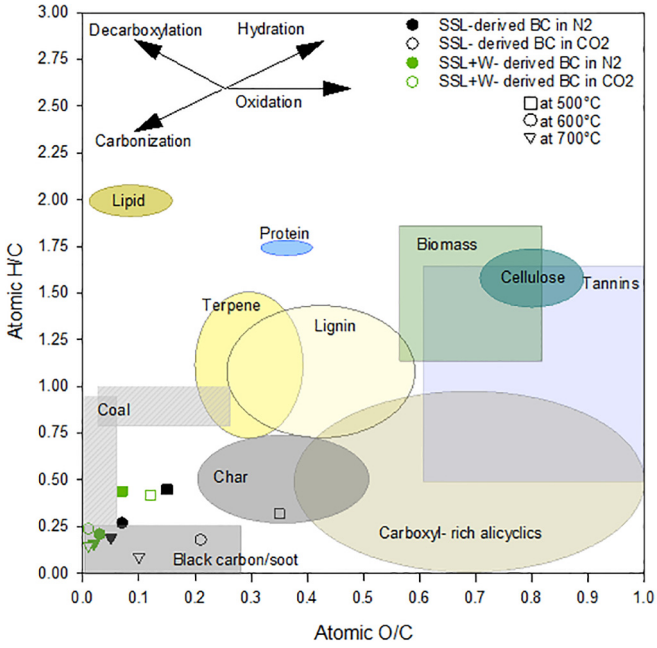

Fig. 2. SSL-derived and SSL + wicker-derived biochars (SSL + W) made in nitrogen (N_2) or carbon dioxide (CO_2) atmosphere showed in the van Krevelen plot.

3.2. Sorption isotherms

The isotherms of PHE and PYR adsorption on the SSL-derived biochars produced at the different temperatures are shown in Fig. 3. Four nonlinear models (Freundlich, Langmuir, Temkin, and Dubinin- Radushkevich) were tested to fit the experimental data. PHE and PYR adsorption was best described by the Freundlich model (Table 2, values for other models are presented in E-Supplementary data).

In previous studies (Hale et al., 2011; Jin et al., 2017a, 2018; Qiu et al., 2014; Wang et al., 2016; Zhang et al., 2014; Zielińska and Oleszczuk, 2015a), the Freundlich model was also the best to describe the experimental data regarding to the adsorption of PAHs by biochars produced at different temperatures and from different feedstocks. In Freundlich model, it is assumed that the adsorption of compounds on an adsorbent occurring by forming several layers. Different types of sorption sites, differing from one another in their amount and free enthalpy, participate in the adsorption (Zielińska and Oleszczuk, 2015a). Furthermore, for the biochars BCSL500N2 and BCSL700N2 the adsorption of PHE was also well described by the Dubinin-Radushkevich model (DRM) and the Langmuir model (LM). In DRM it is accepted that the adsorption sites are heterogeneous and that adsorption is dependent on the degree of microporosity to the greatest extent, while in LM it is assumed that the adsorption sites on the sorbent are homogeneous, interactions between the adsorbates do not occur, and the sorbate forms a monolayer on the surface of the adsorbent. The fit to the different models indicates that the adsorption of studied compounds on the adsorbents investigated occurred according to various mechanisms.

The log K_d partition coefficient ($C_w = 0.01\ S_w$) determined from the Freundlich equation for the SSL-derived biochars ranged from 5.15 to 5.60 for PHE and from 5.53 to 5.66 for PYR, depending on the pyrolysis temperature. The values of the normalized log K_{oc} coefficient ($C_w = 0.01\ S_w$) ranged from 3.72 to 4.21 for PHE and from 4.11 to 4.28 for PYR. The obtained values of log K_d and log K_{oc} were similar to the previous studies concerning SSL-derived biochars (Zielińska and Oleszczuk, 2015a) as well as biochars produced from other feedstocks (Han et al., 2018; Jin et al., 2018). Both for PHE and PYR, the highest adsorption capacity (log K_{oc}) was observed for the biochars produced at the highest temperature (Table 2, Fig. 4). At the same time, these

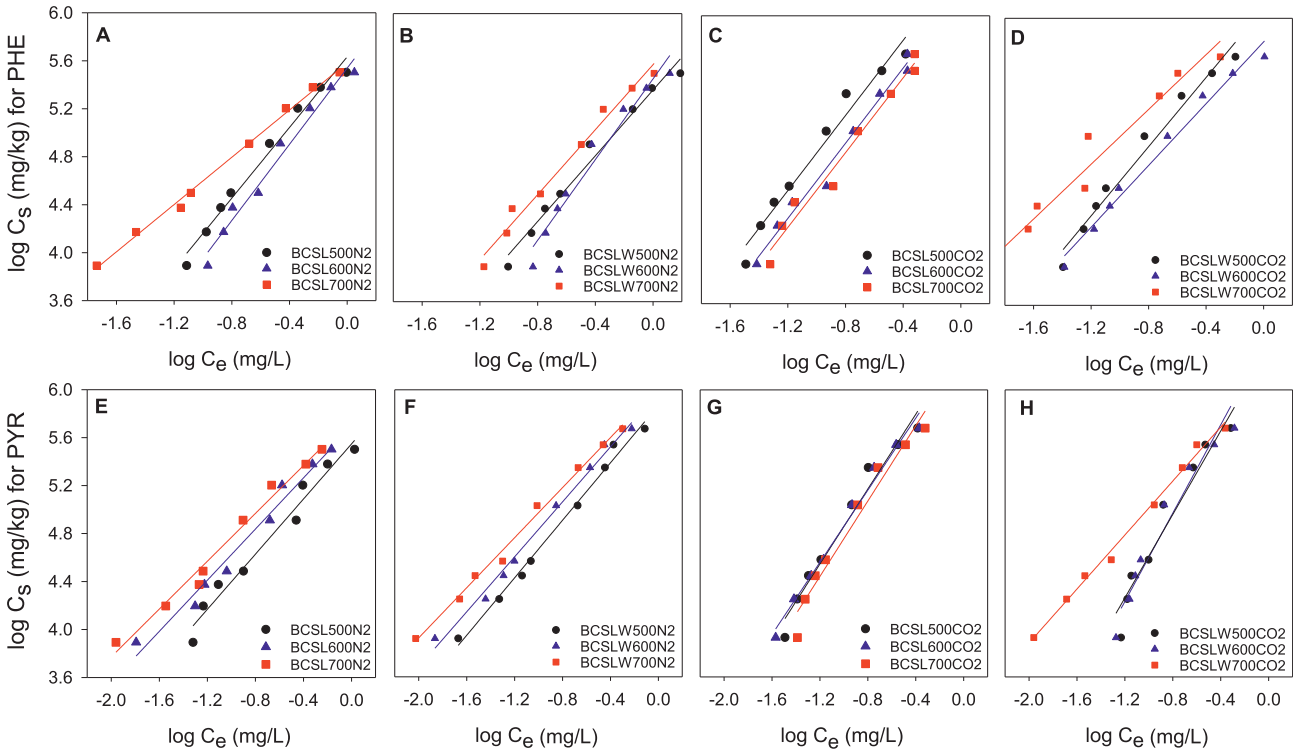

Fig. 3. Effect of pyrolysis temperature of phenanthrene (A, B, C, D) and pyrene (E, F, G, H) adsorption by SSL-derived biochars. A, B, E, F – pyrolysis in N_2 atmosphere, C, D, G, H – pyrolysis in CO_2 atmosphere.

biochars were characterized by the highest specific surface area (Fig. 1), which could explain the high capacity of adsorption of both PHE and PYR on these biochars (Han et al., 2018; Qiu et al., 2014; Wang et al., 2016). PYR was adsorbed on the biochars more efficiently than PHE because it is characterized by a more hydrophobic nature than PHE and therefore by higher affinity for the hydrophobic and aromatic surface of biochar (Zielińska and Oleszczuk, 2015a).

The nonlinear coefficients (n) of the SSL-derived biochars ranged from 0.63 to 1.02 for PHE and from 0.79 to 0.87 for PYR (Table 2). It is an index of diversity of the free sorption energies on a heterogeneous sorbent (Schwarzenbach et al., 2016). A value of $n > 1$ was only observed for the biochar BCSL700N2. When $n > 1$, the isotherm is convex upward and it can be concluded that the increased presence of the sorbate in the sorbent increases the free energies of further adsorption (Schwarzenbach et al., 2016). In all the other biochars $n < 1$ was observed. When $n < 1$, the isotherm is concave downward, which is a common case for biochars. This type of isotherm is connected with decreasing free energy during the adsorption. The n coefficient is also related to the heterogeneity of the sorbent's surface. The lower the value of n, the more heterogeneous the surface is (Zielińska and Oleszczuk, 2015a). Thus, the lower values of n during PHE adsorption (except for BCSL700N2), compared to the n values for PYR adsorption, suggest that the sorption sites for PHE on the biochars varied more than the adsorption sites for PYR.

Based on the calculated correlations between the PHE adsorption parameter (log K_{oc}) and the physical and chemical properties of the biochars (S_{BET}, d, V_t, V_{micro}, V_{meso}, pH, ash content, content of C, OC, H, N, and O, the value of the O/C, H/C, (O + N)/C ratios), a statistically significant relationship was found between log K_{OC} and the average pore size (d) (0.997, $P < 0.05$). For PYR, a statistically significant relationships were observed between log K_{oc} and S_{BET} (1.000, $P < 0.05$) as well as a negative relationship with N (−1.000, $P < 0.05$). These correlations suggest that the adsorption of both PHE and PYR on the biochars followed the pore filling mechanism. It is assumed that the pore filling is the main mechanism of adsorption of various organic compounds by biochars and other carbon adsorbents (Hu et al., 2018; Jin et al., 2018; Wang et al., 2016). The negative relationship between log K_{oc} and N content may suggests the repulsive action between the aromatic structures of PHE and PYR and the polar functional groups containing nitrogen atoms, due to which the molecules had impeded access to the surface of the biochars and their pores (Jin et al., 2018; Wang et al., 2006). The FTIR spectra for the biochars investigated (please see the E-Supplementary data) confirm the occurrence of peaks in the 500–700 and 1000–1300 cm^{-1} band, which are responsible for the presence of carbonitrates and nitrates (Socrates, 2001) on the surface of the SSL-derived biochars.

The n coefficients calculated from the Freundlich model for PHE (Table 2) were significantly negatively correlated with the content of carbon (C) (−1.000, $P < 0.05$) and organic carbon (OC) (−1.000, $P < 0.05$). Wang et al. (2016) found that the presence of carbon has a significant effect on increased aromaticity of biochar, which in turn results in an increase in isotherm nonlinearity (a low value of n). However, no significant correlations were found between the n values and the biochar properties for PYR.

3.3. Effect of biomass in SSL on PHE and PYR adsorption

The addition of biomass to the sewage sludge before pyrolysis decreased PHE and PYR affinity (log K_{oc}) to the biochars. The adsorption of PHE and PYR (based on log K_{oc}) on the biochars with biomass was lower from 9 to 14% and from 4 to 6%, respectively, than on the biochars produced from SSL alone (with N_2 as carrier gas) (Table 2). Similarly, as for the biochars obtained from SSL alone, the biochars produced at a temperature of 700 °C exhibited the highest affinity for PHE and PYR. At the same time, these biochars were characterized by the greatest specific surface area (S_{BET}), pore diameter (d), total pore volume (V_t), and mesoporosity (V_{meso}) as well as by the highest hydrophobicity (O/C) and aromaticity (H/C), and the lowest polarity ((O + N)/C), which can explain their lower capacity in relation to the biochars produced at the lower temperatures.

Table 2
Freundlich isotherm parameters and concentration-dependent distribution coefficients (K_{OC}) for phenanthrene (Phe) and pyrene (Pyr) adsorption onto biochars. BCSL- biochars made of sewage sludge only in different temperatures (500, 600 or 700 °C) and different atmospheres (nitrogen- N_2 or carbon dioxide- CO_2).

Adsorbent	$\log K_F$	K_F[a]	n[b]	R^2_{adj}[c]	K_d[d]	$\log K_d$	K_{OC}[e]	$\log K_{OC}$			$\log K_{OC}/\log K_{ow}$[f]
					$C_w = 0.01\ S_w$[g]	$C_w = 0.01\ S_w$	$C_w = 0.01\ S_w$	$C_w = 0.01\ S_w$	$C_w = 0.1\ S_w$	$C_w = 1\ S_w$	$C_w = 0.01\ S_w$
Phenanthrene (Phe)											
$BCSL500N_2$	5.63 ± 0.05	429896.44	0.68 ± 0.02	0.984	196342.03	5.29	7493.97	3.87	6.28	7.28	0.848
$BCSL600N_2$	5.54 ± 0.03	345604.96	0.63 ± 0.01	0.977	139971.52	5.15	5301.95	3.72	6.18	7.18	0.815
$BCSL700N_2$	5.58 ± 0.04	383371.62	1.02 ± 0.03	0.996	397990.98	5.60	16311.11	4.21	6.26	7.26	0.922
$BCSLW500N_2$	5.36 ± 0.05	231120.70	0.73 ± 0.02	0.980	119595.55	5.08	2705.78	3.43	5.78	6.78	0.751
$BCSLW600N_2$	5.46 ± 0.02	286031.48	0.59 ± 0.02	0.962	106337.80	5.03	2368.33	3.37	5.86	6.86	0.738
$BCSLW700N_2$	5.57 ± 0.03	375775.37	0.74 ± 0.03	0.983	198471.44	5.30	4314.60	3.63	5.97	6.97	0.795
$BCSL500CO_2$	5.35 ± 0.02	223358.40	0.96 ± 0.03	0.985	200734.62	5.30	8193.25	3.91	6.02	7.02	0.856
$BCSL600CO_2$	5.83 ± 0.01	672479.29	0.80 ± 0.02	0.981	417322.01	5.62	16759.92	4.22	6.49	7.49	0.924
$BCSL700CO_2$	6.17 ± 0.01	1468902.54	0.71 ± 0.01	0.955	726945.24	5.86	32893.45	4.52	6.88	7.88	0.988
$BCSLW500CO_2$	5.88 ± 0.04	753393.42	0.75 ± 0.01	0.976	413306.14	5.62	9350.82	3.97	6.29	7.29	0.869
$BCSLW600CO_2$	5.62 ± 0.03	416294.24	0.84 ± 0.02	0.983	284827.35	5.45	5640.15	3.75	5.98	6.98	0.821
$BCSLW700CO_2$	5.94 ± 0.04	874049.52	0.95 ± 0.04	0.955	778075.41	5.89	16484.65	4.22	6.33	7.33	0.923
Pyrene (Pyr)											
$BCSL500N_2$	5.55 ± 0.03	353107.30	0.87 ± 0.04	0.971	339582.99	5.53	12961.18	4.11	5.26	6.26	0.802
$BCSL600N_2$	5.61 ± 0.02	403205.31	0.79 ± 0.03	0.955	379114.94	5.58	14360.41	4.16	5.31	6.31	0.810
$BCSL700N_2$	5.68 ± 0.03	481551.09	0.86 ± 0.02	0.979	462226.47	5.66	18943.71	4.28	5.43	6.43	0.834
$BCSLW500N_2$	5.55 ± 0.02	350989.74	0.82 ± 0.03	0.957	332340.37	5.52	7519.01	3.88	5.03	6.03	0.756
$BCSLW600N_2$	5.68 ± 0.03	475427.82	0.84 ± 0.04	0.969	452761.06	5.66	10083.77	4.00	5.16	6.16	0.780
$BCSLW700N_2$	5.70 ± 0.06	502984.63	0.94 ± 0.04	0.976	494377.84	5.69	10747.34	4.03	5.17	6.17	0.786
$BCSL500CO_2$	6.19 ± 0.04	1539200.80	0.70 ± 0.01	0.971	1405226.33	6.15	57356.18	4.76	5.93	6.93	0.928
$BCSL600CO_2$	5.98 ± 0.03	944590.49	0.70 ± 0.02	0.984	862302.55	5.94	34630.62	4.54	5.71	6.71	0.885
$BCSL700CO_2$	5.90 ± 0.02	790739.60	0.70 ± 0.02	0.975	722574.64	5.86	32695.68	4.51	5.68	6.68	0.880
$BCSLW500CO_2$	5.99 ± 0.01	984059.24	0.60 ± 0.02	0.979	873045.33	5.94	19752.16	4.30	5.48	6.48	0.837
$BCSLW600CO_2$	5.92 ± 0.04	824728.32	0.63 ± 0.01	0.972	738188.04	5.87	14617.59	4.16	5.34	6.34	0.812
$BCSLW700CO_2$	5.80 ± 0.03	628952.77	0.90 ± 0.02	0.961	609616.80	5.79	12915.61	4.11	5.26	6.26	0.801

[a] K_F is the sorption affinity coefficient $((mg\,kg^{-1})\,(mg\,L^{-1})^{-n}$ with standard deviation.
[b] Nonlinearity index (dimensionless) with standard deviation.
[c] Adjusted coeffcient of determination: $R^2_{adj} = 1 - [(1 - R^2)(m - 1)(m - b - 1)^{-1}]$, where m is the number of data points used for the fitting and b the number of coefficients in the fitting equation.
[d] Sorption distribution coefficient (K_d) with units of $(mL\,g^{-1})$.
[e] K_{OC} $(mL\,g^{-1})$ is the organic carbon normalized.
[f] K_{OW} is the octanol - water partition coefficient equal 4.57 for Phe and 5.13 for Pyr.
[g] S_w is the water solubility of Phe (1150 $\mu g\,L^{-1}$) or Pyr (135 $\mu g\,L^{-1}$).

The observed lower affinity of PHE and PYR (Table 2, Fig. 4) for the SSL-derived biochars with biomass addition relative to the biochars produced from SSL alone could have been associated with the occurrence of the so-called steric hindrance effect (Jin et al., 2018). This resulted from the lower pore diameter (d) as well as the lower total volume of pores (V_t) and mesopores (V_{meso}) in the biochar obtained from the mixture of SSL and biomass in comparison to the biochar produced from SSL alone (Fig. 1). In this situation, the sorbates cannot enter a large amount of biochar pores.

The changes in the *n* coefficient, indicating the degree of heterogeneity of the surface after adding willow to SSL, were not regular (Table 2). For PHE, after the biomass was added to SSL, *n* increased for the biochars produced at 500 °C and decreased for the biochars produced at 600 and 700 °C relative to the corresponding biochars obtained from SSL alone. An opposite trend was noted for PYR. Differences between compounds indicates that both compounds can behave differently and that the differences between them are probably determined by the different molecule size (Wang et al., 2006) and the differences in hydrophobicity (Zielińska and Oleszczuk, 2015a).

No statistically significant relationships were found between the sorption coefficient ($\log K_{oc}$) for PHE and the properties of the biochars produced with willow addition. However, significant relationships were found between $\log K_{oc}$ for PYR and pH (0.999, $P < 0.05$), H content (−1.000, $P < 0.05$), O/C (−1.000, $P < 0.05$), and H/C (−1.000, $P < 0.05$). The high negative relationship of $\log K_{oc}$ with O/C and H/C as well as the absence of relationships with the surface properties of the biochars may suggest that the sorption of PYR on the SSL-derived biochars with willow addition occurred due to the hydrophobic interactions (Schwarzenbach et al., 2016) and the π-π electron-donor–acceptor (EDA) interactions. It is accepted (Ok et al., 2016; Rao et al., 2017; Wang et al., 2016) that these are the main mechanisms responsible for interactions of PAH molecules with biomass-derived biochar. Thus, the addition of willow changed the sorption mechanism from the pore filling-based mechanism (for the sewage sludge-derived biochars) to the one related to the biochar surface chemistry. This change in the mechanism could have been the immediate cause for the decreased adsorption on the SSL- and willow-derived biochars. This is due to the weak nature of the hydrophobic and π-π interactions because the higher the surface aromaticity is, the stronger these interactions are (Han et al., 2018). Nonetheless, the increase in surface aromaticity was not probably significant enough for the sorbent to form strong interactions with the sorbate.

3.4. Influence of CO_2 atmosphere on sorption by SSL- derived biochars

The change in the pyrolysis atmosphere from N_2 to CO_2 had a significant impact on increasing the capacity of the biochars to PHE and PYR. Generally, the SSL-derived biochars produced in an atmosphere of CO_2 showed the $\log K_{oc}$ coefficient from 7% to 12% (for PHE) and from 6 to 16% (for PYR) higher compared to the biochars produced in N_2 atmosphere. The biochar produced at the highest temperature best adsorbed PHE, whereas PYR was best adsorbed by the biochar produced at the lowest temperature (based on $\log K_{oc}$). These differences confirm again that the adsorption of both compounds can occur by different interactions between biochar and investigated compounds. Although PHE and PYR are similar to each other in their chemical structure, there

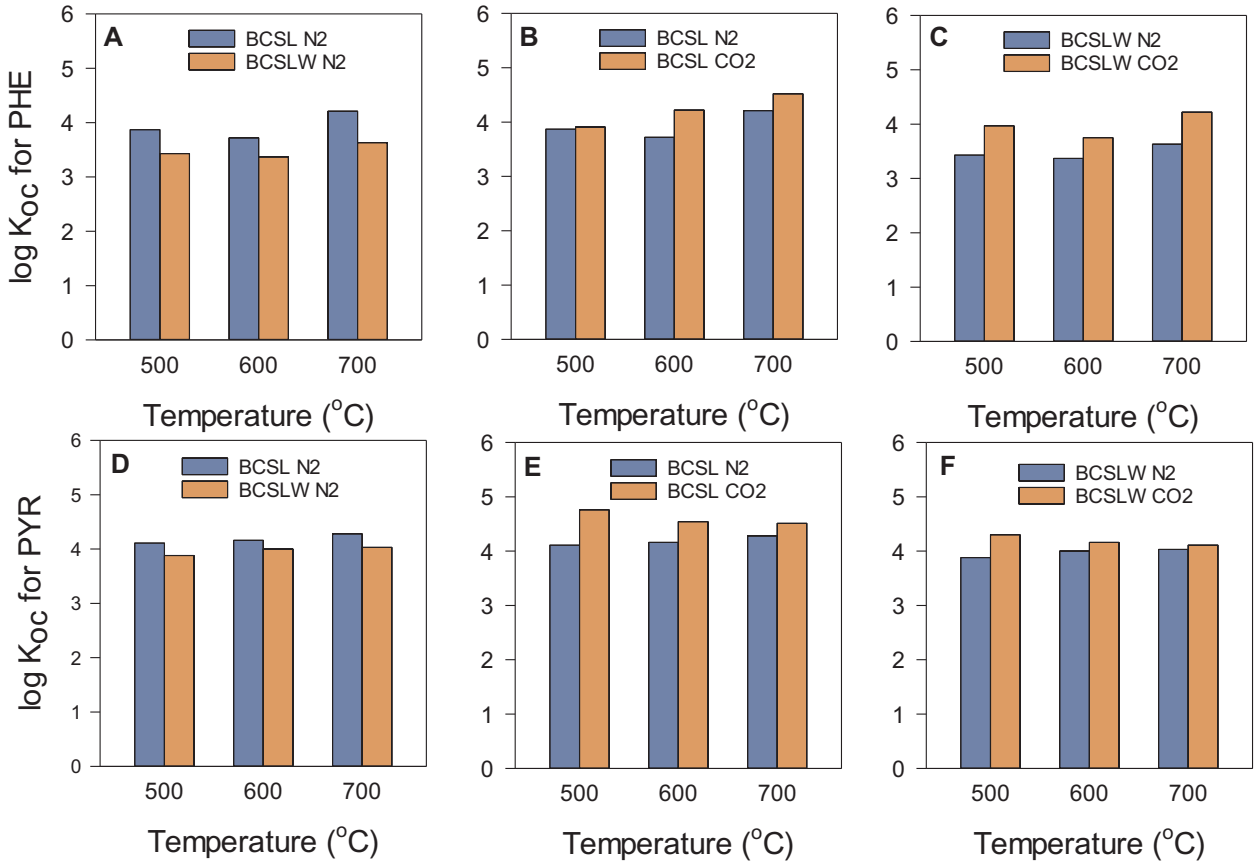

Fig. 4. Effect of willow and CO_2 on log K_{oc} values for PHE (top panel) and PYR (bottom panel) determined for biochars produced from sewage sludge and in N_2 or CO_2 atmosphere.

are subtle differences between them (the number of aromatic rings and hence a different molecular mass and molecule shape – the PHE molecule is more elongated, while the PYR molecule is closer to a spherical shape) (Wang et al., 2006), which results in differences in the way in which they are adsorbed.

The change in gas from N_2 to CO_2 during pyrolysis caused an increase in the *n* parameter for PHE and the biochars produced at the temperatures of 500 and 600 °C as well as its decrease for the biochars obtained at a temperature of 700 °C. These differences may indicate that the presence of CO_2 and pyrolysis temperature promote establishing homogeneous active sites on the biochar's surface which show affinity for PHE. At a higher temperature, this effect is opposite because more aromatic structures are formed and their presence contributes to an increase in adsorption nonlinearity (Wang et al., 2016). On the other hand, the change in the atmosphere resulted in a decrease in the *n* parameter for PYR, which is evidence of increased diversity of adsorption sites for PYR. It was also observed that the *n* parameter became uniform for the biochars produced at the different temperatures.

Based on the analysis of the relationships between log K_{oc} for PHE and the biochar properties, statistically significant correlations were found for PHE and pH (1.000, $P < 0.05$), ash content (1.000, $P < 0.05$), hydrogen (−0.999, $P < 0.05$), and oxygen (−1.000, $P < 0.05$) as well as for O/C (−0.999, $P < 0.05$) and (O + N)/C (−0.999, $P < 0.05$). Negative correlations of log K_{oc} with H and O content as well as with O/C and (O + N)/C ratios are a typical (Jin et al., 2017b; Sun et al., 2013; Wang et al., 2006; Zielińska and Oleszczuk, 2015a) resulting from the presence of functional groups (containing O and H) on the surface, which impede access of hydrophobic molecules (such as PHE and PYR) to the adsorbent's surface. This may contribute to a decrease in the adsorption capacity (Jin et al., 2018; Wang et al., 2006).

The increase in the specific surface area of the biochars produced in an atmosphere of CO_2 (in comparison to N_2) (Fig. 1A), together with a significant increase in their aromaticity (a 29–53% reduction in H/C, Fig. 1H), was probably the effect of increased efficiency of adsorption of the compounds studied by the biochars produced in an atmosphere of CO_2 as compared to N_2 (Fig. 4). In such case, adsorption is the result of three concurrently occurring mechanisms, i.e. pore filling, hydrophobic interaction (Jin et al., 2018) and π-π EDA interaction (Jin et al., 2018; Park et al., 2013). The higher the aromaticity of the surface, the stronger the interactions between the matrix and the sorbate are (Han et al., 2018); thus, the significantly increased surface aromaticity (low H/C values) contributed to the more efficient adsorption. The previous studies (Zielińska and Oleszczuk, 2015a) also confirmed the important role of aromaticity in PYR adsorption by sewage sludge-derived biochars and also in the adsorption of other organic compounds by biochar (Chen et al., 2008; Zhang et al., 2014). Moreover, the very good fit of the experimental data regarding PHE sorption on two biochars produced in a CO_2 atmosphere (BCSL600CO2 and BCSL700CO2) to DRM may indicate that adsorption is also associated with pore filling.

3.5. Influence of CO_2 on adsorption by biochars with wicker addition

The change of the carrier gas from N_2 to CO_2 also increased the adsorption capacity of the SSL-derived biochars with willow addition (Fig. 4). The biochars produced with the addition of willow in CO_2 were characterized by higher log K_{oc} values from 11 to 16% (PHE) and from 2 to 11% (PYR) than the same biochars produced in an atmosphere of N_2. The increase in the sorption capacity was higher for PHE than PYR. But a reverse trend was observed for the biochars produced from SSL alone in an N_2 atmosphere. PHE was better adsorbed by the biochar produced at the highest temperature, while PYR was best adsorbed by

BCSL500N2 (based on log K_{oc}) but, in turn, adsorption was the same for the biochars produced from SSL alone in CO_2.

The value of the *n* parameter for PHE sorption ranged from 0.75 to 0.95 and increased as a result of CO_2 application, that is, the change of the atmosphere promoted homogenization of the sorption sites. On the other hand, the *n* parameter for PYR sorption ranged from 0.60 to 0.90 and its value decreased in comparison with the biochars produced in N_2. Therefore, as a result of the action of CO_2 on the mixture of SSL with biomass, the sorption sites became more heterogeneous for PYR, while for PHE more homogenous.

The biochars produced from the mixture of SSL with biomass in a CO_2 atmosphere were characterized by the greatest specific surface area (Fig. 1A). Nevertheless, this did not translate into the highest adsorption of PHE and PYR (Table 2, Fig. 4). Despite that the biochar BCSLWCO2 adsorbed better than the biochars produced from SSL alone (in an N_2 atmosphere) and from the mixture of SSL with willow (in an N_2 atmosphere), the best adsorption effects were still achieved for the biochars obtained from SSL alone in an atmosphere of CO_2 (which were characterized by a lower specific surface area than BCSLWCO2). It should therefore be presumed that the aromaticity of the biochars, which was highest (the lowest H/C values) in the case of the biochars obtained from SSL in a CO_2 atmosphere, plays an important role here.

4. Conclusions

Willow in SSL reduced adsorption of PHE and PYR by the biochars, which was associated with the decrease in the pore size, with small changes in the surface chemistry. The observed increased adsorption after change of N_2 to CO_2 was however related to the increase mainly in the aromatic nature of the biochar surface. The use of carbon dioxide as the atmosphere during pyrolysis instead of nitrogen beneficially affects the adsorption properties of biochars obtained both from sewage sludge and from a mixture of sewage sludge with willow in relation to PHE and PYR.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2019.02.021.

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SUPPORTING INFORMATION

ADSORPTION CAPACITY OF PHENANTHRENE AND PYRENE TO ENGINEERED CARBON-BASED ADSORBENTS PRODUCED FROM SEWAGE SLUDGE OR SEWAGE SLUDGE-BIOMASS MIXTURE IN VARIOUS GASEOUS CONDITIONS

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Table S1. Linear parameters of Langmuir, Temkin and Dubinin-Radushkevich isotherms for phenanthrene (PHE) and pyrene (PYR) adsorption onto sewage sludge (SS), biochar at 500 ºC (BC500), biochar at 600 ºC (BC600) and biochar at 700 ºC (BC700).

	Langmuir			Temkin			Dubinin- Radushkevich		
Adsorbent	Q_L (µg g^{-1})	K_L (L μg^{-1})	R^2	Q (J mol^{-1})	A (L μg^{-1})	R^2	Q_D (µg g^{-1})	E (kJ mol^{-1})	R^2
PHE									
BCSL500N2	-57534.39	-1.78	0.925	0.02	9.85	0.926	357.63	2.31	0.990
BCSL600N2	-51155.74	-1.46	0.896	0.02	7.28	0.919	351.06	2.06	0.975
BCSL700N2	524745.24	0.82	0.995	0.03	29.18	0.845	227.09	3.61	0.922
BCSLW500N2	-66559.06	-1.19	0.942	0.02	7.41	0.930	291.91	2.13	0.978
BCSLW600N2	-40482.74	-1.36	0.853	0.02	5.70	0.959	401.46	1.80	0.999
BCSLW700N2	-77736.05	-1.57	0.922	0.02	11.04	0.900	308.66	2.47	0.969
BCSL500CO2	-176202.26	-0.93	0.924	0.03	13.65	0.905	240.09	2.78	0.960
BCSL600CO2	-95415.59	-2.58	0.962	0.02	21.67	0.918	355.61	3.03	0.983
BCSL700CO2	-55533.29	-4.73	0.924	0.02	25.66	0.961	554.56	2.98	0.994
BCSLW500CO2	-67179.04	-3.02	0.915	0.02	19.13	0.946	412.53	2.84	0.999
BCSLW600CO2	-103601.51	-1.85	0.967	0.02	16.75	0.930	307.65	2.85	0.983
BCSLW700CO2	-169789.08	-3.40	0.955	0.03	48.23	0.913	364.33	3.77	0.961
PYR									
BCSL500N2	-142257.72	-1.35	0.890	0.03	16.14	0.867	269.23	2.89	0.949
BCSL600N2	-176230.87	-1.03	0.893	0.03	14.78	0.782	251.16	2.86	0.891
BCSL700N2	-289672.02	-0.97	0.973	0.03	21.88	0.794	257.23	3.22	0.921
BCSLW500N2	-192294.61	-0.92	0.945	0.03	14.39	0.773	236.03	2.86	0.890
BCSLW600N2	-183632.17	-1.30	0.929	0.03	19.60	0.794	264.47	3.09	0.919
BCSLW700N2	-734895.14	-0.54	0.943	0.03	30.60	0.777	249.49	3.56	0.919
BCSL500CO2	-59229.83	-4.44	0.895	0.02	24.71	0.939	542.73	2.97	0.995
BCSL600CO2	-84379.42	-2.53	0.945	0.02	18.74	0.829	375.57	2.88	0.954
BCSL700CO2	-82691.09	-2.28	0.881	0.02	16.63	0.838	366.88	2.78	0.951
BCSLW500CO2	-55234.44	-2.44	0.855	0.02	12.68	0.857	451.62	2.46	0.967
BCSLW600CO2	-59607.24	-2.29	0.807	0.02	12.68	0.876	433.73	2.47	0.967
BCSLW700CO2	-737708.62	-0.57	0.954	0.03	31.45	0.757	261.91	3.57	0.898

Table S2. Relationship between linear isotherm of Freundlich parameters for phenanthrene adsorption onto SSL-derived biochars (pyrolyzed in nitrogen or carbon dioxide at 500, 600 and 700°C) and its physico-chemical and structural properties.

(PHE)	pH	Ash content (%)	C (%)	OC (%)	H (%)	N (%)	O (%)	O/C	H/C	(O+N)/C	S_{BET} (m^2/g)	d (nm)	V_t (m^3/g)	V_{micro} (m^3/g)	V_{meso} (m^3/g)
SSL- derived biochars made in N_2															
K_F	-0.850	-0.531	-0.031	-0.031	0.605	0.333	0.800	0.794	0.719	0.774	-0.346	0.315	-0.229	-0.483	-0.189
n	0.476	0.815	-1.000	-1.000	-0.760	-0.922	-0.553	-0.561	-0.652	-0.587	0.916	0.966	0.958	0.846	0.969
Log K_d	0.301	0.690	-0.976	-0.976	-0.623	-0.832	-0.385	-0.394	-0.497	-0.423	0.824	0.997	0.887	0.729	0.905
Log K_{OC}	0.306	0.694	-0.978	-0.978	-0.627	-0.835	-0.390	-0.399	-0.501	-0.428	0.827	0.997	0.889	0.733	0.908
SSL-derived biochars made in CO_2															
K_F	0.987	0.988	-0.879	-0.879	-0.975	-0.995	-0.982	-0.974	-0.960	-0.976	0.997	0.053	0.847	0.311	0.899
n	-0.987	-0.986	0.685	0.685	0.996	0.912	0.991	0.996	0.999	0.995	-0.922	-0.364	-0.636	0.004	-0.716
Log K_d	0.996	0.996	-0.741	-0.741	-0.999	-0.942	-0.999	-0.999	-0.999	-0.999	0.950	0.287	0.697	0.077	0.770
Log K_{OC}	1.000	1.000	-0.782	-0.782	-0.999	-0.961	-1.000	-0.999	-0.994	-0.999	0.968	0.227	0.740	0.139	0.808
SSL+ wicker- derived biochars made in N_2															
K_F	0.907	0.954	1.000	1.000	-0.883	-1.000	-0.979	-0.891	-0.881	-0.980	0.960	0.415	0.966	0.301	0.968
n	-0.235	-0.105	0.102	0.186	0.287	-0.188	0.005	0.270	0.291	0.004	-0.086	0.974	-0.063	-0.876	0.436
Log K_d	0.544	0.650	0.850	0.850	-0.498	-0.842	-0.722	-0.513	-0.494	-0.723	0.664	0.835	0.681	-0.252	0.953
Log K_{OC}	0.504	0.613	0.824	0.824	-0.456	-0.816	-0.689	-0.472	-0.453	-0.690	0.628	0.860	0.646	-0.298	0.938
SSL+ wicker- derived biochars made in CO_2															
K_F	0.364	0.499	-0.735	-0.729	-0.203	0.17	-0.034	0.263	-0.067	0.329	0.354	-0.095	0.579	0.329	0.727
n	0.998	0.978	0.417	0.425	-0.994	-0.884	-0.961	-0.836	-0.969	-0.796	0.999	-0.976	0.955	1.000	0.878
Log K_d	0.695	0.796	-0.416	-0.408	-0.566	-0.224	-0.418	-0.130	-0.447	-0.061	0.688	-0.472	0.849	0.669	0.936
Log K_{OC}	0.625	0.735	-0.499	-0.492	-0.486	-0.132	-0.331	-0.037	-0.362	0.032	0.617	-0.388	0.796	0.596	0.899

Statistically significant differences ($p<0.05$) are presented in red.

Table S3. Relationship between linear isotherm of Freundlich parameters for pyrene adsorption onto SSL-derived biochars made in nitrogen or carbon dioxide atmosphere, at 500, 600 and 700°C, and physico-chemical and structural properties of sorbents.

(PYR)	pH	Ash content (%)	C (%)	OC (%)	H (%)	N (%)	O (%)	O/C	H/C	(O+N)/C	S_{BET} (m^2/g)	d (nm)	V_t (m^3/g)	V_{micro} (m^3/g)	V_{meso} (m^3/g)
SSL- derived biochars made in N_2															
K_F	0.848	0.995	-0.883	-0.883	-0.982	-0.993	-0.892	-0.896	-0.941	-0.910	0.995	0.713	0.975	0.999	0.964
n	-0.521	-0.091	-0.479	-0.479	0.180	-0.129	0.442	0.434	0.327	0.404	0.115	0.710	0.235	-0.036	0.275
Log K_d	0.845	0.994	-0.886	-0.886	-0.980	-0.994	-0.889	-0.893	-0.939	-0.907	0.995	0.717	0.976	0.999	0.966
Log K_{OC}	0.786	0.978	-0.928	-0.928	-0.956	-1.000	-0.838	-0.844	-0.900	-0.860	1.000	0.784	0.993	0.988	0.987
SSL- derived biochars made in CO_2															
K_F	-0.947	-0.945	0.554	0.554	0.966	0.831	0.956	0.967	0.979	0.694	-0.844	-0.514	-0.499	0.171	-0.589
n	0.828	0.831	-0.998	-0.998	-0.788	-0.945	-0.811	-0.787	-0.751	-0.793	0.936	-0.375	0.992	0.683	1.000
Log K_d	-0.965	-0.964	0.606	0.606	0.981	0.864	0.972	0.981	0.990	0.979	-0.877	-0.459	-0.553	0.108	-0.639
Log K_{OC}	-0.909	-0.906	0.466	0.466	0.934	0.769	0.920	0.935	0.954	0.932	-0.785	-0.599	-0.407	0.271	-0.503
SSL+ wicker- derived biochars made in N_2															
K_F	0.998	0.983	0.880	0.887	-1.000	-0.886	-0.959	-1.000	-1.000	-0.959	0.979	-0.063	0.974	0.715	0.735
n	0.787	0.861	0.975	0.971	-0.753	-0.972	-0.908	-0.764	-0.750	-0.908	0.871	0.611	0.882	0.076	1.000
Log K_d	0.998	0.982	0.877	0.885	-1.000	-0.884	-0.958	-1.000	-1.000	-0.957	0.978	-0.068	0.973	0.718	0.732
Log K_{OC}	0.999	0.986	0.888	0.896	-1.000	-0.895	-0.964	-1.000	-1.000	-0.964	0.982	-0.044	0.978	0.701	0.748
SSL+ wicker- derived biochars made in CO_2															
K_F	-0.998	-0.979	-0.415	-0.423	0.994	0.884	0.960	0.835	0.969	0.795	-0.999	0.976	-0.955	-1.000	-0.878
n	0.950	0.986	0.055	0.063	-0.885	-0.652	-0.793	-0.577	-0.812	-0.519	0.947	-0.828	0.997	0.938	0.992
Log K_d	-0.997	-0.974	-0.435	-0.442	0.996	0.893	0.966	0.846	0.974	0.808	-0.998	0.980	-0.949	-0.999	-0.868
Log K_{OC}	-0.939	-0.876	-0.662	-0.669	0.983	0.982	1.000	0.959	0.999	0.937	-0.942	0.997	-0.827	-0.951	-0.701

Statistically significant differences ($p<0.05$) are presented in red.

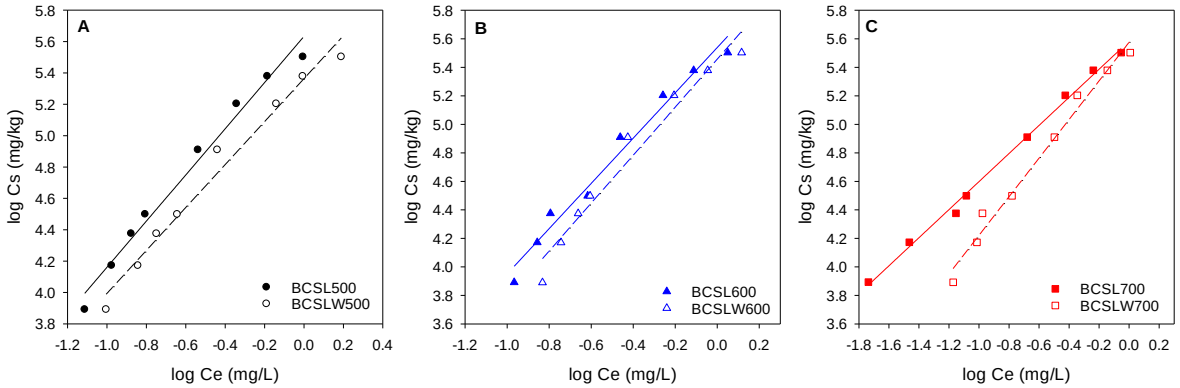

Fig. S1. Effect of wicker addition during pyrolysis on phenanthrene adsorption by SSL-derived biochars

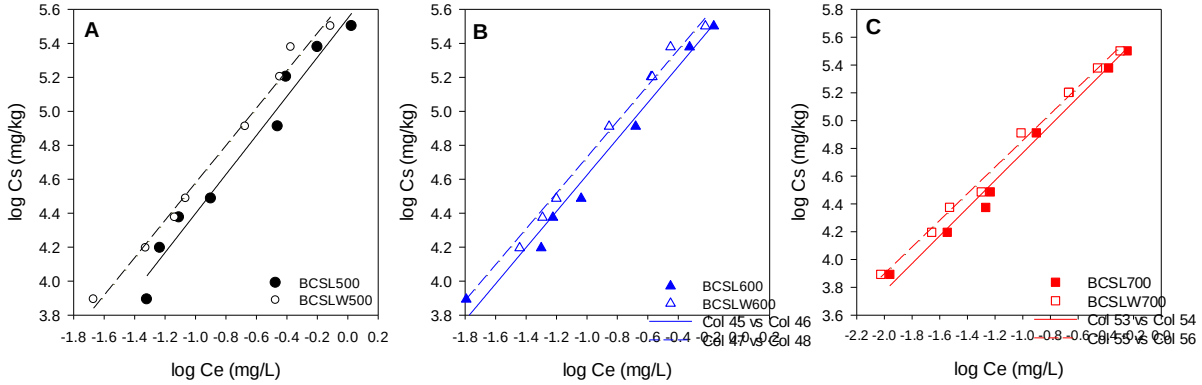

Fig. S2. Effect of wicker addition during pyrolysis on pyrene adsorption by SSL-derived biochars.

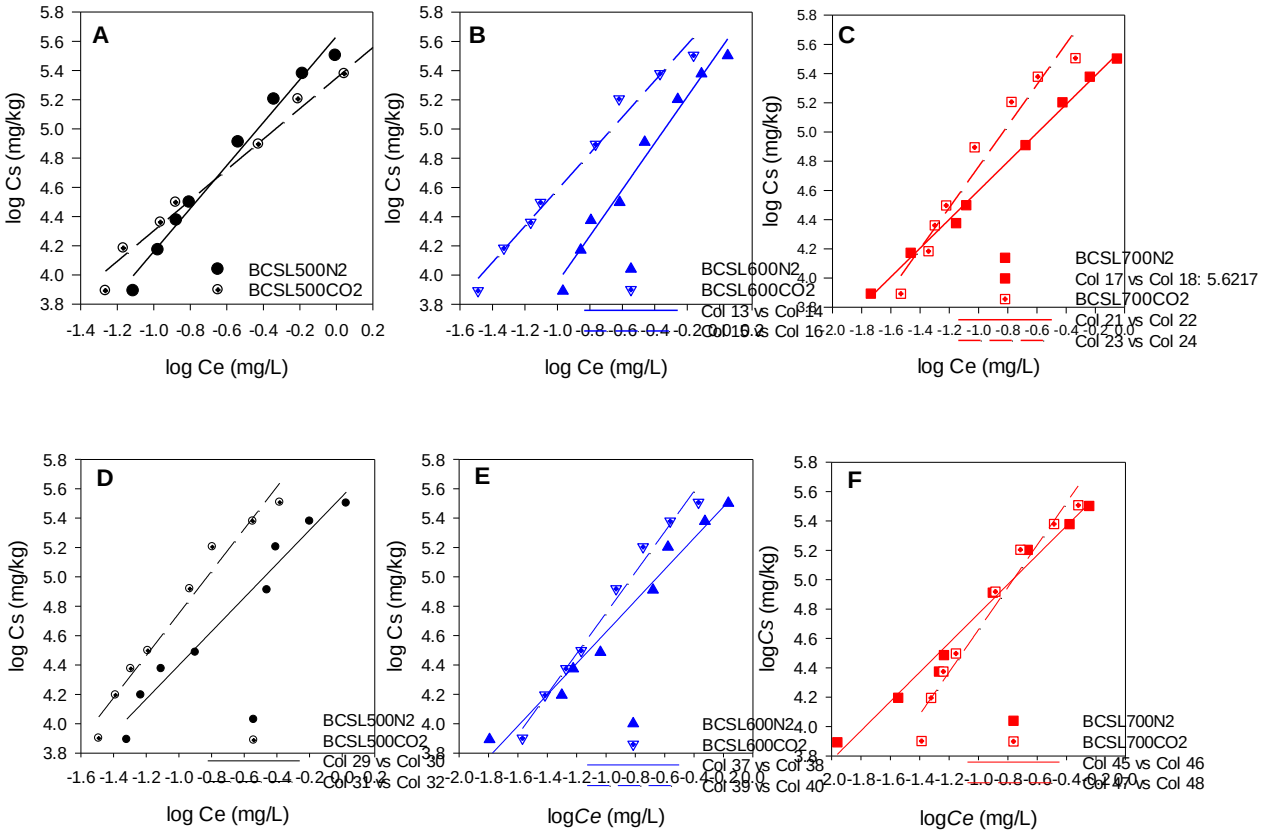

Fig. S3. Effect of CO_2 atmosphere compared to N_2 on adsorption of phenanthrene (A, B, C) and pyrene (D, E, F) by SSL-derived biochars.

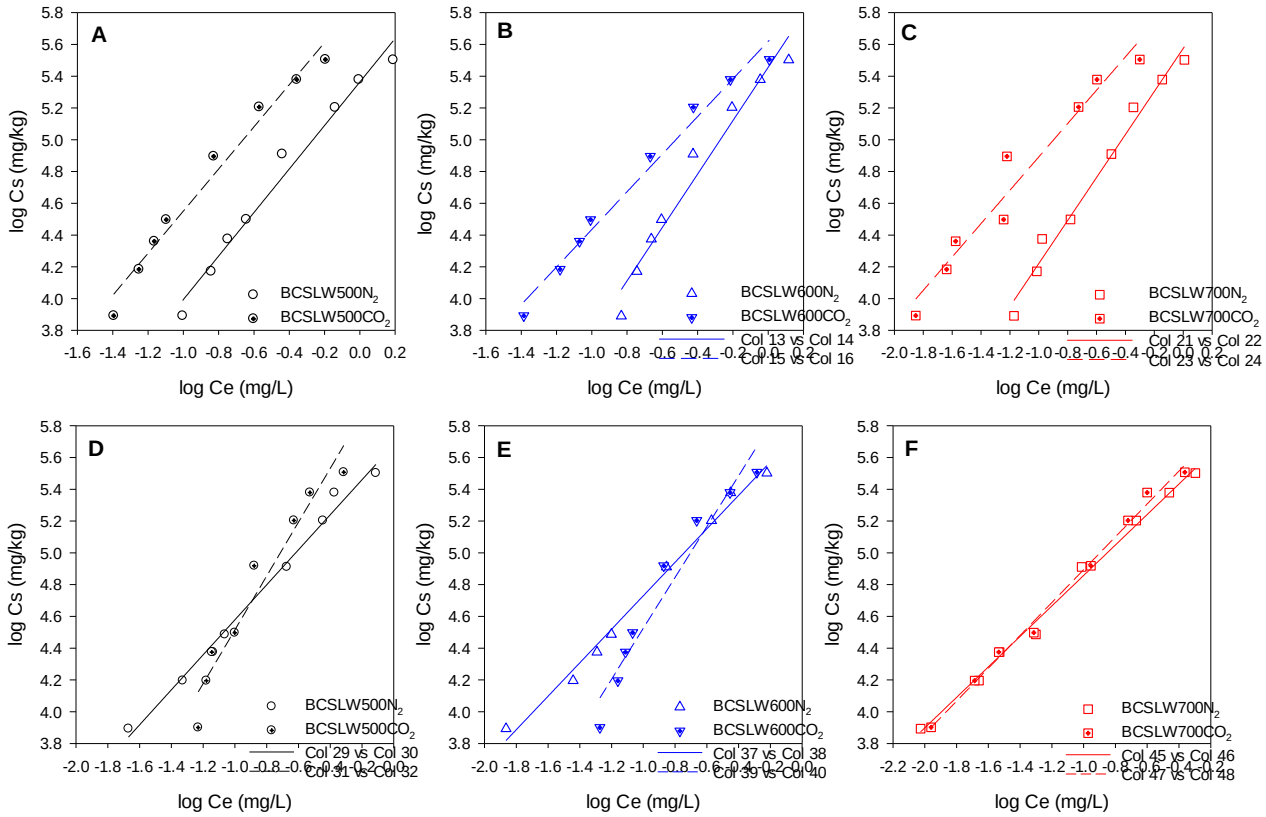

Fig. S4. Effect of CO_2 atmosphere compared to N_2 on adsorption of phenanthrene (A, B, C) and pyrene (D, E, F) by SSL-derived biochars pyrolyzed with addition of willow.

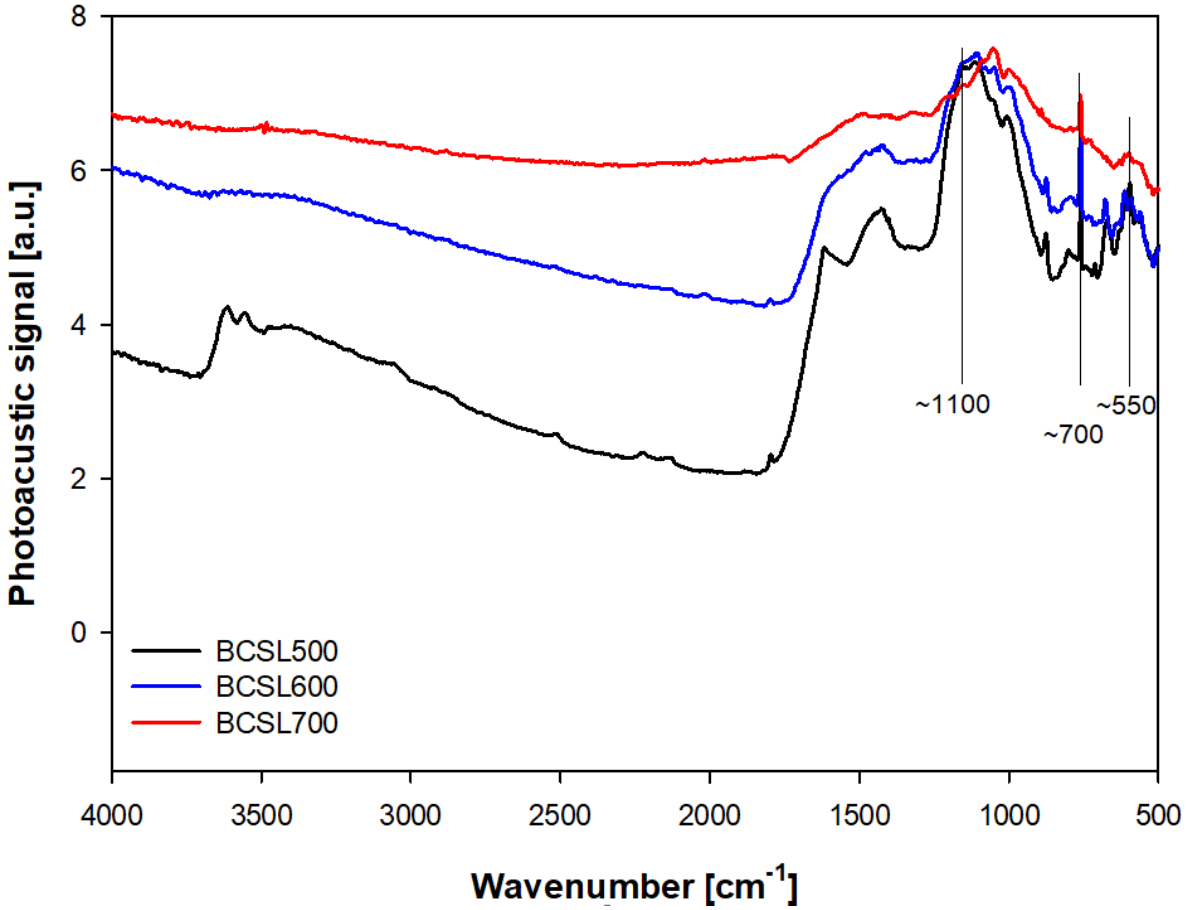

FIGURE S5. FTIR spectra of SSL-derived biochars pyrolyzed at 500, 600 and 700°C in N_2 atmosphere.

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The dark side of black gold: Ecotoxicological aspects of biochar and biochar-amended soils,

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Review

THE DARK SIDE OF BLACK GOLD: Ecotoxicological aspects of biochar and biochar-amended soils

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ABSTRACT

Biochar, a product of biomass pyrolysis, is characterized by significant surface area, porosity, high water holding capacity, and environmental persistence. It is perceived as a material that can counteract climate change due to its high carbon stability and is also considered suitable for soil amendment (fertility improvement, soil remediation). However, biochar can have a toxic effect on organisms as harmful substances may be present in it. This paper reviews the literature regarding the current knowledge of harmful substances in biochar and their potential negative impact on organisms from different trophic levels. The effects of biochar on the content and toxicity of harmful substances in biochar-amended soils are also reviewed. Application of biochar into soil does not usually have a toxic effect and very often stimulate plants, bacteria activity and invertebrates. The effect however is strictly determined by type of biochar (especially the feedstock used and pyrolysis temperature) as well as contaminants content. The pH, electrical conductivity, polycyclic aromatic hydrocarbons as well as heavy metals are the main factor usually responsible for biochar toxicity.

1. Introduction

Biochar is a solid product of biomass and waste pyrolysis (Inyang et al., 2016; Lee et al., 2019), i.e., anaerobic (or with a small amount of oxygen) organic matter decomposition at relatively low temperatures of <700 °C (Ok et al., 2016). A variety of plant components, animal waste, or even industrial waste can be used to produce biochar (Inyang et al., 2016). The structure of biochar is porous and its composition primarily comprises carbon. Although biochar is similar to charcoal, the two materials are generally distinguished since charcoal is used as a fuel during the burning process, whereas biochar is usually used to improve environmental conditions or for applications other than as fuel (Ok et al., 2016). Some of the environmental applications of biochar include (1) serving as a pollutant adsorbent (Inyang et al., 2016; Ok et al., 2016; Ahmad et al., 2014; Shaheen et al., 2018); (2) improving the quality of soil by influencing: pH, water holding capacity (WHC), cation exchange capacity (CEC), the structure of microbial communities, and other soil properties important in agriculture (Ok et al., 2016; Kavitha et al., 2018; El-Naggar et al., 2019a); (3) immobilizing carbon in soil for a long time, which contributes to a decrease in atmospheric carbon dioxide (CO_2) concentrations (Ok et al., 2016), (4) serving as a catalyst support (Xiong et al., 2017); and (4) improve soil aggregate stability (Heikkinen et al., 2019; El-Naggar et al., 2019b).

A significant challenge in the use of biochar is that toxic substances can be released from the biochar matrix (e.g., due to biochar aging) and affect living organisms. The issue of contaminants in biochar is discussed

Abbreviations: HAc, Acetic acid; CEC, Cation exchange capacity; BCR, Community Bureau of Reference; DTPA, Diethylenetriaminepentaacetic acid; EPR, Electron paramagnetic resonance; EDA, Electron-Donor–Acceptor; EBC, European Biochar Certificate; FRs, Free radicals; C_{free}, Freely dissolved concentration; GI, Germination index; GR, Germination rate; EC_{50}, Half maximal effective concentration; HMs, Heavy metals; IBI, International Biochar Initiative; MARA, Microbial assay for risk assessment; NAP, Naphthalene; PFCs, Perfluorochemicals; PFOS, Perfluorooctanesulfonic acid; PFOA, Perfluorooctanoic acid; POPs, Persistent organic pollutants; PCBs, Polychlorinated biphenyls; PCDD/Fs, Polychlorinated dibenzo-p-dioxins and -furans; PAHs, Polycyclic Aromatic Hydrocarbons; POM, Polyoxymethylene; ROS, Reactive oxygen species; RBP, Residues from biogas production; TEQ, Toxic equivalent; TEF, Toxicity equivalency factor; VOCs, Volatile Organic Compounds; WHC, Water holding capacity.

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thoroughly in the next section of this review.

In addition to containing environmental toxins, biochar can substantially influence the conditions of the environment where it is located (e.g., soil), causing changes in the physical, chemical, or biological properties of the environment that can have an indirect harmful effect on organisms. Therefore, the negative impact of biochar on organisms may not result directly from the harmful substances but from the environmental changes which biochar induces. These changes can lead to unfavorable conditions for some organisms. Furthermore, when affected by environmental conditions, biochar undergoes chemical, physical, and biological changes over time (Xiong et al., 2017; Heikkinen et al., 2019), which can also influence its toxicity toward various organisms (Kavitha et al., 2018). This environmental aging can cause a decrease in biochar's affinity for contaminants, releasing them into the environment where they can come into contact with organisms. The properties of biochar can also be modified by the aging process, contributing to changes in soil properties that adversely affect organisms (Yang et al., 2019). Although the latter topic is extremely important in the context of the long-term effects of biochar on the environment and the biological world, few investigations on it exist in literature.

Awareness of the degree of the toxic effects on organisms caused by biochar-amended soil is very important because an unconscious use of a material that may contain harmful substances poses a risk of creating ecological imbalance and subsequently ecosystem impoverishment. Apart from the environmental impact, in the case of agricultural soils, toxicity can also impact the obtained yield, leading to negative economic outcomes. Therefore, it is crucial to identify the effects of different types of biochar on soil organisms.

1.1. Contaminants in biochar

Toxic substances present in biochar can be divided into categories based on their chemical character or origin. In terms of chemical character, biochar contaminants are either organic or inorganic; in terms of origin, contaminants can be distinguished as either byproducts of pyrolysis (i.e., they are formed during biochar production) or components of the feedstock used to produce biochars that remain, often more concentrated, after the pyrolysis process (Fig. 1).

An essential issue regarding the presence of contaminants in biochar is their availability to organisms and thus their potential toxicity. The total amount of contaminants in biochar is not equivalent to the amount that can cause toxic effects. A fraction of contaminants is bound so strongly to biochar that it cannot be taken up and absorbed by plants, microorganisms, or animals. Only the contaminants that are capable of penetrating into an organism can cause toxic effects (Alexander, 2000; Alexander, 1995). Therefore, rather than measuring the total fraction of contaminants, researchers are increasingly choosing to measure the bioavailable fraction (Wang et al., 2017; Cipullo et al., 2018; Oleszczuk, 2007), which is directly available to organisms. The bioaccessible fraction is another quantity used to describe biochar contaminants: this fraction may become bioavailable if an organism gains access to it as a result of external factors, such as a change in environmental conditions (e.g., pH), a change in the material structure (e.g., due to the action of organisms), or the presence of other substances (e.g., surface active ones) (Cipullo et al., 2018; Oleszczuk, 2007).

1.1.1. Polycyclic aromatic hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are the most frequently occurring contaminants in biochar. PAHs are hydrocarbon compounds containing two or more condensed aromatic rings. They are formed during pyrolysis due to the aromatization and carbonization of organic matter as well as the attachment of hydrocarbon radicals and synthesis into heavier aromatic molecules (Wang et al., 2017). PAHs are classified as persistent organic pollutants (POPs) (Schwarzenbach et al., n.d.) and have carcinogenic, mutagenic, and teratogenic properties (Wang et al., 2017).

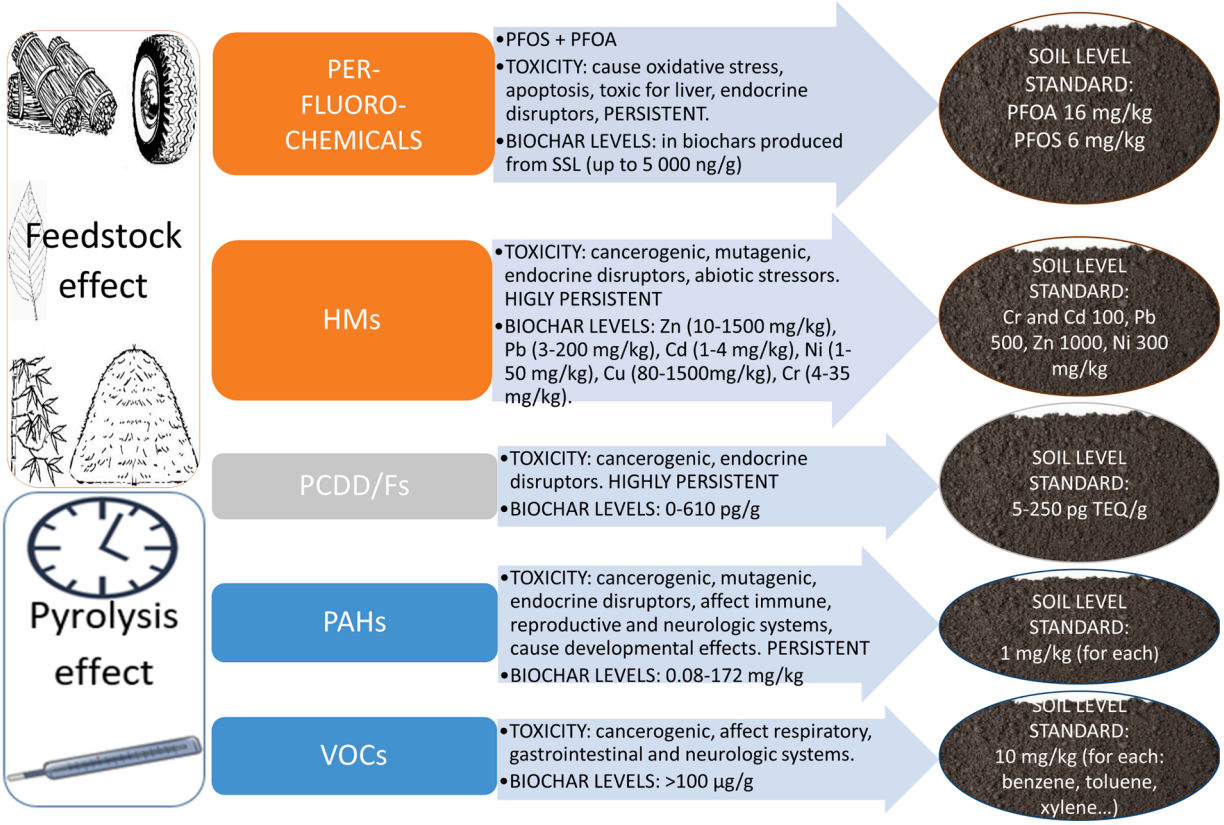

Fig. 1. Contaminants present in biochar regarding to their effect towards living organisms (Kim et al., 2015a; Antoniadis et al., 2019; Weidemann et al., 2018).

Standards for biochar PAH content have been determined by the International Biochar Initiative (IBI) and European Biochar Certificate (EBC). According to IBI's guidelines, the maximum permissible content of Σ16 PAHs (US EPA) in biochar ranges from 6 to 300 mg/kg. Biochar with a PAH content higher than 300 mg/kg is not recommended for use as a soil amendment (IBI International Biochar Initiative Guidelines, n. d.). EBC's recommendations are more rigorous and cover both premium and basic grades of biochar. Biochar with a PAH content lower than 4 mg/kg is considered to be premium grade, whereas basic grade biochar has a PAH content of up to 12 mg/kg (International Biochar Initiative, 2014). The basic and premium grades are intended to distinguish between biochars with an acceptable (basic) PAH content, i.e., an elevated content that does not significantly affect the environment, and a low (premium) PAH content.

The content of PAHs in biochar is affected by a range of factors associated with the transformation process of organic matter into biochar, such as substrate residence time in the oven, oven heating rate, and pyrolysis temperature. Research shows that biochars with lower PAH content are produced during slow pyrolysis than during fast pyrolysis (Wang et al., 2017).

A number of both experimental (Yang et al., 2019; Weidemann et al., 2018; Zielińska and Oleszczuk, 2016; Taherymoosavi et al., 2017; Stefaniuk et al., 2016; De la Rosa et al., 2019; la Rosa et al., 2016; Hale et al., 2012; Oleszczuk et al., 2013; Wiedner et al., 2013; Buss et al., 2015; Khalid and Klarup, 2015; Kołtowski and Oleszczuk, 2015; Gascó et al., 2016; Gondek et al., 2016; Lyu et al., 2016; Visioli et al., 2016; Hilber et al., 2017a; Sigmund et al., 2017; Oleszczuk and Kołtowski, 2018; Liu et al., 2019a) and review (Wang et al., 2017; Dutta et al., 2017; Hilber et al., 2017b; Liu et al., 2018; Raclavská et al., 2018) papers on PAH content in biochars produced from various feedstocks and under different conditions have been published since 2010. In this paper, we present a brief discussion of the topic and previous studies have been referenced for further information. Table 1 presents the total PAH content in biochars produced from a variety of feedstocks at different pyrolysis temperatures. The content of Σ16 EPA PAHs in biochars ranges from 80 μg/kg (Freddo et al., 2012) to as high as 172,000 μg/kg (Khalid and Klarup, 2015) (median value of about 1810 μg/kg). Feedstocks containing plant biomass contribute to reduced PAH content in the obtained biochar as such biomass contains few PAH precursors and leads to the production of biochar with a PAH content primarily comprising light naphthalene (NAP) (Kończak et al., 2019).

Although numerous studies have investigated the total PAH content in biochars, this quantity is not the most reliable indicator of the toxicity or environmental risk associated with the presence of PAHs in biochar (Alexander, 2000; Alexander, 1995). It is the bioavailable fraction of a contaminant, rather than the total amount, that is directly responsible for its toxicity; however, the bioavailable fraction of PAHs in biochar is not legally regulated, and few measurements of this quantity have been reported (Wang et al., 2017; Hale et al., 2012). Existing research demonstrates that the bioavailable fraction of PAHs in biochar does not usually exceed 200 ng/L (Zielińska and Oleszczuk, 2016; Hale et al., 2012; Hilber et al., 2017a). For instance, Hale et al. (2012) determined the bioavailable PAH content in biochars derived from different substrates (23 substrates) at temperatures ranging from 250 to 900 °C. The bioavailable fraction (called the freely dissolved concentration, C_{free}) was determined using polyoxymethylene (POM) passive samplers. The concentration of bioavailable PAHs in biochars ranged from 0.17 to 10.0 ng/L. The lowest (0.17 ng/L) and highest (10.0 ng/L) contents of bioavailable PAHs were found in biochar derived from pine wood and food waste, respectively (Hale et al., 2012). Hilber et al. (2017a) and Zielińska and Oleszczuk (2016) determined (using POM method) the concentrations of bioavailable PAHs in biochars derived from wood, *Miscanthus*, sugar beet, green garden waste, sewage sludge, lignite, and coffee dregs; the concentration ranges were similar to those reported by Hale et al. (2012), ranging from 12 to 85 ng/L. Naphthalene accounted for up to 90% of the bioavailable PAH content. Biochar derived from green garden waste had the highest content of bioavailable PAHs (85 ng/L) (Hilber et al., 2017a). Sewage sludge biochars (produced 500–700 °C) were characterized by concentrations of C_{free} PAHs ranging from 81 to 126 ng/L; in this case, 3-ring PAHs were predominant among the bioavailable PAHs (Zielińska and Oleszczuk, 2016). To date, the highest content of bioavailable PAHs (POM method) has been reported by Kołtowski and Oleszczuk (2018) in biochar produced from *Miscanthus*, for which C_{free} of Σ16 PAHs was nearly 1 μg/L. PAHs containing 2 rings were predominant in the bioavailable fraction, constituting 47% of all the identified compounds. Such a high content of bioavailable PAHs in this biochar was due to its low specific surface area (0.76 m^2/g), which facilitated the migration of PAHs to the extraction solution. Biochars specific surface area is considered one of the main properties driving the adsorption mechanisms (hydrophobic interactions and π-π electron donor- acceptor interactions) between biochar and organic pollutants such as PAHs. Low specific surface area leads then to obtaining less sorption sites for PAHs.

The pyrolysis temperature is another important factor which determines PAHs content in biochar. Biochars produced in the 400–600 °C temperature range are characterized by higher PAH concentrations than biochars obtained at lower or higher temperatures (Table 1). During the gasification process, however, biochars with relatively high PAH concentrations are obtained despite the high temperatures used (Visioli et al., 2016). Biochar produced via gasification had a higher content of bioavailable PAHs (162 ng/L) than biochar obtained from food waste via pyrolysis. Hale et al. (2012) also observed that an increase in pyrolysis temperature decreased the concentration of bioavailable PAHs (with some exceptions). An increase in temperature enhances the aromaticity of the biochar surface; hence, the attraction between PAH and biochar is higher and the bioavailability of the PAHs is lower due to the greater hydrophobic and π-π electron- donor- acceptor (EDA) interactions strengths. The gas used to produce biochar can also influence the PAH content. For example, biochars produced in an atmosphere of nitrogen contain more PAHs than biochars produced in an atmosphere of CO_2 (Kończak et al., 2019; Kwon et al., 2015). A similar PAH reduction effect is achieved owing to the use of CO_2 rather than air during gasification (Lee et al., 2017).

The bioavailable PAH content in biochar is low compared to, for example, the bioavailable PAH content in urban sediments (0.08–342 μg/L) (Hale et al., 2012). Such a large difference between the total biochar content of PAHs and their bioavailable content results from the very strong bonds (physical: occlusion in biochar pores and chemical: based on π-π interactions) formed between biochar and PAHs generated during pyrolysis. Thus far, however, no study clearly indicates that biochar toxicity is determined by PAHs (total or bioavailable). This may confirm the presumptions of some researchers that PAHs are so strongly bound to biochar that they have no toxic effect on organisms, because the bioavailable fraction is too low to cause toxicity. Nevertheless, PAHs can be released from biochar and pose a potential threat, particularly in the case of repeated biochar application in soils. A lack of comprehensive studies also exists on biochar aging in the context of the bioavailability of PAHs. It is therefore very important, given the current stage of the development of biochar production technology and use, to monitor and regulate the bioavailable content of PAHs in biochars, especially those used for fertilization and food production purposes.

1.1.2. Polychlorinated dibenzo-p-dioxins, -furans

Polychlorinated dibenzo-p-dioxins and -furans (PCDD/Fs) exist in insignificant quantities in biochar (Hilber et al., 2017b). PCDD/Fs are formed in biochar during pyrolysis of feedstocks containing significant amounts of chlorine, such as food waste (Hilber et al., 2017b). Due to the varying toxicities of PCDD/Fs, their concentrations are expressed as toxic equivalent (TEQ). TEQ is calculated by multiplying the mass of a given congener by its toxicity equivalency factor (TEF) according to the following equation (Eq. 1):

Table 1
Total and bioavailable PAH content in biochars derived from different feedstock.

Material	Temperature (°C)	Production conditions	PAHs	Total PAHs concentration (μg kg^{-1})	Dominant PAHs	Bioavailable PAHs	Reference
Municipal solid waste	450 550 650		16 US EPA	1200 <500 <500	2- ring (NAP) — —	N/D	(Taherymoosavi et al., 2017)
Residues of biogas production (different types)	400 600 800		16 US EPA	1474-3100 2800-4500 2800-4874	2- ring, 3- ring	N/D	(Stefaniuk et al., 2016)
Sewage sludge	500 600 700		16 US EPA	560- 766 566- 978 488-1118	3- ring (PHE)	93-126 ng/L 95-115 ng/L 81-107 ng/L	(Zielińska and Oleszczuk, 2015)
Sewage sludge	600 600	Residence time 20min	16 US EPA	1942 1820	3- ring (PHE, ANT) and NAP 3- ring (PHE, ANT) and NAP	N/D	(De la Rosa et al., 2019)
Sewage sludge	500 600 700			2263 1730 1449	3- ring (PHE)	44 ng/L 51 ng/L 46 ng/L	
Sewage sludge + willow (8:2)	500 600 700	Atmosphere: N_2 Residence time: 3h		1290 1343 1517	3- ring (PHE) 2- ring	37 ng/L 36 ng/L 40 ng/L	
Sewage sludge + willow (6:4)	500 600 700		16 US EPA	1079 1109 1354	2- ring	31 ng/L 30 ng/L 34 ng/L	(Kończak et al., 2019)
Sewage sludge	500 600 700	Atmosphere: CO_2 Residence time: 3h		1482 1125 715	2- ring 3- ring	38 ng/L 39 ng/L 36 ng/L	
Sewage sludge + willow (6:4)	500 600 700			797 818 938	2- ring	25 ng/L 24 ng/L 24 ng/L	
Sewage sludge	200 300 400 500 600 700	Residence time: 6h	16 US EPA	1640 2260 2990 70390 1240 180	3- ring (PHE) and NAP 3- ring (PHE, ACY) and NAP 3- ring (PHE) and NAP 3- ring (FLU, PHE) and NAP 3- ring (ACY) 3- ring (PHE) and NAP	N/D	(Luo et al., 2014)
Corn stalk	200 300 400 500 600 700		16 US EPA	760 5320 3580 3290 570 360	3- (PHE) and 4- ring (PYR) 2- ring 2- ring 2- ring 3- ring 3- ring		
Miscanthus Andersson		Thermally treated 100-300 °C		39900 500-26000	4-ring		
Willow (*Salix viminalis*)	650	Thermally treated 100-300 °C	16 US EPA	3500 0-3000	4-ring	—	(Kołtowski and Oleszczuk, 2015)
Wheat straw (*Triticum* L.)		Thermally treated 100-300 °C		19900 1000-3200	3-ring		
Wood Rice husk Softwood Rice Bamboo Redwood Maize Rice Bamboo Redwood Maize	450 — 500 300 600 	Residence time: 48h — Residence time: 12h Residence time: 2.5h 	16 US EPA	9556 64650 8700 2270 2470 4540 5660 1150 1060 80 1470	2- ring 2- ring 2- ring 4- ring (PYR) 2- ring 2- ring 2- ring 4- ring (PYR) 4- ring (PYR) 4- ring (FLT) 4- ring (PYR)	N/D	(Quilliam et al., 2013) (Freddo et al., 2012)
Conifer wood				21060	4- ring (PYR) and PHE		
Poplar wood Grape marc	1200, gasification	—	16 US EPA	15660 3810	4- ring (PYR) 3- ring (ACY)	N/D	(Visioli et al., 2016)
Wheat straw				15840	4- ring (PYR, FLT)		

(*continued on next page*)

Table 1 (*continued*)

Material	Temperature (°C)	Production conditions	PAHs	Total PAHs concentration (μg kg^{-1})	Dominant PAHs	Bioavailable PAHs	Reference
Wood chips	620	Residence time: 20min	16 US EPA	2613	2- ring	N/D	(la Rosa et al., 2016)
Paper sludge	500			1774	2- ring		
Sewage sludge	600			959	2- ring		
Grapevine wood	— (kiln pyrolysis)	N/D		15367	2- ring		
Sawdust	250	Residence time: 3h	16 US EPA	190	3- ring (PHE)	N/D	(Lyu et al., 2016)
	300			400	2- ring		
	400			860	3- ring (PHE)		
	500			650	2- ring		
	700			590	2- ring		
Hardwood sawdust	500	N/D	16 US EPA	3600	N/D	N/D	(Fabbri et al., 2013)
Wood waste	475			3800			
	550			1200			
Hardwood	—			1400			
Wood chips/ manure	—			1800			
Macadamia nut shells	—			19000			
Distillers grain	350			5000			
	400			2200			
Wood waste	400			8800			
Wood chips	800, gasification	N/D	16 US EPA	172000	2- ring	N/D	(Khalid and Klarup, 2015)
Wood chips and *Arundo donax* chips				117000	2- ring		
Softwood pellets	550	Residence time: 20min Some biochars went through re-condensation	16 US EPA	6090-53420	2- ring, 3- ring (PHE)	<0.001-2.040 μg/g	(Buss et al., 2015)
Vegetable waste	200	Residence time: 2h	16 US EPA	330	3- ring	N/D	(Yang et al., 2019)
	500			340	2- ring		
Pine cones	200			6930	3- ring		
	500			1600	2- ring		
Vegetable waste + + pine cones	200			2600	3- ring		
	500			3823	2- ring		

$$TEQ = \sum_{i=1}^{i=17} (m_i \cdot TEF_i)$$

where: m_i- concentration, content or mass of the congener,

TEF_i- toxicity equivalency factor based on studies by Van den Berg et al. (Van den Berg et al., 1998).

The limits established by IBI and EBC for PCDD/Fs in biochar are 17 and 20 ng/kg TEQ, respectively. Hale et al. (2012) measured 130 toxic and non-toxic PCDD/F congeners in biochars produced from food waste, digested dairy manure, pine wood, and lodgepole pine. Their concentrations ranged from 84 to 92 ng/kg. In the same study, in biochars obtained from food waste, digested dairy manure, pine wood, lodgepole pine, laurel oak, eastern gamma grass, switch grass, and paper mill waste, 17 toxic congeners were identified (0.5–13.3 ng/kg), for which the TEQ ranged from 0.005 to 1.20 ng/kg TEQ (Hale et al., 2012). The highest TEQ was observed for biochar produced from food waste at 300 °C, which also contained a higher chlorine content than the other pyrolyzed materials. The bioavailable content (C_{free}) of dioxins was below the detection limit, which may indicate that these compounds have a limited effect on biochar toxicity. Wiedner et al. (Wiedner et al., 2013) compared the dioxin content in biochars and hydrochars and observed dioxins above the detection limit (14.2 ng/kg TEQ) only for sewage sludge hydrochar. Wiedner et al. (2013) explained the presence of dioxins by noting their occurrence in the sewage sludge prior to processing; the low hydrochar production temperature (<250 °C) prevented them from degrading. Weidemann et al. (2018) found only monochlorinated dibenzofuran in biochars produced from softwood, wheat straw, or anaerobic digestate at 550 °C, while the concentrations of other congeners were below the detection limit (<0.3 pg/g). Lyu et al. (2016) determined values ranging from 50 to 610 pg/g for the total content of PCDD/Fs in sawdust biochars produced at temperatures ranging from 250 to 700 °C. However, the TEQ concentrations were significantly lower, ranging from 1.7 to 9.6 pg/g TEQ. Based on the above studies (Weidemann et al., 2018; Hale et al., 2012; Wiedner et al., 2013; Lyu et al., 2016), it can be concluded that the level of biochar contamination with PCDD/Fs is low and poses a rather marginal risk for environmental use of biochar, though it cannot be ruled out that environmental contamination may occur during repeated application of biochar contaminated with these compounds.

1.1.3. *Volatile organic compounds*

Volatile organic compounds (VOCs) are also among the biochar contaminants that can have potentially toxic impacts on organisms (a carcinogenic effect as well as effects relating to the respiratory, digestive, and nervous systems) (Ghidotti et al., 2017). VOCs include chemicals such as acetic, formic, butyric and propionic acids, methanol, phenol, methylated phenols, and cresols (Buss et al., 2015; Aller, 2016; Spokas et al., 2011). These compounds are formed during pyrolysis as a result of thermochemical transformations of biomass, wherein larger organic molecules break down into compounds with lower molecular masses. VOCs are then deposited on biochar or inside biochar pores (Buss et al., 2015). Spokas et al. (2011) investigated biochars produced under different conditions from various materials (more than 30) in terms of their VOC content. Acetone, benzene, methyl ethyl ketone, toluene, methyl acetate, and propanal were identified in more than half of the biochars tested, while other VOCs were less common. A broad study on the formation of VOCs and their presence in biochars was also conducted by Buss et al. (2015). They tested biochars produced from softwood pellets at 550 °C. Re-condensation of VOCs was observed on two of the biochars tested, which were then found to have a high VOC content. Phenols (2-methylphenol, 3/4-methylphenol, 3,4 dimethylphenol, 4-ethylphenol, 4-ethyl-3-methylphenol, and 2,4-dimethylphenol), organic acids (acetic acid, methanol, formic acid, butyric acid, and propionic acid), and cresols have been most commonly extracted with water in biochar, all at concentrations of >100 μg/g biochar. Ghidotti et al. (2017) studied corn stalk biochars produced at temperatures ranging from 350 to 650 °C and identified VOCs including benzenes, biphenyls, indanes, benzonitrile, benzofurans, aldehydes, and

ketones. They observed that a higher degree of biochar carbonization generated a smaller amount of VOCs. Rombolà et al. (2015) found VOCs in biochar obtained from poultry litter at 400 °C, identifying substances such as cyclopentenones, furans, guaiacol, pyrroles, pyridines, indole, and acetic acid.

It is suggested to produce highly carbonized biochars in order to obtain low VOCs- contaminated materials. According to Spokas et al. (2011) there is also an influence of partial aerobic conditions during production of biochar, which lead to lower VOCs content in the material.

1.1.4. Heavy metals

Among inorganic biochar contaminants, heavy metals (HMs) are of the greatest interest in the context of biochar toxicity. The chemical composition of the feedstock from which biochar is produced, that is, the presence of HMs in the original material, is responsible for their presence in biochar. For many HMs, their concentrations in biochar are usually higher than those in feedstock due to the partial mineralization of organic substances during the pyrolysis process (the concentration effect). Under specific conditions some metals can be converted to volatile forms that are released during pyrolysis, e.g. Hg or Cd (He et al., 2019; Zielińska and Oleszczuk, 2015). Sewage sludge is a substrate that contains substantial amounts of HMs (from several to as high as 4000 mg/kg dm). Biochar with a high HM content is also obtained from plants grown on HM-contaminated soils. HMs in biochar include essential HMs, such as Co, Cr, Cu, Fe, Mn, Ni, and Zn, and non-essential HMs, such as Pb, Cd, and Hg. In small quantities, essential HMs are necessary micronutrients, but in large amounts they cause a toxic effect. On the contrary, non-essential HMs are highly toxic regardless of the amount (Heavy Metals in the Environment: Origin, Interaction and Remediation, n.d.). It has been found that more than 98% elements included to HMs groups become more concentrated in biochar during pyrolysis compared to feedstock (Hilber et al., 2017b; Lehmann and Joseph, 2015). Due to mass loss during organic matter decomposition, the concentration of HMs in biochar can be up to 4–6 times higher than their concentrations in the original material before pyrolysis. EBC and IBI have defined standards for HMs content in biochar, which are presented in Table 2.

Taherymoosavi et al. (2017) determined the total content of HMs in biochars produced from municipal solid waste at temperatures ranging from 450 to 650 °C. The highest concentrations were found for Zn (735–987 mg/kg depending on pyrolysis temperature) and Pb (160–193 mg/kg), and hence, the tested biochars did not meet EBC standards. The Cd content in biochar was also determined to be high (1–3 mg/kg). The concentrations of the other metals (Ni, Hg, Cr, and As) were within EBC's recommended range for basic grade biochar (Table 2).

Literature describes a range of methods for determining the bioavailability of HMs in different environmental matrices (Dean, 2009). The simplest methods are those involving the extraction of metals using one solvent (e.g., water or diethylenetriaminepentaacetic acid, DTPA) (Dean, 2009). The difference between water and DTPA used for HMs extractants is that DTPA extracts not only water-soluble part but also HMs bound to soil mineral particles because of its chelating properties. Although methods based on a procedure known as sequential extraction are more complicated, they provide a wider spectrum for the assessment of the risks related to metals. Sequential extraction involves the successive leaching of metals from the matrix using increasingly stronger extractants. Thus, it is possible to determine not only the bioavailable fraction but also the fraction bound with varying bond strengths to the different matrix components. In terms of defining the bioavailable fraction, the method proposed by the Community Bureau of Reference (BCR) is frequently used. This method considers the following fractions to be bioavailable to organisms: (1) acid soluble and exchangeable, bound to carbonates (F1, leached with acetic acid) and (2) reducible, bound to Fe and Mn oxides (F2, leached with hydrochloric hydroxylamine). The other two fractions, i.e., the oxidizable fraction (F3) and the residual fraction (F4), are considered not easily available or unavailable due to their strong binding to organic matter and sulfides, respectively, as well as to silicate minerals. Some consider only the fraction F1 to be bioavailable, fractions F2 and F3 not easily available, and F4 a fraction incorporated into the matrix structure.

Yang et al. (2018) used DTPA method to determine the bioavailable fraction of HMs (Cd, Cu, Pb and Zn) in biochars obtained from biosolids from wastewater treatment plants in 500 or 700 °C. The DTPA extracted HMs content ranged from 2% to 10% in 500 °C biochar and from 1% to 10% in 700 °C biochar. Visioli et al. (2016) determined the bioavailable fraction of HMs in biochars obtained through gasification using the DTPA-based method. The DTPA-extracted fraction ranged from 18% to 36% of the total HM content, depending on the feedstock from which biochar was produced. Also using the DTPA method, He et al. (2019) studied the bioavailability of HMs in biochars derived from *Avicennia marina* at 300–800 °C. The bioavailable fraction varied from 5% to 90%, a significantly wider range than that reported by Visioli et al. (2016). The type and pyrolysis temperature determined the availability of metals. With the exception of Pb, Cd, and Cu, there was a decrease in the bioavailability of metals (Cr, Ni, As, Mn, Co, and Zn) with increasing pyrolysis temperature, which was caused by transformations to inorganic forms, such as oxides and sulfides. Luo et al. (2014), using the DTPA method, identified bioavailable HMs in biochars produced from corn stalk or sewage sludge at temperatures ranging from 200 to 700 °C. The bioavailable concentrations of individual metals, depending on temperature, were determined for corn stalk and sewage sludge biochars, respectively, as follows: Cu (30–170 μg/L, 29.5–365.0 μg/L), Zn (35–115 μg/L, 220–970 μg/L), Pb (1.95–9.50 μg/L, 27.5–115.0 μg/L), Cd (0.45–0.75 μg/L, 0.30–10.50 μg/L), Cr (3.0–11.5 μg/L, 8–11 μg/L), and Ni (6.5–32.5 μg/L, 14.0–46.5 μg/L). Sewage sludge biochars were characterized by a higher content of metals than corn stalk biochars due to the higher metal content in the feedstock. In biochars produced at temperatures >500 °C, higher concentrations of bioavailable metals were found than in biochars produced at lower temperatures (200, 300, 400, and 500 °C). This was probably due to a higher total metal concentration in biochars produced at >500 °C, resulting from higher mass losses. The plant available fraction in corn stalk biochars was usually the highest for biochars produced at 400-500 °C and ranged from 3% to 6% of total Cu content, from 1% to 2% of total Zn content, from 3% to 5% of total Pb content, 1% of total Cr content and from 3% to 7% of total Ni content. Cd only did not follow this trend. The highest contribution of Cd in DTPA fraction was determined for corn stalk biochar produced at 300 °C (100%) and in 400 °C (79%) as well as for corn stalk biochars regardless of temperature and ranged from 21 to 100%. In sewage sludge biochars the DTPA- extracted HMs ranged from less than 1% to 4% of total Cu content, from less than 1% to 2% of total Zn content, from 2% to 3% of total Pb content, from less than 1% to 30% of total Cd content, 0.1% of total Cr content and from less than 1% to 2% of total Ni content. The contribution of bioavailable fraction usually increased with increasing temperature of feedstock pyrolysis. Wang et al. (2016) studied sewage sludge biochars produced at 700 °C and pre-heated at 160–220 °C and found bioavailable fractions ranging from 0% to 58%. Bioavailability decreased as the thermal treatment temperature of the

Table 2
Guidelines for heavy metal content in biochars.

Heavy metals (mg/kg)	EBC guidelines (EBC, 2012)		IBI guidelines (IBI International Biochar Initiative Guidelines, n.d.)
	basic grade biochar	Premium grade biochar	
Pb	<150	<120	121-300
Cd	<1.5	<1	1.4-39
Cu	<100	<100	143-6000
Ni	<50	<30	47-420
Hg	<1	<1	0.8-17
Zn	<400	<400	416-7400
Cr	<90	<80	93-1200
As	<13	<13	N/D

materials before pyrolysis was increased. They attributed this trend to the conversion of metals to more stable forms due to pre-heating. Zeng et al. (2018) studied bioavailable HMs using the DTPA method in biochars obtained from swine and goat manure at 200–800 °C and reported bioavailable fractions ranging from 3.5% to 37.3%.

The methods using sequential extraction allow us to obtain more precise information on not only the bioavailability of metals but also their potential interactions with environmental components. The bioavailable fraction (F1 + F2) in biochars measured by different authors (Wang et al., 2019a; Wang et al., 2019b; Chen et al., 2018; Devi and Saroha, 2014) using the BCR method ranged from 0% to 70% and was largely determined by the HM content in the feedstock. For example, in biochars produced from textile dyeing sludge (at 300–700 °C), Wang et al. (2019a) determined bioavailable fractions between 1% and 43%. Wang et al. (2019b) found a similar range of bioavailable fractions (from 0% to 58%) in biochars obtained from sewage sludge and

Table 3
Heavy metal content in biochars produced from various feedstocks.

Material	Pyrolysis conditions	Total content (mg/kg)	Bioavailable content	Reference
Conifer wood	1200 °C gasifier	Zn 272, Cu 111, Pb 6.34, Ni 85.4, Cr 114, Cd 0.34, Co 2.05, Hg <0.001	DTPA method (% of total content): Zn 4.7, Cu 10.1, Pb 3.6, Ni 0.7, Cr 1, Cd 12.7, Co <3.1, Hg 0	(Visioli et al., 2016)
Poplar wood		Zn 180, Cu 34.9, Pb 10.7, Ni 18.9, Cr 23.5, Cd 1.56, Co 2.74, Hg <0.001	DTPA method (% of total content): Zn 6.2, Cu 3.3, Pb 4.8, Ni 1.6, Cr <0.2, Cd 14.3, Co <3.1, Hg 0	
Grape marc		Zn 282, Cu 69, Pb 5.23, Ni 16.2, Cr 30.3, Cd 0.47, Co 1.29, Hg <0.001	DTPA method (% of total content): Zn 2.2, Cu 1.8, Pb <2, Ni 0.7, Cr <0.2, Cd 8, Co <3.1, Hg 0	
Wheat straw		Zn 183, Cu 26.5, Pb 6.92, Ni 19.4, Cr 29.5, Cd 0.41, Co 2.43, Hg <0.001	DTPA method (% of total content): Zn 4.1, Cu 5.5, Pb 2, Ni 1.8, Cr <0.2, Cd 17.8, Co <3.1, Hg 0	
Pneumatophores of *Avicennia marina* after phytoremediation	300-800 °C	Zn 83.53-307.5, Cu 30.25-62.74, Pb 1.37-98.59, Ni 1.68-3.17, Cr 1.89-3.14, Cd 0.012-0.943, As 4.33-6.29, Co 1.93-3.49, Mn 131.2-250.4	DTPA method (% of total content): Zn 8-30, Cu 2-21, Pb 9.5-88.0, Ni 8-21, Cr 0-5, Cd 11-38, As 3-35, Co 4-35, Mn 18-68	(He et al., 2019)
Corn stalk	200-700 °C Residence time: 6h	Zn 60-133, Cu 20-57, Pb 1.5-3.5, Ni 4-10, Cr 8-21, Cd <0.014	DTPA method (μg/L): Zn 35-115, Cu 30-170, Pb 1.95-9.50, Ni 6.5-32.5, Cr 3.0-11.5, Cd 0.45-0.75.	(Luo et al., 2014)
Sewage sludge		Zn 735-986, Cu 149-202, Pb 55-74, Ni 41-56, Cr 180-247, Cd 1	DTPA method (μg/L): Zn 220-970, Cu 29.5-365.0, Pb 27.5-115.0, Ni 14.0-46.5, Cr 8-11, Cd 0.30-10.50	
Cotton + + sewage sludge	350-750 °C Residence time: 2h	Zn 350-700, Cu 260-400, Pb 40-50, Ni 10-30, Cr 75-120, Cd 1.3-2.0	BCR (% of total content): Zn 7-77, Cu 0-53, Pb 0-29, Ni 1-72, Cr 1-52, Cd 1-35	(Wang et al., 2019b)
Cotton stalk + + sewage sludge	300-600 °C	Zn 410-580, Cu 220-310, Pb 22-25, Ni 8-21, Cr 16-25, Mn 320-440	BCR (% of total content): Zn 30-35, Cu 0-4, Pb 7-9, Ni 45-56, Cr 8-9, Mn 48-57	(Wang et al., 2018)
Sewage sludge		Zn 900-990, Cu 500-560, Pb 36-46, Ni 22-37, Cr 45-55, Mn 670-760	BCR (% of total content): Zn 40-80, Cu 10-27, Pb 19-28, Ni 42-90, Cr 9-30, Mn 44-90	
Sewage sludge	180-300 °C Hydrothermal treatment	Zn 41.4-54.6, Cu 2.7-11.6, Pb 6.6-7.6, Cd 0.2-0.3	BCR (% of total content): Zn 7-10, Cu 12-32, Pb 14-18, Cd 34-45	(Chen et al., 2018)
	550-850 °C pyrolysis	Zn 24.2-60.7, Cu 1.9-2.6, Pb 8.4-10.6, Cd 0.13-0.15	BCR (% of total content): Zn 18-23, Cu 1-3, Pb 18-23, Cd 30-33	
Paper mill effluent treatment plant sludge	200-700 °C	Zn 200-325, Cu 90-154, Pb 20-33, Ni 17-25, Cr 15-17	BCR (% of total content for biochars produced in 500-700 °C): Zn 1-2, Cu 2-3, Pb 2.5-4.0, Ni 6-8, Cr 3.0-11.5, Cd 0.45-0.75	(Devi and Saroha, 2014)
Sewage sludge	700 °C (most of biochars were previously thermally treated)	Zn 3925-4321, Cu 5513-6258, Pb 115-132, Ni 672-710, Cr 3317-3485, Cd 0.45-1.88	BCR (% of total content): Zn 28-58, Cu 0-4, Pb 0-1, Ni 22-47, Cr 0, Cd 12-38	(Wang et al., 2016)
Sewage sludge (different types)	300 °C Residence time: 15 min	Zn 732-2321, Cu 100-467, Pb 22-81, Cd 1.12-3.87	Water extractable (mg/kg): Zn 0.65-3.75, Cu 0, Pb 0.40-1.00, Cd 0.05-0.10	(Gondek and Mierzwa-Hersztek, n.d.)
Sewage sludge	300-900 °C Residence time: 2h	Zn 730-1510, Cu 10-180, Pb 76-143, Ni 0-140, Cr 190-800, Cd 50-170, As 10-70, Ba 20-280, Co 0-270	Water extractable (mg/L): Zn 0-10, Cu 0-0.22, Pb 0-0.66, Co 0-0.08, Cr 0-0.38, Cd 0-0.01, As 0-0.97, Ba 0-0.72	(Phoungthong et al., 2018)
Swine manure	200-800 °C Residence time: 1h	Zn 795-1464, Cu 702-1335, Pb 18-35, Ni 59.5-115.0, Cr 14-31, Cd 0.66-1.15	DTPA method (mg/kg): Zn 19-206, Cu 68-240, Pb 0.5-2.8, Ni 11.2-15.6, Cr 0.4-0.5, Cd 0-0.16 BCR method (mg/kg): Zn 259-544, Cu 13-292, Pb 5.5-8.0, Ni 23-25, Cr 1.4-3.8, Cd 0.1-0.7	(Zeng et al., 2018)
Goat manure		Zn 113-229, Cu 21-40, Pb 27-52, Ni 60-116, Cr 15.5-32.0, Cd 1.0-2.6	DTPA method (mg/kg):5-42, Cu 0.1-1.9, 1.9-5.0, Ni 11.0-12.4, Cr 0.3-0.7, Cd 0.2-0.6 BCR method (mg/kg): Zn 24.0-80.2, Cu 0-0.4, Pb 2.7-9.3, Ni 20.0-24.5, Cr 1.0-2.9, Cd 0-0.8	
Residues from biogas production (different type)	400-800 °C	Zn 13.3-168.0, Cu 20.4-77.7, Pb 3.3-25.9, Ni 0-33.6, Cr 4.2-38.9, Cd 1.9-8.7	N/D	(Stefaniuk et al., 2016)
Municipal solid waste	450-650 °C	Zn 735-987, Pb 160-193, Cd <1-3, Hg <1, Ni 18-45, Cr 29-35, As 7-8	N/D	(Taherymoosavi et al., 2017)
Biosolids from wastewater treatment plant	500 °C	Cd 1.54, Cu 464, Pb 43, Zn 913	DTPA method (% of total content): Cd 4, Cu 10, Pb 2, Zn 3	(Yang et al., 2018)
	700 °C	Cd 1.71, Cu 431, Pb 45, Zn 888	DTPA method (% of total content): Cd 5, Cu 10, Pb 1, Zn 4	

co-pyrolyzed sewage sludge and cotton stalk at 300–600 °C. Biochars produced at 600 °C usually had a lower F1 + F2 fraction than those produced at lower temperatures. The addition of cotton stalk to sewage sludge significantly reduced metal bioavailability relative to biochars obtained from sewage sludge alone. Using the BCR method, Chen et al. (2018) determined HMs in sewage sludge biochars (550–850 °C) and hydrochars (180–300 °C). The bioavailable fractions of metals in hydrochars and biochars were similar and ranged from 6% to 40% and 2% to 35%, respectively. Increase of the production temperature decreased the bioavailability of Cd and Pb in hydrochars; however, a reverse trend was observed for Cu and Zn, wherein an elevated process temperature increased their bioavailable fraction. In the case of biochar, increased pyrolysis temperature decreased the bioavailability of Cu and Pb. A relatively low bioavailable fraction (2%–19%) compared to the previously cited study was found by Devi and Saroha (2014) in biochars produced from paper mill sludge at 500–700 °C. They observed that, generally, the bioavailable fraction of HMs in biochars decreased with increasing pyrolysis temperature.

The experimental data (Table 3) shows that both the material from which biochar is produced and the pyrolysis temperature have an impact on the bioavailable fraction of HMs in biochar. With increasing pyrolysis temperature, the bioavailable fraction usually decreases and the fraction strongly bound to the matrix increases. Bioavailability also changes depending on the type of HMs, but no clear trends that would allow us to draw more concrete conclusions can be observed.

1.1.5. Other contaminants

Apart from the abovementioned classic pollutants that can occur in biochar, there are a few studies showing that biochar can contain other potentially hazardous compounds depending on its feedstock. One example is perfluorochemicals (PFCs), which are resistant to chemical and thermal degradation and are used in various consumer products. Wastewater treatment plants are considered to be an important pathway for environmental contamination by PFCs, and high concentrations of PFCs are frequently found in sewage sludge. Sewage sludge is an increasingly popular material used to produce biochar; sewage sludge coupled with the high thermal stability of PFCs poses a risk of these compounds being present in sewage sludge biochars. In their analysis of sewage sludge biochars, Kim et al. (2015b) found concentrations of perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) in biochar ranging from 10.6 to 11.5 ng/g and 4.8 to 6.3 ng/g, respectively. Although the total amounts of PFOA and PFOS in biochar decreased by up to 50% based on the total weight loss of biochar during pyrolysis.

Free radicals (FRs), which can potentially contribute to biochar toxicity, have been observed to form in biochar during the pyrolysis of organic matter (Liao et al., 2014). Chemisorption and electron transfer are recognized mechanisms for the formation of FRs. The process of FR formation in biochar is described in detail in a previous study (Ruan et al., 2019). FRs were found to generate reactive oxygen species (ROS), which can damage DNA under in vitro conditions and in cell structures (Gehling and Dellinger, 2013). Liao et al. (2014) investigated the content of FRs in biochars obtained from various types of biomass and from major components of biomass (i.e., lignin and cellulose). They observed that lignin-derived biochar generated higher electron paramagnetic resonance (EPR) signals than cellulose biochar, and no signal was obtained for the original materials. Increase of the pyrolysis temperature enhanced the EPR signal for both biochars. Zeng et al. (Zielińska and Oleszczuk, 2015) noted significant germination inhibition, root and shoot growth retardation, and plasma membrane damage in the case of biochars containing large amounts of FRs. Zhang et al. (Liao et al., 2014; Zhang et al., 2019a) also observed a significant effect of pyrolysis temperature on FR formation in biochar produced from pine needles at 300–600 °C. They further found that FR-containing biochar induced the production of extracellular ROS (such as ·OH) in water, which would also induce the production of interior cellular ROS in aquatic organisms. Moreover, the EPR signal decreased only by 10% after a month. Lieke et al. (2018) did not observe significant changes in the intensity of the EPR signal in biochar even after one year, which indicates the high persistence of FRs in biochar. They suggested that the neurotoxic effect of biochar on *Caenorhabditis elegans* was associated with the presence of FRs in biochar, identified by EPR.

1.2. Contaminants in biochar-amended soil

Biochar application to soil can lead to increased levels of toxic compounds in the soil due to their presence in biochar (Aller, 2016). There is an underlying risk of the migration of toxic soil contaminants to plants, soil organisms, and other environmental elements. Biochar, however, has strong adsorption properties due to its large specific surface area, a well-developed pore network, and the presence of numerous functional groups (Anyika et al., 2014). Thus, although there is a risk of the spread of the contaminants in biochar into the surrounding environment, the significant affinity of biochar for these contaminants tends to reduce their spread. It can therefore be presumed that not only will contaminants originating from biochar have reduced mobility in soil but also biochar can also decrease the mobility of contaminants already present in the soil. This phenomenon is used in remediation techniques and is widely described in literature (Ahmad et al., 2014; Fang et al., 2018; Qi et al., 2017; Derakhshan Nejad et al., 2018). However, there is sparse information available on the persistence of native contaminants after biochar incorporation in soils. Despite the significant affinity of biochar for contaminants, there is still a risk that owing to various environmental processes (e.g., biochar biodegradation), changes may occur that could affect the properties of biochar responsible for binding contaminants; in this case, previously unavailable contaminants can be mobilized. This is a new issue that has not yet been adequately addressed in literature.

1.2.1. Polycyclic aromatic hydrocarbons

Bioavailability and bioaccessibility of PAHs in soil are affected by their concentrations, the concentrations of other contaminants, soil type (especially in the context of soil organic matter content), and other environmental conditions (such as pH, temperature, and moisture content). Owing to biochar's large specific surface area and the aromatic nature of its surface (and thus its strong sorption capacity), it is assumed that the addition of biochar in soil will not increase the bioavailable PAH content in soil and may in fact reduce the bioavailable content, even if the total PAH content increases. Although a number of studies have reported the total PAH content in biochar-amended soils, few have investigated the bioavailable fraction.

De la Rosa et al. (2016) produced biochars at temperatures ranging from 500 to 620 °C using wood chips, paper sludge, or sewage sludge as feedstock and added biochars in soil at rates of 10–40 t/ha. After 79 days of incubation, the Σ16 PAH content in the soil ranged from 50 to 2710 μg/kg. Application of biochars produced from wood chips and paper sludge at the lowest application rate resulted in virtually no change in the total PAH concentration relative to the control soil (59 μg/kg). For all biochars with the exception of sewage sludge and kiln wood biochars, more PAHs were incorporated into the soil as the biochar application rate increased. Soil incubated with kiln wood biochar, applied at a rate of 10 t/ha, contained the greatest amount of PAHs (2710 μg/kg) amongst all the treatments studied, with fluoranthene, phenanthrene, and pyrene identified as the dominant PAHs. After biochar amendment the PAHs content was from 19% to almost 26-times (for wood biochar) higher compared to control soil. Maienza et al. (2017) investigated the effect on PAHs content of biochar produced from orchard pruning waste in 500 °C added to vineyard soil. They found that although the application of biochar increased the total PAHs content in the soil, it did not exceed the threshold limits defined by the Italian environmental legislation. After one year, the PAHs content significantly decreased; Maienza et al. (2017) attributed the decrease to leaching,

photodegradation, biodegradation, bioaccumulation, and/or volatilization. Kuśmierz et al. (2016) studied the persistence of PAHs in soil (Podzolic, loamy sand) amended with biochar (30 or 45 t/ha) produced from wheat straw at 650 °C. The experiment was conducted under field conditions over an 18 month period. Biochar increased the soil content of Σ16 PAHs by factors of 2.2 (30 t/ha) and 5 (45 t/ha). However, 105 days after biochar incorporation, the total content of PAHs was comparable to the PAHs content in the control soil. Biochar amendment, even at the highest rate, did not result in an increase in the soil PAHs content above the permissible limits throughout the duration of the experiment (Kuśmierz et al., 2016). Likewise, in the studies by de Resende et al. (2018) and Rombolà et al. (2019), the PAHs content decreased to the level of the control soil after 3 to 5 years. The content of PAHs increased above that in the control soil (Rombolà et al., 2019) only when the soil was amended with biochar twice (year after year). In no case, however, were the European limits for PAHs content exceeded (de Resende et al., 2018; Rombolà et al., 2019). While de Resende et al. (2018) investigated the persistence of PAHs in tropical soil amended with biochars (16 t/ha) obtained from savannah wood at 350 °C or from eucalyptus at 450 °C, Rombolà et al. (2019) studied their persistence in vineyard soil amended with biochar (16.5 t/ha) produced from orchard pruning biomass (apple, grapevine, pear, and peach) at 500 °C. An increase in soil PAHs concentration immediately after biochar application was also observed by Quilliam et al. (2013). Similarly to the abovementioned studies, the soil content of Σ16 PAHs did not exceed the European threshold limits. In this study, biochar produced from rice husk or wood at 450 °C was applied at a rate of 25 or 50 t/ ha to a Eutric Cambisol with sandy clay loam texture.

To date, only a single study of bioavailable PAHs in biochar-amended soils has been reported, a field experiment conducted by Oleszczuk et al. (2016). This study evaluated changes in PAH C_{free} in soil amended with biochar (obtained from wheat straw at 650 °C) at rates of 30 and 45 t/ha for a duration of nearly two years. A significant decrease in PAHs C_{free}, which ranged from 26% to 36%, was recorded 105 days after biochar incorporation. No significant change was observed in subsequent years. At the completion of the experiment (after 851 days), PAHs C_{free} was lower by 40%–42% in soil with biochar relative to that in the control soil. However, PAHs degradation was observed to decrease in the presence of biochar, which may contribute to the increased persistence of PAHs in soil.

1.2.2. Heavy metals

Biochar is typically added to soils contaminated with metals in order to immobilize the contaminants (Ahmad et al., 2014; Rajapaksha et al., 2016; Vithanage et al., 2017). In recent years, biochar production from more controversial materials (e.g., waste or sewage sludge) has become increasingly common. In biochar produced from these feedstocks, metals present in the feedstock become more concentrated after pyrolysis (Zielińska and Oleszczuk, 2015), and consequently may cause environmental effects after biochar is added to soil. To date, research on this phenomenon has been scarce. de Figueiredo et al. (2019a) produced biochar from sewage sludge at temperatures of 300 or 500 °C and applied biochar to tropical soil at a rate of 0.7% (w/w). The addition of biochar did not change the bioavailable fraction (extracted with DTPA) of HMs (Pb, Cr, Cu, Mn, Zn, and Co) in the soil. Khanmohammadi et al. (2017) applied biochar produced from sewage sludge at 350 °C at a rate of 14.5 t/ha to two calcareous soils. The bioavailable fractions (extracted with DTPA) of Fe and Zn increased by 7.6%–8.6% and 21.0%–35.5%, respectively, in comparison to biochar-unamended soil. Biochar addition did not affect the bioavailable content of Cu and Mn but caused a decrease of 5.4%–22.3% in the bioavailable fraction of Pb. Méndez et al. (2012) produced biochar from sewage sludge at 500 °C. The biochar was added to soil (Haplic Cambisol, sandy loam) at two rates (4% and 8% based on mass). The experiment was carried out for 200 days. The addition of biochar to soil generally increased the bioavailable fraction content (DTPA and $CaCl_2$- methods). It was found that the DTPA extracted Cu content was from 1.5 to 1.6- times higher, Ni content was from 3.0 to 3.5- times higher, Pb content was from 1.6 to 1.8- times higher and Zn content was from 1.2 to 1.5- times higher compared to soil without biochar addition. However, Cd content was from 7% to 12% lower in soil with biochar compared to control soil. The content of HMs in soil with biochar based on the extraction with $CaCl_2$ was higher for all HMs cases than control soil. The $CaCl_2$- extracted fractions increased with biochar addition from 1.2 to 3.0- times, from 3.6 to 5.1-times, from 1.2 to 1.3- times, from 2.5 to 3.4- times and from 1.9 to 2.9- times in case of Cu, Cd, Ni, Pb and Zn respectively.

1.3. Toxicity of biochars

Tests on living organisms are an important part of research on the toxicity of materials. Such analysis is complementary to chemical analysis and therefore necessary to properly assess the risks involved in incorporating materials into the environment (Oleszczuk et al., 2013). By applying both chemical analysis and toxicological evaluation, comprehensive risk assessment as well as an analysis of relationships between biochar properties, such as the content of toxic compounds, and the toxic effects of biochar can be conducted.

Previous studies (Kavitha et al., 2018; Lehmann et al., 2011; Domene, 2016; Buss et al., 2016) have demonstrated that biochar can affect microorganisms, plants, and soil invertebrates differently. The toxic effects of biochar on organisms can be associated with the abovementioned toxic contaminants in biochar (Tables 1 and 3), but they can also result from the dose of biochar, physicochemical properties such as size of the particles of applied biochar (Prodana et al., 2019), high pH and/or salinity (Lehmann et al., 2011), strong capacity to adsorb nutrients and thus reduce their availability (Ren et al., 2018), and water retention capacity leading to impeded access to water for plants (Ren et al., 2018).

On the other hand, biochar can stimulate the growth and development of organisms due to its macro- and micronutrient content and the partial bioavailability of carbon contained in its matrix. Furthermore, owing to its porous structure (together with its nutrient content), biochar can provide excellent environment for the functioning of microorganisms and other small soil organisms (Anyika et al., 2014; Ren et al., 2018; Palansooriya et al., 2019). The positive impact of biochar on organisms has been described in many previous reviews (El-Naggar et al., 2019a; Lehmann et al., 2011; Domene, 2016; Palansooriya et al., 2019; Warnock et al., 2007); the following sections in this review focus on its toxic effects (Fig. 2). Toxicity is usually measured by liquid and solid phase tests. Liquid phase tests examine the effect of water extract from the studied material eg. biochar on organisms, while solid phase tests investigate the direct effect of studied material or the material mixed with solid matrix (e.g. artificial soil) to tested organisms.

1.3.1. Toxicity to microorganisms

Ecotoxicological tests involving microorganisms are commonly employed to assess the risk related to biochar use, since these organisms are widespread in soil and exert a great influence on the functioning of the entire ecosystem. Biochar may induce a positive effect on the reproduction of microorganisms (Palansooriya et al., 2019; Sun et al., 2013) by providing both protection and a source of food (Palansooriya et al., 2019; Hammer et al., 2014; Ennis et al., 2012). However, evidence pointing to negative impacts of biochar on soil microorganisms has also been reported (Anjum et al., 2014). It should be emphasized that the impact of biochar may significantly differ depending on the organism/strain. For example, in one study (Kavitha et al., 2018) a greater diversity of bacteria was observed in biochar-amended soil than in biochar-unamended soil. Nonetheless, in the case of fungi, no similar relationship was observed. In other studies (Kavitha et al., 2018; Sun et al., 2013), biochar (derived from corncob, temperature not given) promoted an increase in biomass of both bacteria and fungi after application to soil. Han et al. (2016) conversely found that biochar

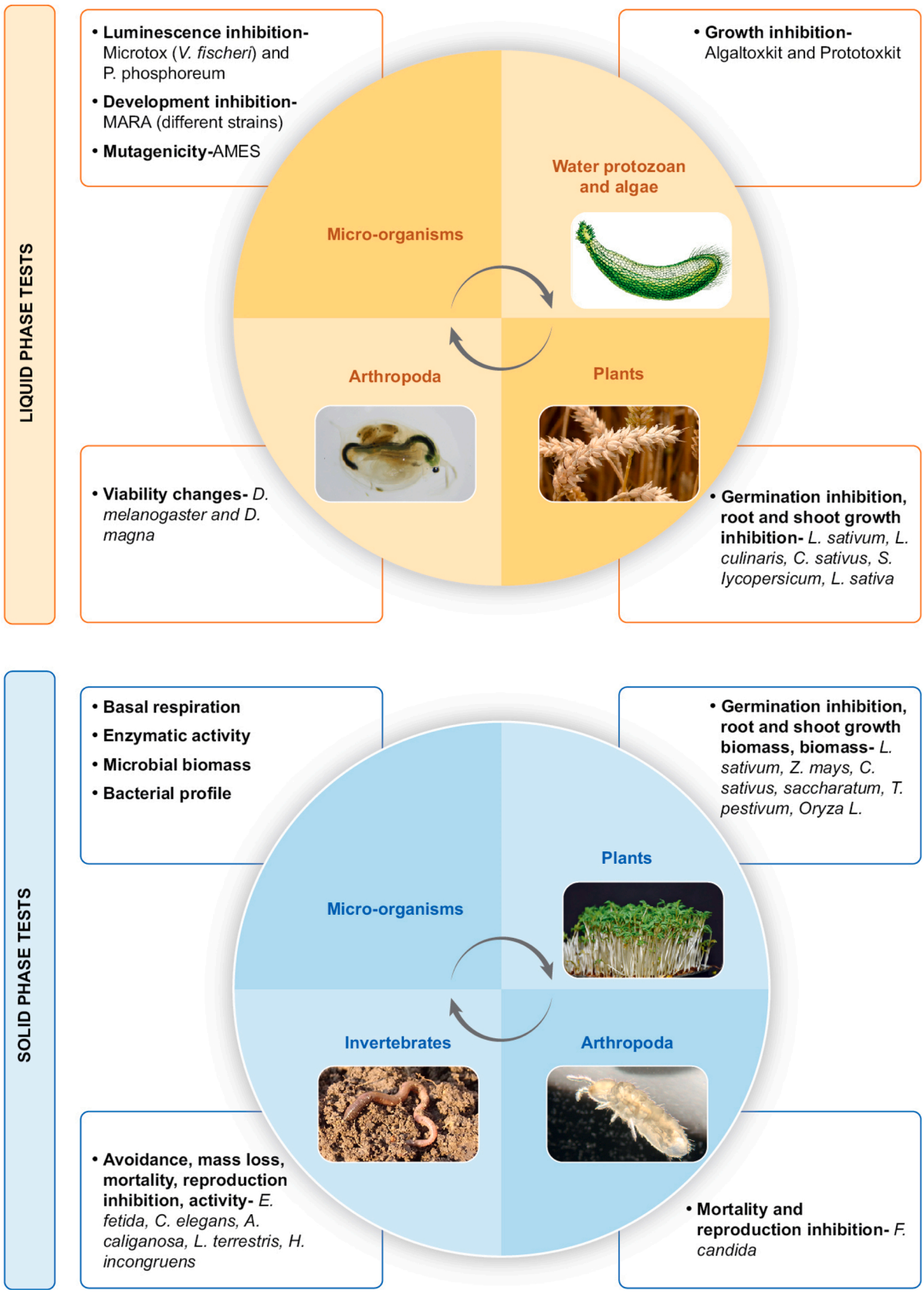

Fig. 2. Ecotoxicological methods and endpoints used for biochar toxicity assessment.

produced from pyrolyzed switchgrass (*Panicum virgatum* L.) at temperatures of 500 or 700 °C negatively impacted a colony of mycorrhizal fungi in soil (Han et al., 2016).

The above cited studies evaluated the numbers of organisms and their viability. There are, however, tests that facilitate the comparison of results between studies conducted by different research groups. The test using *Vibrio fischeri* bacteria, which exhibit bioluminescence, is one of the most popular ecotoxicological assays using microorganisms as test organisms. The decrease or disappearance of luminescence in the test solution relative to the control provides a measure of the toxic effect of a tested substance, extract, or mixture. Although this test is very common, few studies have employed it for the evaluation of the toxic effects of biochar. Gondek et al. (2016) studied extracts of biochars produced from a mixture of sewage sludge with wheat straw, bark, or sawdust at temperatures of 300 or 600 °C and tested their toxicity toward *V. fischeri*. In all cases, no toxic effect was observed. Nevertheless, a number of diverse negative impacts of biochar extracts on *V. fischeri* have been reported. Lyu et al. (2016) produced biochars from pine tree wood sawdust at temperatures between 250 and 700 °C. The half maximal effective concentration (EC_{50}) associated with luminescence inhibition ranged from 0.39 to 6.00 g/L, with the greatest toxic effects resulting from biochar produced at 300 °C. In the 400–700 °C temperature range, the toxicity decreased. Lyu et al. (2016) explained the high toxicity of biochars produced at low temperatures by noting their higher content of 5- and 6-ring PAHs compared to biochars produced at higher temperatures. Moreover, they did not exclude the impact of other substances (e.g., phenols) that can form during pyrolysis at a low temperature (Buss et al., 2015). The study investigated the toxicity of aqueous extracts of four biochars produced from *Miscanthus*, coconut shell, wicker (*Salix viminalis*), and wheat straw (*Triticum L.*) at 650 °C (Oleszczuk et al., 2013). *Miscanthus* biochar exhibited the highest toxicity, causing almost complete luminescence inhibition (99%). Biochar made from wheat straw was also moderately toxic toward the test bacteria (85%), whereas biochar obtained from wicker was less toxic, inhibiting less than half of the luminescence (40%). Biochar derived from coconut shell was the least toxic, inhibiting only 12% of the *V. fischeri* luminescence (Oleszczuk et al., 2013). The same biochars were subsequently subjected to a post-treatment that comprised drying at 100, 200, and 300 °C (Kołtowski and Oleszczuk, 2015). A distinct decrease in toxicity with increased biochar drying temperature (and thus a decreased PAH concentration in biochar) was observed in the case of *Miscanthus* biochar. The toxicity of biochar made from wheat straw increased with increasing drying temperature, despite the observed decrease in PAH content. This effect was attributed to the possible presence of other toxic substances that re-condensed on biochar during the drying process. Moreover, due to the drying, the content of carbon, whose presence reduces the bioavailability of harmful substances, decreased, which could also have caused a toxic effect (Kołtowski and Oleszczuk, 2015). Stefaniuk et al. (Stefaniuk et al., 2016) prepared aqueous extracts of biochars produced from residues from biogas production (RBP) at 400–800 °C and studied their effects on *V. fischeri*. The toxicities of the extracts toward *V. fischeri* varied depending on the origin of the feedstock used to produce biochar. The extract of biochar obtained from non-separated RBP under mesophilic conditions proved to be the most toxic. All biochars exhibited enhanced toxicity as the pyrolysis temperature increased; notably, biochar produced at the lowest applied temperature (400 °C) was the most toxic, causing bacteria luminescence inhibition of more than 90%. Elevated hydrophobicity with increasing pyrolysis temperature, which leads to increased affinity of the biochar surface for organic substances and reduces their mobility and hence toxicity, may be responsible for this effect.

Zhang et al. (2019a) produced biochar from pine needles (*Pinus massoniana Lamb*) at different temperatures in the range of 300–600 °C and investigated its effect on the luminescence of the bacterium *Photobacterium phosphoreum*. Biochar was applied at rates between 125 and 2000 mg/L. Higher luminescence inhibition was observed with increasing biochar concentration as well as increasing pyrolysis temperature from 300 to 500 °C. Zhang et al. (2019a) suggested that FRs occurring in biochar were the source of the toxic effect.

Another ecotoxicological test that uses bacteria is a less popular assay called microbial assay for risk assessment (MARA) (Wadhia, 2013). This assay examines the growth of 11 bacterial strains in the presence of a potentially toxic agent. The MARA test was used (Oleszczuk et al., 2013) to evaluate the toxicity of aqueous extracts of biochars obtained from wicker, wheat straw, *Miscanthus*, and coconut shell. The test showed no significant differences between the individual biochars. Nonetheless, particular strains were observed to exhibit varying sensitivity to the biochars tested, which confirmed a previous field study wherein biochars had also been found to have a diverse impact on soil microbiology (Oleszczuk et al., 2014). Coconut shell biochar was the most toxic (causing a toxic effect on all test strains), particularly toward the strains of *Enterococcus casseliflavus*, *Serratia rubidaea*, and *Pichia anomalia* (Oleszczuk et al., 2013).

Biochar has also been investigated for its genotoxicity, cytotoxicity, and mutagenicity. Using the AMES test (the mutagenicity test using *Salmonella typhimurium* strains), Anjum et al. (2014) studied aqueous extracts of biochar produced from hemp bedding or commercial wood pellets at 500 °C. Although both extracts showed mutagenic properties, the hemp bedding extract was more mutagenic. The observed toxicity was attributed to the high PAHs content in biochars. Piterina et al. (2017) used the AMES test to investigate the mutagenicity of dimethylsulfoxide extracts (1:2, v/v) of biochars produced from pig manure, cow manure, calf manure, sawdust, *Miscanthus*, or solid municipal waste at temperatures ranging from 400 to 800 °C. Pig manure biochar exhibited the highest mutagenicity (54% mutagenic potency) among the biochars tested. Biochars derived from *Miscanthus* and sawdust were the least mutagenic (1% mutagenic potency).

Busch et al. (2013) studied hydrochars obtained from maize silage, food leftovers, cut grass, sewage sludge, and digestates from a biogas plant at 170–230 °C as well as biochars produced from food leftovers, digestates from a biogas plant, olive residues, poplar wood chips, wheat straw, and *Miscanthus* at 400–1000 °C. To evaluate their mutagenicity, the *Tradescantia* micronucleus test was applied: chromosomal aberrations in *Tradescantia* pollen cells in the form of micronuclei were microscopically evaluated after exposure to extracts of hydrochars and biochars. Pollen cells of *Tradescantia* flowers were also examined to determine the number of micronuclei after hydrochar or biochar exposure. Hydrochars from hydrothermal carbonization generally exhibited negative results. In the biochar experiments, a genotoxic effect was only observed for the extract of *Miscanthus* biochar, which was also found to have an enhanced concentration of naphthalene.

Lyu et al. (2016) applied the AhR agonistic activity test using the H4IIE-luc cell line to study the mutagenicity of biochars produced from pine tree sawdust at 250–700 °C. The H4IIE bioassay measures the catalytic activity of the cytochrome P4501A (CYP1A), a mixed-function oxidase (MFO) enzyme, as 7-ethoxyresorufin-O-deethylase (EROD) activity in cultured rat liver cells exposed to environmental extracts. EROD is induced and the H4IIE bioassay is consequently useful for characterizing the presence of certain PAHs and related compounds (e.g., nitrogen heterocyclics as well as sulfur-, oxygen-, nitro-, amino-, and alkyl-substituted PAHs) and polyhalogenated hydrocarbons (e.g., PCDD/Fs, PCBs, and naphthalenes) in environmental samples. Biochar produced at 300 °C exhibited the most potent AhR agonistic ability, followed by biochars produced at 250 and 400 °C. Above 400 °C, toxicity decreased with increasing temperature. Exposition of biochar produced at 700 °C to the extract did not cause any significant induction of AhR-mediated expression of the reporter gene. The TEQ concentrations determined by the H4IIE-luc assay were higher than those calculated based on the analytical data. This indicates that there are other AhR ligands produced in addition to PAHs and PCDD/Fs during pyrolysis, and they need to be identified.

Liu et al. (2019b) studied the effect of ball milling of biochar on its

cytotoxic properties toward *Streptomyces coelicolor* M145. Biochar produced from a poplar tree feedstock at 500 °C was ball milled; the hydrodynamic diameters of pristine and ball-milled biochar were 1829 ± 114.6 and 490.5 ± 35.5 nm, respectively. Pristine biochar applied at a rate of 10 mg/L had no toxic effect toward *S. coelicolor*. After ball milling, the physicochemical properties of biochar changed significantly, which according to Liu et al. (2019b) resulted in cell damage. Application of ball-milled biochar stimulated antibiotic production; the highest rate (10 mg/l) caused massive cell disruption and the survival rate of *S. coelicolor* cells was only 68.2% (Liu et al., 2019b).

1.3.2. Aqueous algae and protozoa

Several studies have examined the effects of aqueous biochar extracts on algae and protozoa. Understanding the toxicity of biochar toward aquatic organisms is important since contaminants may be leached from biochar-amended soil into water, potentially leading to negative impacts on organisms there. Oleszczuk et al. (2013) investigated the toxicity of four biochars produced from coconut shell, willow, wheat straw, and *Miscanthus* by employing two tests: Algaltoxkit (chronic toxicity microbioassay: a 72 h growth inhibition test based on the green algae *Selenastrum capricornutum*, also called *Raphidocelis subcapitata* or *Pseudokirchneriella subcapitata*) and Protoxkit (chronic toxicity microbioassay: a 72 h growth inhibition test based on the protozoa *Tetrahymena thermophila*). Biochar derived from coconut shell had the greatest negative effect on the test organisms, whereas *Miscanthus* biochar exhibited the lowest toxicity toward algae and protozoa. Oleszczuk et al. (2013) did not indicate a potential toxic factor. Zhang et al. (2019a) studied biochar produced from pine needles (*Pinus massoniana Lamb*) at various temperatures ranging from 300 to 600 °C and its effect on the growth and chlorophyll content of the algae *Scenedesmus obliquus*. The concentration of biochar in the water ranged from 50 to 800 mg/L. The EC_{50} values determined for *S. obliquus* growth were 133.6, 72.1, 60.6, and 80.6 mg/L for biochars produced at 300, 400, 500, and 600 °C, respectively. Biochar produced at 500 °C had the greatest toxic effect on the chlorophyll content.

1.3.3. Phytotoxicity

The majority of studies on biochar address its effects on plant growth and development. The toxicity of biochar toward plants is investigated by exposing organisms to both biochar leachates and biochars in the solid phase (usually mixed with soil). Leachates enable the determination of biochar's direct toxicity as well as the potential impact of leaching various biochar constituents. Solid phase tests, on the other hand, are a better approximation of real conditions in the environment, although the effect of biochar on plants may vary depending on the type of soil.

Biochar toxicity toward plants often varies substantially depending on the feedstock from which biochar is manufactured, pyrolysis temperature (and other process parameters), and biochar application rate. Benavente et al. (2018) prepared aqueous extracts of biochar produced from the organic fraction of urban waste at 300 °C (with a residence time of 1 or 5 h) or 500 °C (with residence time of 1 h) and measured their effects on germination of *Lepidium sativum*. Biochars produced at 300 °C exhibited a toxic effect that increased when the residence time during pyrolysis was extended (the germination index (GI) was 40% and 62%, respectively, for biochar produced from 1 and 5 h pyrolysis). In contrast, biochar produced at 500 °C stimulated *L. sativum* germination. This effect occurred owing to the decreasing content of soluble organic carbon, volatile matter, and toxic PAHs in biochars produced at higher temperatures. Gondek and Mierzwa-Hersztek (n.d.) studied aqueous extracts of biochars produced from sewage sludge at 300 °C and their effect on the growth of *L. sativum* roots. Two of the biochar extracts had no effect on root growth, while one extract caused a toxic effect that manifested in a 25% root growth inhibition. No significant relationship between toxicity and content of Cd, Cu, Pb and Zn in biochar (determined by a 24-h extraction of a sample with redistilled water) was observed.

Oleszczuk et al. (2013) reported varying impacts of feedstock on biochar phytotoxicity. They evaluated the toxicity of biochars obtained from *Miscanthus*, wicker, coconut shell, and wheat straw. At all application rates tested (1%, 5%, and 10%), wicker biochar stimulated the growth of *L. sativum* roots. Coconut shell biochar applied at rates of 1% and 5% also stimulated root growth, but application at the higher rate of 10% caused a toxic effect. The other two biochars inhibited root growth by 33% and 48% when applied at rates of 5% and 10%, respectively, while a slight stimulating effect was observed for the application rate of 1%.

Stefaniuk et al. (2016) compared the phytotoxicity of aqueous and solid phase extracts of biochar (applied at rates of 0.5% and 5.0% in OECD soil) toward *L. sativum*. Differences were observed depending on the type of feedstock used to produce biochar, the feedstock preparation method (separation into solid and liquid phases vs. no separation), and the pyrolysis temperature. Biochar produced from RBP and applied at a rate of 5% inhibited germination and root growth. In contrast, biochars produced at 400 and 600 °C and applied at the lower rate of 0.5% stimulated *L. sativum* root growth. Liquid-phase toxicity analysis confirmed observations of high biochar toxicity in the solid phase. Undetermined components leached from biochar were the main source of the toxic effects. For the other biochars studied, a trend was observed wherein an increase in pyrolysis temperature led to elevated biochar toxicity. Biochar obtained from separated RBP under mesophilic conditions stimulated root growth regardless of the pyrolysis temperature and the rate applied. The negative impact of biochar on *L. sativum* growth was attributed to the presence of both PAHs and HMs in biochar as well as its high salinity (>19.4) and pH (>10.8). Gascó et al. (2016) investigated the toxic effects on plants (*L. sativum*, *Lens culinaris* Medikus, *Cucumis sativus*, *Solanum lycopersicum*, and *Lactuca sativa*) of aqueous extracts of biochars obtained from mixed wood sievings (at a temperature of 620 °C), a mixture of paper sludge and wheat husks (500 °C), and sewage sludge (600 °C). The effect of biochar was observed to vary depending on the plant species: all biochars stimulated the growth of *L. culinaris*, whereas wood biochar had a toxic effect on *L. sativum, C. sativus*, and *S. lycopersicum*. The highest toxicity observed (a GI of 49%) was exhibited by biochar from paper sludge and wheat husks toward *L. sativa*. According to Gascó et al. (2016), the toxic effect was caused by high pH and the presence of HMs in biochar.

The toxic effects of biochar can be significantly reduced using an appropriate application rate. Application of biochar at excessively high rates is the main reason for the commonly observed toxic effects of biochar on plants. It is accepted that realistic biochar application rates, i.e., <1%, do not usually cause toxicity toward plants (Lehmann and Stephen, 2009). Intani et al. (2019) studied the toxicity toward *L. sativum* of biochar produced from corncob at 450 °C and added to soil at rates of 10, 20, and 30 t/ha (~1%). While the lowest biochar application rate did not significantly influence the germination rate (GR), the higher rates led to toxic effects (GR was 79% and 30%, respectively, for rates of 20 and 30 t/ha). Biochar post-treatments of heating to 105 °C or washing with deionized water resulted in reduced biochar toxicity; washing with water was more effective than heating for reducing the toxic effects. Aqueous extracts of pristine biochar were also subjected to the test with *L. sativum* and were found to cause similar effects on plants as the solid phase of pristine biochar. Intani et al. (2019) suggested that water-soluble phenols, organic acids, ketones, and alcohols as well as the soluble fraction of PAHs may have been responsible for the toxicity. The salinity of biochar and its content of volatile compounds were also indicated as factors that could have negative impacts on plants. Visioli et al. (2016) produced gasified biochars from conifer wood, poplar wood, grape marc, and wheat straw and studied their effects on the growth of *C. sativus*, *L. sativum*, and *S. saccharatum* at application rates ranging from 0.5% to 50%. Biochar from grape marc exhibited the greatest toxic effects, but significant toxicity was only seen at a rate of 10%, which is higher than typical rates in the environment. Biochar

produced from conifer wood and poplar wood had the lowest toxicity toward all plants studied. The reason for biochar's toxic activity could have been the presence of HMs (but not PAHs) as well as its salinity and pH, similar to previous studies (Visioli et al., 2016). Liao et al. (2014) studied the effects of corn biochar (500 °C) at different application rates (from 0.03% to 0.36%) on germination as well as the shoot and root length of *Z. mays*, *T. aestivum*, and *Oryza* L. Increase of the application rate led to increased toxicity. Corn and wheat growth were stimulated by low application rates (0.03% and 0.06%). Liao et al. (2014) suggest that this stimulation was associated with the incorporation of additional micro- and macronutrients in the soil with biochar. Application rates above 0.06% caused plant germination and growth inhibition. Potential toxic effects caused by HMs and PAHs were ruled out based on an investigation of the content of these contaminants in biochar. Ultimately, FRs present in biochar were concluded to be responsible for the observed toxicity (Liao et al., 2014).

Rogovska et al. evaluated the effects of aqueous extracts of biochars of different origin on *Zea mays* germination and growth. Biochars were produced from hardwood (450–500 °C) or corn (500 or 732 °C) via fast pyrolysis, from hardwood (500 °C) via slow pyrolysis, and from switchgrass (850 °C) or corn (845 °C) via gasification (Rogovska et al., 2012). Extracts of all types of corn biochars exhibited a toxic effect (shoot length inhibition), which was attributed to PAHs or other potentially phytotoxic organic substances occurring in biochars. The plant toxicity was also evaluated based on the length of the radicle (the embryonic root of the plant, growing downward in the soil). A toxic effect was observed for an extract of corn biochar produced at 500 and 845 °C. Biochar produced using fast pyrolysis promoted radicle growth, which was rationalized by the higher content of nutrients in the biochar extract relative to deionized water. Free et al. (2010) investigated the effect of biochar produced at 550 °C from biosolid, corn stover, eucalyptus, fresh pine, or willow on *Z. mays* growth. Biochars were applied at a rate of 10 t/ha in two different sandy soils; biochar amendment did not affect the seed germination, coleoptile and root dry weight, or coleoptile length of *Z. mays*. Busch et al. (2013) produced hydrochar from maize silage (230 °C) and biochar from poplar wood chips and investigated their effects on the growth of *Z. mays* and *Brassica rapa*, adding the hydrochars and biochars to a standard soil (unfertilized turf-based substrate) at rates ranging from 1.25% to 25% (v/v). Hydrochar addition at rates above 2.5% caused inhibition of germination and biomass growth. Addition of biochar to the soil at a rate of 1.25% did not significantly affect the test plants, while rates ≥5% stimulated plant growth (measured as a greater amount of biomass compared to the control).

Phoungthong et al. (2018) studied the toxicity toward *Triticum aestivum* of extracts of sewage sludge biochars produced at 300 to 900 °C, using deionized water, H_2SO_4/HNO_3 solution (60:40 w/w), and acetic acid (HAc) as extractants. The HAc solution used in the assay simulated municipal sewage sludge, whereas the H_2SO_4/HNO_3 solution simulated acid rain. The toxicities of the aqueous and acid extracts were similar, while the GI values differed between the extracts depending on the biochar production temperature. The extracts of biochar produced at 500 °C, which stimulated germination, were the least toxic, while the extracts of biochar produced at 400 °C were the most toxic (GI of 65%) (Phoungthong et al., 2018). Kong et al. (2019) prepared aqueous extracts of sewage sludge biochars produced at various temperatures ranging from 300 to 700 °C and studied their effect on seed germination as well as root and shoot length of *T. aestivum*. The aqueous extracts were obtained using different biochar/distilled water ratios (1:70 and 1:7 w/v). The extracts did not influence seed germination, whereas root growth inhibition was enhanced for biochars produced at higher pyrolysis temperatures (from 5.98% to 18.32%). In contrast, the inhibition of shoot growth was similar (about 8%) regardless of the pyrolysis temperature. The toxic effect of the biochar extracts was linked to the presence of HMs in biochars.

1.3.4. Toxicity toward Arthropoda

Collembolans, common soil organisms from the phylum Arthropoda, are a frequent subject of ecotoxicological studies in soils (Domene, 2016), but fewer studies have examined these organisms in the context of biochar toxic effects. The activity of Collembolans is associated with organic matter transformation (Domene, 2016). Conti et al. (2018) investigated the toxicity of gasified biochars toward *Folsomia candida* and found that among the biochars studied (produced from conifer wood, poplar wood, grape marc, and wheat straw), only grape marc biochar applied at rates ranging from 10% to 50% (w/w) was toxic toward *F. candida* (100% mortality). The negative impact of biochar on reproduction was more varied but less adverse. The values of half maximal effective concentration (EC_{50}) calculated for individual biochars were 11.2%, 6.2%, 0.2%, and 19.0% (w/w) for biochars produced from conifer wood, poplar wood, grape marc, and wheat straw, respectively. The results of an avoidance test (Conti et al., 2018) obtained for the same experimental design confirmed the previous observations. Biochar from grape marc ($EC_{10} = 3.8\%$) again proved to be the most toxic, while the EC_{10} values for the other biochars ranged between 5.3% and 8.1%. According to Conti et al. (2018), the negative effect of biochars on *F. candida* was caused by (1) their pH (in the cases of avoidance, survival, and reproduction), (2) their HM content (in the case of reproduction), and (3) their PAH content (in the case of reproduction).

In recent years, the use of various wastes for biochar production has been extensively explored (Sun et al., 2017). Waste material requires thorough ecotoxicological analysis due to the possible environmental risk associated with an elevated content of HMs (Mierzwa-Hersztek et al., 2018) and a generally higher content of other organic contaminants (Chen et al., 2019). Stefaniuk et al. (2016) investigated the effects of biochar produced from RBP on mortality and reproduction of *F. candida*. Biochar from non-separated RBP under mesophilic conditions had the greatest toxic effect, which was also confirmed for other test organisms employed in this study (Stefaniuk et al., 2016). Depending on the pyrolysis temperature used to produce biochar, the *F. candida* mortality ranged from 20% to 50% and reproduction inhibition ranged from 70% to 100%. The toxic effect increased with the pyrolysis temperature. The toxicity toward *F. candida* was attributed to the high pH of biochars as well as their high salinity and PAH content (Stefaniuk et al., 2016). Raclavská et al. (2018) studied the toxicity of biochars produced from beverage cartons (Tetrapak) at 400–700 °C and found that *F. candida* reproduction inhibition ranged from 12% to 90% depending on the application rate of biochar (0.5%–100%). Increase of the biochar application rate led to increased toxicity. Biochars produced at 500 and 600 °C were the most toxic toward *F. candida*.

Other less common ecotoxicological tests are also applied to test biochar toxicity. Yang et al. (2019) studied the effect of biochar on the viability of *Drosophila melanogaster*. Aqueous extracts (1:10, w/v) were prepared from biochars produced at 200 or 500 °C from vegetable waste, pine cones, or a mixture of vegetable waste and pine cones (1:1). The extracts were diluted to obtain biochar concentrations of 1.5, 3, and 5 mg/mL. Depending on the type of substrate used to produce biochar and the extract dilution, biochars had varying impacts on the viability of *D. melanogaster*. The extracts with the lowest concentration of biochar obtained from vegetable waste or pine cones slightly stimulated the viability of *D. melanogaster* (regardless of the pyrolysis temperature). The extract of biochar produced from the mixture of vegetable waste and pine cones exhibited a toxic effect at the lowest biochar concentration. A slightly different effect was observed for the higher biochar concentrations: biochars derived from the single substrates were more toxic than those obtained from their mixture. No relationship was found between the toxic effect and HM and PAH contents in biochars. It was suggested that other toxic substances (such as VOCs, other PAHs, and/or dioxins unidentified in the study) could be responsible for the toxic activity. However, if this were the case, one would expect an increase in toxic effect with increasing biochar application rate, which was not observed

for biochars obtained from the waste mixtures.

Aqueous extracts of biochars derived from various feedstocks were also evaluated for their toxicity toward *Dapnia magna* (Oleszczuk et al., 2013). The commercially available test Daphtoxkit F, which is based on immobility or mortality of the test organisms during 48 h in accordance with OECD Guideline 202 and ISO 6341, was used in this study. All raw biochar extracts caused 100% mortality of the test organisms. Differences between biochars were observed only after 10-fold dilution of the extract. *Miscanthus* biochar was the most toxic, causing mortality in nearly 80% of individuals. The toxicity of the 10-fold diluted extracts of biochars produced from wicker and wheat straw ranged from 18% to 28%. The 10-fold diluted extract of biochar obtained from coconut husks caused no toxic effect on *D. magna*. A relationship was established between the extent of biochar toxicity toward *D. magna* and the biochar PAH content.

1.3.5. Toxicity toward invertebrates

Earthworms are an important element of the soil ecosystem (Domene, 2016). They transform soil organic matter and mix it with mineral particles, aerate soil, and influence other soil organisms (Nahmani et al., 2007; Hirano and Tamae, 2011; Li et al., 2011; Blouin et al., 2013). Biochar toxicity toward earthworms has been previously studied (Lehmann et al., 2011; Domene, 2016; Ren et al., 2018; Weyers and Spokas, 2011). Nevertheless, the large majority of studies are conducted in contaminated soils in which biochar is used as a toxicity-reducing agent or in natural soils. Relatively few studies exist concerning the use of OECD soil as an inert matrix in tests with earthworms. Such tests allow the comparison of studies conducted in a variety of media. They also facilitate the intercomparison of biochars, which is frequently problematic when a natural soil is used in a study, due to the complicated nature of the soil containing other elements that may also cause toxic effects.

An important factor in determining the toxic or stimulating effect of biochar is its application rate. Biochar added to soil at high rates can be toxic to earthworms (Weyers and Spokas, 2011) due to (1) a change in the pH of the environment, to which earthworms are sensitive; (2) physical injuries arising from the impact of a dry material on animal systems; and (3) bioavailable contaminants present in biochar. In a study by Li et al. (2011), an avoidance test was conducted on *Eisenia fetida* based on the fact that organisms have the ability to avoid unfavorable conditions. Biochar produced from apple wood at 525 °C was mixed with artificial soil at application rates of 1%, 10%, and 20%. Earthworms avoided soil amended with biochar at application rates of 10% and 20%, but no avoidance effect was observed for the lowest rate of 1%. Moreover, *E. fetida* earthworms were characterized by a lower weight in soil with a 10% and 20% rate of biochar addition than that of *E. fetida* in the control soil. A biochar application rate of 1% did not result in weight loss, which is consistent with the avoidance test results. Li et al. (2011) suggested that biochar's high WHC, which reduced water availability and desiccated earthworms leading to a toxic effect, was responsible for the avoidance of biochar by earthworms. In soil with pre-wetted biochar, no differences were observed between the control soil and biochar-amended soil (Li et al., 2011). Zhang et al. (2019b) studied the toxicity toward *E. fetida* of biochar produced from wheat straw at 500 °C and added to OECD soil at rates ranging from 1% to 10% (w/w DM) over 28 days. *E. fetida* mortality did not differ significantly between the soil with biochar and the control soil without biochar. In soil amended with biochar at low rates (1%–3%), the earthworm weight was even observed to increase, whereas the 10% application rate caused a decrease in the earthworm weight, compared to that in the control. Biochar was not found to affect *E. fetida* reproduction.

Lieke et al. (2018) investigated the effect on the behavior of *Caenorhabditis elegans* of biochar produced from rice straw at 500 °C and applied at four rates ranging from 250 to 2000 mg carbon/L. Biochar applied at rates between 1000 and 2000 mg carbon/L had a neurotoxic effect on *C. elegans*, causing decreased locomotion, frequency of defecation, and chemical sensory ability. In contrast, the lowest application rate (250 mg carbon/L) stimulated the movement of the organisms. The observed negative effect on *C. elegans* was explained by the presence of FRs in biochars.

1.4. Toxicity of biochar-amended soil

When biochar is applied to standardized OECD soil in an experiment, the matrix effect can be determined and biochar toxicity can be compared across studies; when biochar is added to other soils, comparison of results between different research groups is difficult because the matrix effect can significantly influence potential biochar toxicity. Nonetheless, regardless of the method used, research on the toxicity of biochar-amended soils is important for understanding the toxicity of biochars derived from various feedstocks or under different temperature or carrier gas conditions.

1.4.1. Toxicity toward microorganisms and arthropods

Microorganisms are a necessary element of the soil environment, decomposing organic matter and leading to soil enrichment with plant-available nutrients (Kavitha et al., 2018). Microorganism populations in agricultural soil, which requires a suitable level of fertility, are particularly important. Most studies investigating the effects of biochar on microorganisms focus on evaluating the direct impact of biochar on the biological environment (number and structure of microorganisms, enzymatic activity, etc.) (Palansooriya et al., 2019). Only a few studies have evaluated the toxicity of biochar-amended soil using the above-mentioned classical ecotoxicological tests (e.g., Microtox®, MARA, and AMES).

Mierzwa-Hersztek et al. (2016) studied the effects of biochar produced from poultry litter at 300 °C, added to soil (Eutric Cambisols, loamy sand) at a rate of 2.25 or 5 t/ha. The toxicity of aqueous extracts toward *V. fischeri* was evaluated using the Microtox® test. Biochar-amended soil was found to be less toxic toward *V. fischeri* than biochar-unamended soil. Increase in the biochar application rate further reduced the soil toxicity. Hale et al. (2013) observed a similar effect, investigating the impact on toxicity toward *V. fischeri* of soil amended with biochar (applied at rates ranging from 0.5% to 5.0%) produced from corn stover at 600 °C. They found that the toxicity of the aqueous soil extracts decreased with increasing biochar application rate. The decrease in toxicity may have resulted from biochar-induced immobilization of certain compounds present in the soil. Prodana et al. (2019) added pine wood biochar to soil and observed no negative impact on its toxicity toward *V. fischeri*. To date, only the study by Zhang et al. (Mierzwa-Hersztek et al., 2018), which investigated the effect of sewage sludge biochar on soil toxicity toward *V. fischeri*, found evidence of a toxic effect, measuring luminescence inhibition from 31% to 50% compared to the control soil.

More studies have been conducted regarding the effects of biochar on the number and structure of organisms in soil. This topic has been discussed in previous studies (Gondek et al., 2016; Palansooriya et al., 2019; Zhu et al., 2017; Gul et al., 2015). In short, biochar contributes to increased activity, diversity, and occurrence of microorganisms in soil (Maienza et al., 2017; Palansooriya et al., 2019; Benavente et al., 2018; Gomez et al., 2014; Li et al., 2016; Paz-Ferreiro et al., 2015a; El-Naggar et al., 2020), as a result of its various physicochemical properties. It was observed (Li et al., 2019) that in soil amended at a rate of 1% with biochar produced from maize straw at 500 °C, the ratio of the number of gram-positive to gram-negative bacteria was nearly twice that in biochar-unamended soil. Gram-negative bacteria are more sensitive and hence less resistant to environmental stress compared to gram-positive bacteria. Moreover, gram-positive bacteria process several persistent carbon-rich substances (including PAHs), which may stimulate their growth in biochar-amended soils. Gomez et al. (2014), however, observed an opposite effect. In soil amended with biochar (produced from wood at 550 °C) at rates of 5%–10%, gram-negative bacteria

predominated over gram-positive bacteria and fungi. The bacterial profile and its response to the presence of biochar depend on specific biochar properties as well as on soil properties and environmental conditions. Li et al. (2016) observed an increase in soil microbial biomass carbon from 14% to 62% after biochar application, de Figueiredo et al. (2019b) observed increased (33%) soil microbial biomass carbon in soil with sewage sludge-derived biochar addition. Paz-Ferreiro et al. (2015a) determined microbial biomass in soil with four biochars (sewage sludge- derived in 600 °C, deinking sludge- derived in 600 °C, Miscanthus- derived in 450 °C and obtained during gasification in 800 °C from wood). The addition of three biochars (sewage sludge, deinking sludge and Miscanthus- derives) increased soil microbial biomass nearly 2- fold. In other studies biochar did not significantly influence soil microbial biomass (Abujabhah et al., 2016; Giagnoni et al., 2019). de Figueiredo et al. (2019b) also observed increase in the colonization of corn roots (mycorrhizal fungi) by 20 percentage points after adding sewage sludge- derived biochar produced in 300 °C to soil and by 12 percentage points after adding sewage sludge- derived biochar produced in 500 °C.

Basal respiration is a basic test used to evaluate soil microbiological activity. The effect of biochar on soil respiration depends on the biochar type (Šlapáková et al., n.d.). Amendment with some types of biochar contributes to increased basal respiration (Ennis et al., 2012; Mierzwa-Hersztek et al., 2018; Paz-Ferreiro et al., 2015a), meaning that biochar added to soils enhances the effectiveness of organic matter decomposition processes. However, this stimulation effect only lasts 1–12 months (Piterina et al., 2017) and thereafter the rate of decomposition returns to that of the control soil. An excessively high level of soil basal respiration is not desirable due to increased soil CO_2 emissions. One study suggests higher CO_2 emissions are generated from soils amended with low-temperature biochar compared to those from soil amended with high-temperature biochar (Ennis et al., 2012). This is due to the higher content of water-soluble carbon in low-temperature biochar.

Enzymatic activity is not a typical ecotoxicological test, but it can provide information similar to classical ecotoxicological tests, i.e., it allows us to determine the impact of various factors on different groups of organisms responsible for specific soil processes. Biochar is thought (Zhu et al., 2017; Gul et al., 2015) to change enzymatic activity because it adsorbs extracellular enzymes on its surface, it is a source of soluble carbon, and it affects the bioavailability of nutrients (by increasing pH) necessary for microorganism growth and development. Moreover, due to its specific structure, biochar provides optimal living environment for microorganisms (Palansooriya et al., 2019; Mierzwa-Hersztek et al., 2017). Nonetheless, divergent results are present in literature regarding the impact of biochar on enzymatic activity. Giagnoni et al. (2019) observed varying effects of biochar on the activity of arylesterase, arylsulfatase, alkaline phosphomonoesterase, phenol oxidase, phosphodiesterase, cellulase, acid phosphomonoesterase, β-glucosidase, β-galactosidase, urease, and protease. The activity of arylesterase, arylsulfatase, alkaline phosphomonoesterase, phenol oxidase, and phosphodiesterase increased after double application of biochar, whereas the activity of cellulase, acid phosphomonoesterase, β-glucosidase, β-galactosidase, urease, and protease decreased upon biochar addition. The increased activity of phenol oxidase is attributed to the fact that this enzyme is produced by microorganisms capable of degrading aromatic substances (such as PAHs), which are often components of biochar (Giagnoni et al., 2019). In general, changes observed in the enzymatic activity of biochar-amended soil are associated with changes in the availability of building blocks and nutrients (aromatic C as well as N and P) and may also be related to various interactions of soil organisms. Wang et al. (2015) observed that a biochar application rate of 0.5% increased soil enzymatic activity of C-cycling enzymes (b-D-cellobiosidase, b-glucosidase, and a-glucosidase), one C- and N-cycling enzyme (N-acetyl-b-glucosaminidase), and one S-cycling enzyme (sulfatase), whereas higher rates ranging from 1.0% to 5.0% had a different effect. The decrease in activity was caused by excessive biochar porosity and the reactive surface area. In the case of N-cycling enzymes (leucine aminopeptidase and urease), the enzyme activity was enhanced with increasing biochar application rate. On the contrary, Mierzwa-Hersztek et al. (2016) did not observe a significant effect of biochar on enzymatic activity, though they found that dehydrogenase and urease activity was higher for a biochar application rate of 5 t/ha than that for 2.25 t/ha. Apart from the biochar application rate, the conditions in which biochar is produced also influence enzymatic activity. Benavente et al. (2018) observed that biochars produced from urban waste at 300 °C caused an increase in phosphomonoesterase and dehydrogenase activity, regardless of the pyrolysis duration (1 or 5 h), while biochar produced at 500 °C (1 h pyrolysis time) caused a significant decrease in soil dehydrogenase activity. The reduced activity was attributed to the immobilization of nutrients (C and P) in biochar produced at 500 °C, leading to a decrease in enzyme production by soil microorganisms. Paz-Ferreiro et al. (2012) studied the effect of sewage sludge- derived biochar produced in 600 °C on the enzymatic activity of soil. Biochar was added to soil at the rate of 4% or 8% based on weight. The activity of dehydrogenase was higher in treatments with biochar compared to control soil, while the activity of b-glucosidase decreased. The activity of phosphomonoesterase and arylsulphatase was not affected by biochar addition to soil. In different study from Paz-Ferreiro et al. (2015b) biochar from poultry litter produced in 400 °C was incubated with soil (Haplic Acrisol) for 4 months. Biochar addition caused higher activity of cellulase, b-glucosidase and arylsulphatase.

1.4.2. Phytotoxicity

Soil biochar amendment is often considered in terms of its usefulness in agriculture. To that end, toxicity tests of biochar-amended soil on plants are conducted to analyze the impact of biochar on the toxicity of a particular soil. A large number of studies (Table 4) emphasize the benefits for plants that result from biochar-amended soil (Kavitha et al., 2018).

Germination, root and shoot length, and produced plant biomass are the most frequently evaluated ecotoxicological parameters for plants. Many studies addressing the impact of biochar on plant growth and development have been published over the last decade (Gascó et al., 2016; de Figueiredo et al., 2019a; Mierzwa-Hersztek et al., 2018; Mierzwa-Hersztek et al., 2016; Mierzwa-Hersztek et al., 2017; O'Toole et al., 2018; Chi and Liu, 2016; Jeffery et al., 2017; Faria et al., 2018). Contradictory data have been reported regarding the beneficial or adverse effects of biochar on plants. For example, Jeffery et al. (2017) found a varying impact of biochar on plant yield, depending on the type of soil to which biochar was added. They observed that biochar had a positive effect on plant yield when the study was conducted on tropical soils and when the soils used were nutrient-poor with a low pH (acidic soils). However, in temperate zone soils and in soils with a pH optimal for plants (i.e., 6.2–7.0), the effect of biochar amendment was negligible or negative (Jeffery et al., 2017). Mierzwa-Hersztek et al. (2016) investigated the amount of biomass produced for grass (pasture grass mix) grown in soil amended with biochar produced from poultry litter at 300 °C. The amount of biomass produced was 30%–32% higher in the biochar-amended soil than in the biochar-unamended soil. In another study conducted by the same authors (Mierzwa-Hersztek et al., 2017), biochar obtained from wheat straw or *Miscanthus* at a temperature of 300 °C was added to a loamy sand Eutric Cambisol. The yield was found to increase only by 2% and 14% (pasture grass mix with red clover) after application of biochar at rates of 2.25 and 5 t/ha, respectively. Another study (Mierzwa-Hersztek et al., 2018) reported a significant increase in *Poa pratensis* L. biomass after the addition of biochars produced from sewage sludge to sandy acid soil at 300 °C. The application rate of biochar (0.5%, 1%, and 2%) was responsible for the stimulating effect. Plant biomass was higher for soil with a biochar application rate above 1%, and at the highest rate applied, the yield increase ranged between 71% and 122%, depending on the type of sewage sludge from which

Table 4
Phytotoxic properties of biochar-soil mixture.

Biochar	Pyrolysis	Experiment	Soil type	Plant	Effect	Reference
Mixed wood sievings	Temp. 620 °C Residence time: 20 min	Lab Dose: 8% w/w Temp. 28 Duration: 1 month	Haplic Cambisols sandy loam	Lettuce (*Lactuca sativa*) Lentil (*Lens culinaris*)	Lettuce: 28% lower stem lenght, 75% lower root dry weight Lentil: 33% lower aerial biomass 29% higher root dry weight	(Gascó et al., 2016)
Switchgrass					67% higher shoot weight 23% higher root weight	
Hardwood	Temp. 500 or 700 °C	Lab Dose: 10% w/w Duration: 6 weeks	Pool filter + field soil + vermiculite + turface	Leek (*Allium porrum* L. cv. Musselburgh)	6.4% lower shoot height 14% lower root weight	(Han et al., 2016)
Softwood					3.6% higher shoot weight 6.4% lower shoot height 15% lower root weight 1.2% higher shoot weight	
Paper sludge + wheat husks	Temp. 500 °C Residence time: 20 min	Lab Dose: 8% w/w Temp. 28 °C Duration: 1 month	Haplic Cambisols ST- sandy loam SA- sandy	Lettuce (*Lactuca sativa*) Lentil (*Lens culinaris*)	Lettuce: ST- 33% lower stem length. SA- no statistical difference Lentil: ST- 14% lower aerial biomass, 38% lower root dry weight. SA- higher aerial biomass, stem length and root dry weight 15-38%	(Gascó et al., 2016)
Sewage sludge					Lettuce: ST- 32% lower lower stem length, 75% lower root dry weight. SA- 34% higher stem length Lentil: ST- no statistical difference. SA- 21% lower stem length	
Sewage sludge (from three different treatment plants)	Temp. 300 °C Residence time: 15 min	Lab Dose: 0.5 or 1 or 2% w/w Duration: 200 days	Loamy sand	*Poa pratensis* L.	1) From 60% to over 2.5- fold higher biomass depending on biochar dose. The higher dose, the higher biomass. 2) 80% higher biomass 3) 40-80% higher biomass	(Mierzwa-Hersztek et al., 2018)
Wood chip sievings (*Picea abies* + *Fagus sylvatica*)	550-600 °C	Greenhouse Dose: 1.5% (34.26 t/ ha) or 3% (68.53 t/ ha)	Silty sand	Maize (cv. DKC-3399)	6.5-7.9% higher plant growth (stem and leaf)	(Haider et al., 2015)
Wheat straw (*Triticum aestivum* L.)	Temp. 300 °C Residence time: 15 min	Field Dose: 2.25 or 5.00 t/ ha Duration: 18 months	Loamy sand Eutric Cambisol	Perennial grass	No statistical difference	(Mierzwa-Hersztek et al., 2017)
Miscanthus straw (*Miscanthus giganteous)*					14% higher crop yield	
Miscanthus straw (*Miscanthus giganteous)*	Temp. 500-750 °C	Field Dose: 11.4 (0.38% w/w) or 35.0 (1.16% w/w) t/ ha Duration: 4 years	Silty clay loam Albeluvisol	Oat (*Avena sativa*), Barley (*Hordeum vulgare* L.)	No significant differences (grain and straw yield)	(O'Toole et al., 2018)
Acacia (*Acacia spp.*)	Temp. 350-450 °C Residence time: 6 days Earth kilns	Field Dose: 10 t/ ha Duration: 1 year	Acidic Eutric Nitisol	Barley (*Hordeum vulgare* L.)	Higher grain yield from 30 to 79% Higher straw yield from 56 to 89%	(Agegnehu et al., 2016a)
Acacia (*Acacia spp.*)	Temp. 350-450 °C Residence time: 6 days Earth kilns	Field Dose: 10 t/ ha Duration: 1 year	Nitisol	Barley (*Hordeum vulgare* L.)	Higher grain yield from 30 to 67% Higher number of productice tillers from 24 to 26%	(Agegnehu et al., 2016c)
Hardwood + coniferous wood chips	Temp. 750 °C	Field Dose: 8 t/ ha	Haplic Regosol	Grapevines	No statistical differences (shoot diameter and fertility)	(Schmidt et al., 2014)
Waste willow wood (*Salix* spp.)	Temp. 550 °C	Field Dose: 10 t/ ha	Ferralsol	Peanut (*Arachis hypogaea* L.) cv. Menzies	21% higher seed yield 23% higher pod yield 7% higher chlorophyll content	(Agegnehu et al., 2015)
			Dark reddish brown Ferralsol or Red Ferrosol	Maize (*Zea mays*)	29% higher grain yield 17.7% higher biomass	(Agegnehu et al., 2016b)
Sewage sludge	Temp. 300 °C Residence time: 30 min Temp. 500 °C Residence time: 30 min	Field Dose: 15 t/ ha Duration: 2 years	Red Yellow Latosol (Typic Haplustox)	Corn (hybrid LG6030)	42% higher grain yield 50% higher grain yield	(Faria et al., 2018)
Sewage sludge	Temp. 300 or 500 °C Residence time: 30 min	Field Dose: 15 t/ ha Duration: 1 year	Clayey Oxisol (Typic Haplustox)	Corn	45% higher grain yield	(de Figueiredo et al., 2019c)

biochar was produced. Plant yield was positively correlated with pH, salinity, and total nitrogen and carbon content, and negatively correlated with Pb and Cd content. The latter negative relationship may indicate that the metals present in biochar negatively affected the yield to some extent. Faria et al. (Faria et al., 2018) investigated the effect of addition (15 t/ha application rate) of biochar produced from sewage sludge at 300 or 500 °C to Red–Yellow Latosol soil. Biochar addition increased the corn grain yield, and biochar amendment by adding fertilizer based on NPK also enhanced yield, particularly in the case of biochar produced at 300 °C. de Figueiredo et al. (2019a) also observed an increase in corn yield after the addition of sewage sludge biochar produced at 300 or 500 °C to soil.

In addition to studies describing the positive impact of biochar on organisms, numerous studies indicate that under certain conditions, the addition of biochar to soils has a toxic effect on plants. Gascó et al. (2016) investigated the effects on plants (*L. sativum*, *Lens culinaris* Medikus, *Cucumis sativus*, *Solanum lycopersicum*, and *Lactuca sativa*) of three biochars added to Haplic Cambisols, sandy loam-ST, or sandy-SA soil. Biochars were produced from mixed wood sievings at 620 °C, a mixture of paper sludge and wheat husks at 500 °C, and sewage sludge at 600 °C, and applied at a rate of 8%. The results varied depending on the soil and plant species used for the test (Table 4). It was determined that HMs in biochars did not contribute to the toxicity toward plants and suggested that other substances that might have been present in biochar were responsible for the toxicity. O'Toole et al. (2018) investigated application at a rate of 35 t/ha of biochar obtained from *Miscanthus* at 750 °C and observed no impact on the grain and straw yields of barley and oat.

Relatively few studies have been conducted on the effect of biochar on aquatic plants. This type of research is a rather indirect measure of biochar toxicity, allowing the possible impact of surface runoff on aquatic ecosystems to be evaluated. To date, the only study of biochar effects on aquatic plants has been conducted by Chi and Liu (2016) in the context of the possible application of biochar for immobilization of contaminants found in bed sediments. Biochar produced from wheat straw at 400 or 700 °C was added to bed sediments at a rate of 3% (w/w), and its effect on growth and root and shoot biomass of *Vallisneria spiralis* was investigated. No statistically significant difference was observed immediately after biochar application relative to the control experiment, with the exception of shoot biomass. After 54 days, the shoot biomass in the experiment with biochar was lower than that in the control experiment. Biochar produced at 700 °C proved to be the most toxic; it manifested not only in a lower biomass of *V. spiralis* but also in a lower root length relative to the control experiment. According to Chi and Liu (2016), the observed inhibiting effect of biochar on the plants was caused by increased retention of nutrients in the presence of biochars. Consequently, a decrease in nutrient bioavailability and disturbances in the symbiosis of plants with microorganisms occurred due to the sorbing by biochar of substances necessary for chemical communication in symbiotic organisms.

1.4.3. Toxicity toward invertebrates

Invertebrates are components of soil fauna that decompose organic matter and participate in nutrient cycling. Earthworms are the type of invertebrates most frequently studied in the context of biochar-amended soil (Nahmani et al., 2007). Contaminants can be consumed by these organisms in two ways: through the skin (passive diffusion) and through ingestion together with soil particles (Zhang et al., 2019b). It is accepted that the addition of biochar to soil essentially improves the living environment of earthworms due to increased organic matter content and soil aeration as well as due to an increase in the content of trace elements and minerals (Hale et al., 2013). There are, however, studies indicating that biochar can negatively impact earthworms, primarily due to toxic substances present in biochar.

Hale et al. (2013) applied biochar produced from corn stover at 600 °C to uncontaminated soil to determine its effect on *Aporectodea caliginosa* earthworms, with application rates ranging from 0.5% to 5.0%. It was found that rates ranging from 2% to 5% contributed to weight loss in *A. caliginosa*. Elliston and Oliver (2019) conducted an earthworm avoidance test on *E. fetida* in natural soil (Kettering loam) and artificial OECD soil. Biochars produced from rice husk or wheat straw at 550 °C were added to the soils at rates ranging from 5% to 20% (w/w). A positive linear relationship was observed between biochar application rate and avoidance, but only for OECD soil with wheat straw biochar. For the other treatments, i.e., in OECD soil with rice husk biochar and in natural soil with rice husk or wheat straw biochar, no such correlation was found. Moreover, after exposure of over two weeks of *E. fetida* or *Lumbricus terrestris* to the soil with 20% biochar amendment, changes occurred in the structures of some individuals, suggesting a toxic effect of high biochar application rates on the test organisms. A high application rate of wheat straw biochar (10%–20%) in OECD soil also led to a decrease in the weight of *L. terrestris*. In natural soil, 20% addition rates of both biochars caused weight loss in earthworms of both species. Other studies confirm the adverse effect of high biochar application rates on earthworm growth and development (Weyers and Spokas, 2011).

The wide range of effects depending on not only the type of biochar but also the type of soil indicates that soil type must be considered when determining biochar toxicity, since differing soil properties can produce highly divergent results (Jeffery et al., 2017). Van Zwieten et al. (2010) performed an interesting experiment wherein biochar was added to two soils of different pH (acidic and alkaline) and performed an avoidance test with *E. fetida* earthworms. The earthworms preferred the mixture of biochar with acidic soil rather than the mixture with alkaline soil. This is due to the fact that in acidic soil, biochar amendment essentially causes an increase in pH and the creation of more optimal conditions for earthworm growth, whereas in alkaline soil, a further increase in pH after biochar addition is adverse for earthworm growth.

Studies investigating the effects of biochar on other invertebrates are relatively scarce. The effects of biochar on *Heterocypris incongruens* have been studied using the Ostracodtoxkit F test. In a study (Mierzwa-Hersztek et al., 2016) on the impact of poultry litter biochar (produced at 300 °C) on mortality and growth inhibition of *H. incongruens*, a significantly lower toxic effect in biochar-amended soil relative to unamended soil was found. Nonetheless, opposite results were obtained for biochar derived from sewage sludge (Mierzwa-Hersztek et al., 2018). *H. incongruens* growth inhibition ranged from 10% to 44% and depended on both the application rate and the type of sewage sludge from which biochar was produced. The toxic effect was, however, observed to decrease with increasing biochar application rate (for two out of the three biochars tested), which may indicate that nutrient deficiency, rather than the negative impact of biochar, inhibited the growth of the test organisms.

Collembolans from the phylum Arthropoda consume biochar particles, especially when *F. candida* is not given yeasts. This phenomenon may be associated with colonies of the fungus living in biochar that Collembolans feed on (Domene, 2016). Hale et al. (2013) investigated the effect on the toxicity toward *F. candida* of soil amended with corn stover biochar produced at 600 °C. Biochar was added to uncontaminated soil at rates ranging from 0.5% to 5.0%, and no differences in *F. candida* reproduction were observed relative to the unamended soil. Domene et al. (2015) studied the effects of various types of biochar (produced from bull manure with sawdust, corn stalks, digested dairy manure, food waste, oak, paper mill waste, or pine at temperatures of 300, 350, 550, or 600 °C) on Collembolans. High application rates of biochar derived from certain substrates had a clear toxic effect on reproduction (e.g., biochar produced from food waste at 300 °C applied at a rate above 7%). The increased salinity after biochar incorporation in the soil was responsible for the toxic effect. Gruss et al. (2019) added biochar produced from pine and spruce chips at 300 °C to agricultural soil at rates ranging from 1.0% to 50.0%. *F. candida* mortality was less than 20% for all the conditions studied. Biochar application rates

between 5.0% and 50.0% decreased reproduction and led to avoidance of *F. candida*. It was thus concluded that a significant increase in soil pH caused the toxic effect resulting from the high biochar application rates, due to the preference of Collembolans for a pH below 8.

1.5. Conclusions and future studies

Biochar is usually found to have a toxic impact on living organisms in experiments that directly evaluate its toxicity (without soil). High pH, salinity, and sometimes the presence of contaminants in biochar are primarily responsible for the observed toxic effects. However, we obtain a completely different picture of "potential biochar toxicity" when the toxicity of biochar is assessed by mixing with soil (especially at a rate of <1%, approximating actual conditions in the environment). Addition of various types of biochar in soil does not usually have a toxic effect and it even results in a range of desired effects (such as increased plant biomass or a higher number of microorganisms in soil). However, these effects are associated with the type of biochar (the material used for its production and pyrolysis temperature), its application rate, and the type of soil to which biochar is added. Research reveals that the above-mentioned factors have a significant influence on the observed effects. Biochars obtained from plant substrates usually have a much lower content of contaminants compared to biochars produced, e.g., from sewage sludge or animal manure, and hence, their toxicity is lower. Biochar produced at low temperatures (<500 °C) contains more bioavailable nutrients, which contributes to an increase in the amount of microorganisms in the soil and to higher crop yields. The type of soil to which biochar is added is a very important factor, since the physico-chemical properties of the soil can substantially change biochar toxicity. Depending on the particle-size distribution, bulk density, porosity, compaction, or viscosity, the resulting effect may vary. Likewise, different effects can be observed depending on the content of carbon, nitrogen, phosphorus, and other essential macronutrients in the soil as well as its pH and salinity.

In the future, it will be necessary to focus research efforts to determine certain parameters relevant to biochar toxicity, such as the bioavailable fraction of contaminants in biochar-amended soil (rather than their total content); to conduct a greater number of ecotoxicological tests in uncontaminated soils, particularly under field conditions and long-term perspective; and to increase the diversity of test organisms used to evaluate biochar toxic effects. It would also be worthwhile to increase the number of assays. This will provide an insight into the direct toxic mechanism or into mechanisms promoting an enhanced impact of biochar on organisms. Due to the planned use of biochar under a variety of environmental conditions, improved understanding of these mechanisms will be essential in future research.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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EFFECT OF BIOMASS ADDITION BEFORE SEWAGE SLUDGE PYROLYSIS ON THE PERSISTENCE AND BIOAVAILABILITY OF POLYCYCLIC AROMATIC HYDROCARBONS IN BIOCHAR-AMENDED SOIL

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ABSTRACT

There is a lack of studies dealing with the effects of co-pyrolyzed biochars produced from different materials on the persistence of polycyclic aromatic hydrocarbons (PAHs). This research aimed to determine the persistence (based on extractable C_{tot}) and bioavailability (based on freely dissolved content, C_{free}) of PAHs in soil amended with biochar derived from sewage sludge (SL) or a mixture of SL and biomass (SLW). Biochars produced at 500, 600, and 700 °C were applied to the soil (podzolic loamy sand) at a rate of 2% (w/w) and incubated for 180 days. The content and changes of PAHs differed between the experiment with SL, SL-derived biochar, and SLW-derived biochar. In the soil with the SLW-derived biochar, the losses of C_{tot} Σ16 PAHs were lower (between 13 and 38%) than in the soil with the SL-derived biochar (from 27 to 74%). Compared to C_{tot}, a completely different trend was observed for C_{free} PAHs. The decrease of C_{free} PAHs in the soil with the SLW-derived biochar was higher than or similar (from 4 to 18%) to the losses of C_{free} PAHs in the soil with the addition of the SL-derived biochar. The differences between the individual treatments resulted from the difference in the physical and chemical properties of the biochars, which affected the persistence and bioavailability of the studied compounds.

1. Introduction

Due to its volume, odors, and toxic substances, sewage sludge (SL) can be problematic in the disposal. On the other hand, SL can find potential application in agriculture, which is associated with nutrients content (carbon, nitrogen, and phosphorus) [1–4]. Direct application of SL in crop fields maybe hazardous because it involves the incorporation into soil of harmful substances such as heavy metals (HMs) and organic contaminants (e.g. polycyclic aromatic hydrocarbons, pharmaceuticals, endocrine disrupting compunds, or other) as well as biological material (e.g. parasite eggs or weed seeds) [5]. Conversion of SL into biochar through the process of pyrolysis can eliminate some of SL drawbacks [6]. Pyrolysis is the process of thermochemical decomposition of organic matter at 300–900 °C in an oxygen-limited atmosphere [7,8] and is proposed as one of the effective methods of SL utilization [7]. Despite that, polycyclic aromatic hydrocarbons (PAHs) in biochar, whose concentration can be very high depending on the method of biochar production (even 172 mg/kg [9]), could be of concern. During pyrolysis, PAHs in SL are not fully disintegrated [6] or are formed again [10], and as a result they are still present in SL-derived biochar [11]. PAHs are known as toxic, mutagenic and cancerogenic compounds [10,12]. A previous study showed [13] that addition of biomass to SL before pyrolysis reduces the content of PAHs in biochar. The bioavailable (freely dissolved, C_{free}) content of PAHs in biochar also decreases (relative to biochar produced from SL alone) [10]. However, the literature lacks information on the persistence and/or bioavailability of PAHs in the co-pyrolyzed biochar-amended soil.

Biochar has potential application as a soil amendment [14] and hence a low content of both total (C_{tot}) and bioavailable (C_{free}) PAHs is an important aspect of its environmental management. Co-pyrolysis of SL with biomass improves the physico-chemical properties of biochar [1,7,15] and contributes to a reduction of the PAH content and toxicity of biochar [13]. To date, the research about co-pyrolyzed (SL/biomass) biochars has mainly focused on: (1) the optimization of the pyrolysis process and investigation of the properties of the obtained material [16–19], and (2) the adsorption of different compounds [20,21] as well as (3) the content of trace elements and organic compounds in biochars [1,15,22–24]. However, the literature lacks information regarding the

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persistence and bioavailability of organic contaminants in soils amended with biochars produced by co-pyrolysis [25]. The studies concerning the persistence of PAHs in biochars produced from single feedstock show that adding biochar to soil at a rate of about 20 t/ha does not affect significantly the soil content of PAHs over the long term (at least several months) [26–29]. Despite that, the PAH content can sometimes increase after adding biochar, this does not cause the legal limits for the PAH content in soil to be exceeded [30,31]. The absence of a significant increase in PAH concentration in biochar-amended soils can be associated with the strong adsorption of PAHs by biochar due to their penetration into developed pores or through strong π-π interactions, related to the high aromaticity of the biochar surface [29].

Biochar added to the soil can change physico-chemical properties of soil (pH, water content, etc.), thus affecting the mobility/bioavailability of PAHs. Biochar can also change the microbial content and microbial activity of soil by influencing the soil properties [32], which may contribute to quicker or slower biodegradation of PAHs. Application of biochar to soil can also change the adsorption properties of the biochar itself and therefore impact the degree of binding of organic contaminants with its surface [33,34]. Higher elemental carbon content and higher specific surface area, as well as greater differences in pores in SL/biomass co-pyrolyzed biochar, have been found in comparison to biochar derived from SL alone [22,35]. The above adsorption properties of biochar are extremely important in the context of immobilization of organic contaminants and can lead to stronger interactions of PAHs with the biochar surface [36,37]. Given that, the differences in the properties between SL-derived biochar and biochar obtained from SL co-pyrolyzed with biomass (e.g. willow) can affect the persistence (increasing it) and bioavailability (decreasing it) of PAHs in soil. This is an important issue, particularly in the context of environmental use of co-pyrolyzed biochars, which has not been investigated thus far.

This research aimed to determine the total (C_{tot}) and bioavailable (C_{free}) content of PAHs in soil amended with biochar produced from SL or SL with the addition of willow. This study additionally investigated the effect of biochar production temperature (500, 600, and 700 °C), in the context of the feedstock used to produce the biochar, on the persistence and bioavailability of PAHs. We identify two main hypotheses: (1) a persistence of PAHs in soil with SL-derived biochar produced with biomass addition will be higher compared to soil with SL-derived biochar as biochar produced from SL and biomass has more carbon which makes the interaction between PAHs and biochar stronger compared to SL-derived biochar with lower carbon and affinity to PAHs, (2) with the same reason the bioavailability (based of freely dissolved content) PAHs in soil with SL/biomass-derived biochar will be lower compared to soil with SL-derived biochar.

2. Materials and methods

2.1. Pot experiment

Sewage sludge (SL) was collected from municipal wastewater treatment plant (50°20′04″N 23°29′49″E). SL was stabilized biologically by aerobic fermentation. Before the pyrolysis, the SL was mixed, ground, and passed through a 2 mm sieve. Willow (Salix viminalis) was provided by a biomass-producing farm localized in the southeastern part of Poland. Biochars production conditions were described elsewhere [13,35] and briefly presented in Supporting Information (Table S1). The soil used in the pot experiment was obtained from the Bezek Experimental Station localized in Poland (51°12′06′′N 23°16′06′′E). The soil was classified as Podzol lying on marl substrate with the textural composition of loamy sand (Table S2).

The experiment was carried out in plastic containers (20 L). Dry sewage sludge (SL), SL-derived biochar or SL/biomass-derived biochars (SLW-) was added to soil at the dose of 2 % w/w (dry weight) and mixed thoroughly to prepare a homogeneous mixture. The mixture was placed in the containers and watered by deionized water to bring it to 60 % of soil water holding capacity. Soil moisture was maintained by weighing the pots periodically and adding necessary water to keep the constant water holding capacity. The experiment was carried out at 23 ± 2 °C in the dark conditions. Samples (about 50–60 g) were collected at the beginning of the experiment (0) and after 30, 90, and 180 days. Samples were then air-dried for 24 h and stored in the fridge before analysis.

2.2. Organic solvent extractable content (C_{tot}) of PAHs

Soil and SL- and biochar-amended soil (10 g) were extracted using a Soxhlet extractor with hexane for 24 h. Before the extraction deuterated PAHs were added as an internal standard (IS). The solvent was reduced to about 1 mL using rotary vacuum concentrator RVC 2–25CD plus (Martin Christ, Germany). A quantitative analysis of PAHs was carried out on Thermo Scientific Trace 1 300 Gas Chromatograph equipped with a Restek Rxi-5 ms Column (length 30 m, 0.25 mm id and 0.25 μm film thickness). Detailed information about PAHs analysis is presented elsewhere [13].

2.3. Freely dissolved content (C_{free}) of PAHs

Soil, SL- or biochar-amended soil (1 g) were added to 50 mL glass flasks with glass lids. Millipore water (40 mL) with sodium azide (200 mg L^{-1}) (to prevent microorganisms activity) and strips of 55 μm thick polyoxymethylene (POM) passive samplers (0.3 g for all batches) were added to flasks. Before use, POM samplers were cleaned using overnight in methanol, next in heptane, and then were rinsed in Millipore water and dried. Flasks were rolled end over end 28 days at 1 RCF. Next, POM strips were removed, cleaned with Millipore water and wiped with a tissue until they were dry and visibly clean. After 30 days extraction of POM samplers was carried out in 20 mL of 20:80 acetone: heptane for 48 h with deuterated PAHs added as the IS. The solvent was reduced to about 1 mL using rotary vacuum concentrator RVC 2–25CD plus (Martin Christ, Germany). A quantitative analysis of PAHs was carried out on Thermo Scientific Trace 1 300 Gas Chromatograph equipped with a Restek Rxi-5 ms Column (length 30 m, 0.25 mm id and 0.25 μm film thickness) described earlier in detail [6,13].

2.4. Data analysis

Mean values were taken from a triplicate data set. The differences between C_{tot} and C_{free} of all mixtures and the control were evaluated using a one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. The relationships between the properties of SL- or biochar amended soil (total PAHs content, Cfree) were determined by correlation analysis with Statistica 5.0. Significance was set at $P \geq 0.05$. The information about quality control is presented in the Supporting information section. Detailed information about C_{free} PAHs calculation is presented elsewhere [38] and in supporting information.

3. Results and discussion

3.1. Persistence of PAHs in soil and sewage sludge-amended soil

At the beginning of the study, the content of a total of 16 (Σ16) C_{tot} PAHs in the control soil was 40.5 μg/kg (±7.5) (Fig. 1), which characterizes uncontaminated soils [26]. The light PAHs (2–4-ring) predominated (82%) in the soil (Fig. S1). During the experiment, the content of Σ16 C_{tot} PAHs did not undergo significant changes (Fig. 1a). However, for individual C_{tot} PAHs groups specific changes in their content were observed (Fig. S1a–e). After 180 days of the experiment, the content of 2-ring C_{tot} PAHs was lower by 32%, whereas the content of 3-, 4-, and 6-ring PAHs was higher by 42, 33, and 173%, respectively, than at the beginning of the study (Fig. 2b–f). As a result of this changes, the profile of PAHs also changed. The contribution of 2-ring PAHs declined, because of an increase of 3-ring compounds (Fig. S1b).

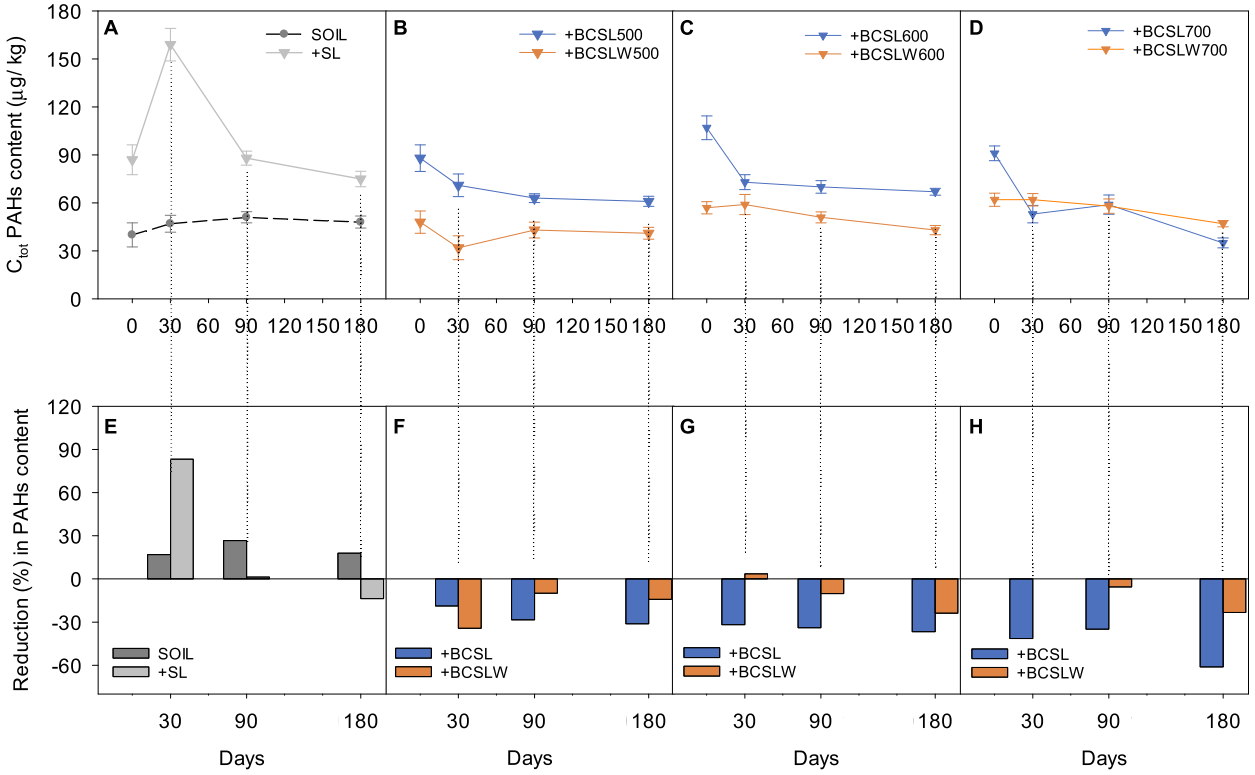

Fig. 1. Changes in organic solvent extractable PAHs content (Σ16 C_{tot}) in soil, sewage sludge (SL-), sewage sludge-derived-biochar- (BCSL-) and sewage sludge-biomass-derived biochar- (BCSLW-) amended soil during the experiment. Values 500, 600 and 700 refer to temperature of biochar production. Biochars produced in 500 (B), 600 (C) or 700 °C (D). Bottom panel presents the reduction of 16 PAHs in particular term compare to the beginning of the experiment.

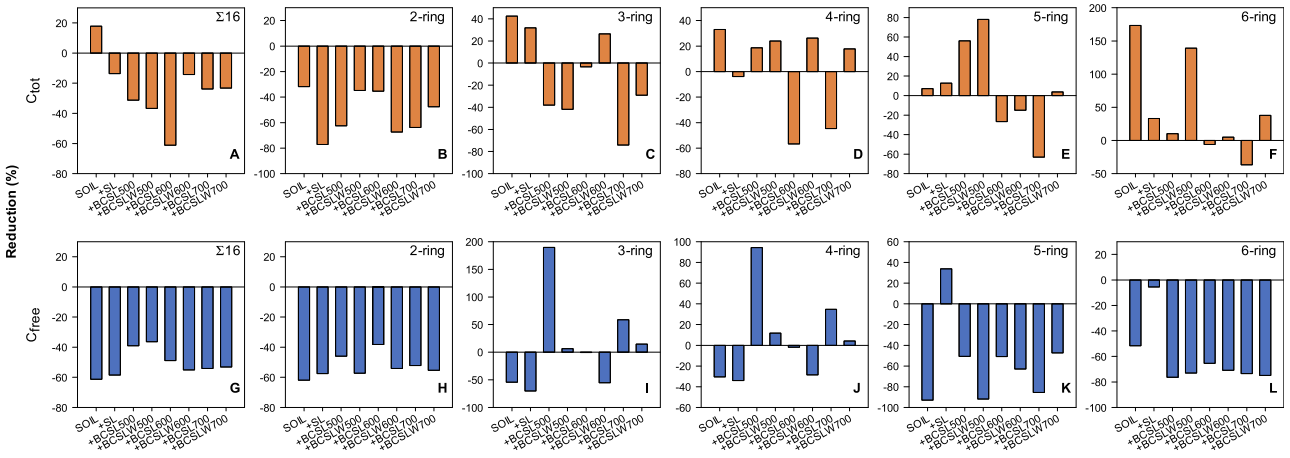

Fig. 2. Reduction (%) in C_{tot} (A-F) and C_{free} (G-L) PAHs group content at the end of the experiment. Positive difference is the increased content while negative one is the loss in PAHs content.

The content of freely dissolved PAHs (Σ16 C_{free} PAHs) in the control soil was at a level of 56.3 ng/L (±1.6) (Fig. 3a,). During the experiment, Σ16 C_{free} PAHs gradually decreased, reaching the level of 21.8 ng/L after 180 days (Fig. 3a). The decreased content of Σ16 C_{free} PAHs was affected by the reduction of all PAH groups. After 180 days the highest reduction was found for 5-ring (93%) and 2-ring (62%) PAHs (Fig. 2 h–l).

Adding SL to the soil increased the content of Σ16 C_{tot} PAHs from 40.5 µg/kg (±7.5) to 86.7 µg/kg (±9.39) (Fig. 1a). An increase in the PAH content after adding SL to the soil has also been observed by other authors [2,3,39–42]. Depending on the degree of contamination of SL and soil by PAHs, such an increase can range from 16 % to even 400 % [2,3,39–42]. The content of PAH groups regarding to number of rings in the soil increased in the range between 70 and 195%. The highest increase was found for 6-ring PAHs (195%), followed by 2-ring (121%) and 3-ring (117%) PAHs (Fig. S1a–f). The soil content of Σ16 C_{tot} PAHs after 30 days from adding sewage sludge increased by 83% (Fig. 1a) and was higher than the content of Σ16 C_{tot} PAHs in the control soil by 236% over the same period. An increase in the content of PAHs several weeks after SL application has been previously observed [3] and is explained by the release of PAHs (initially non-bioavailable/strongly bound to SL) during organic matter mineralization. The increase of Σ16 C_{tot} after 30 days was primarily associated with the content of 3-ring PAHs (Fig. S1b). After 90 days the content of Σ16 PAHs in the SL-amended soil decreased to the level at the beginning of the experiment (Fig. 1a).

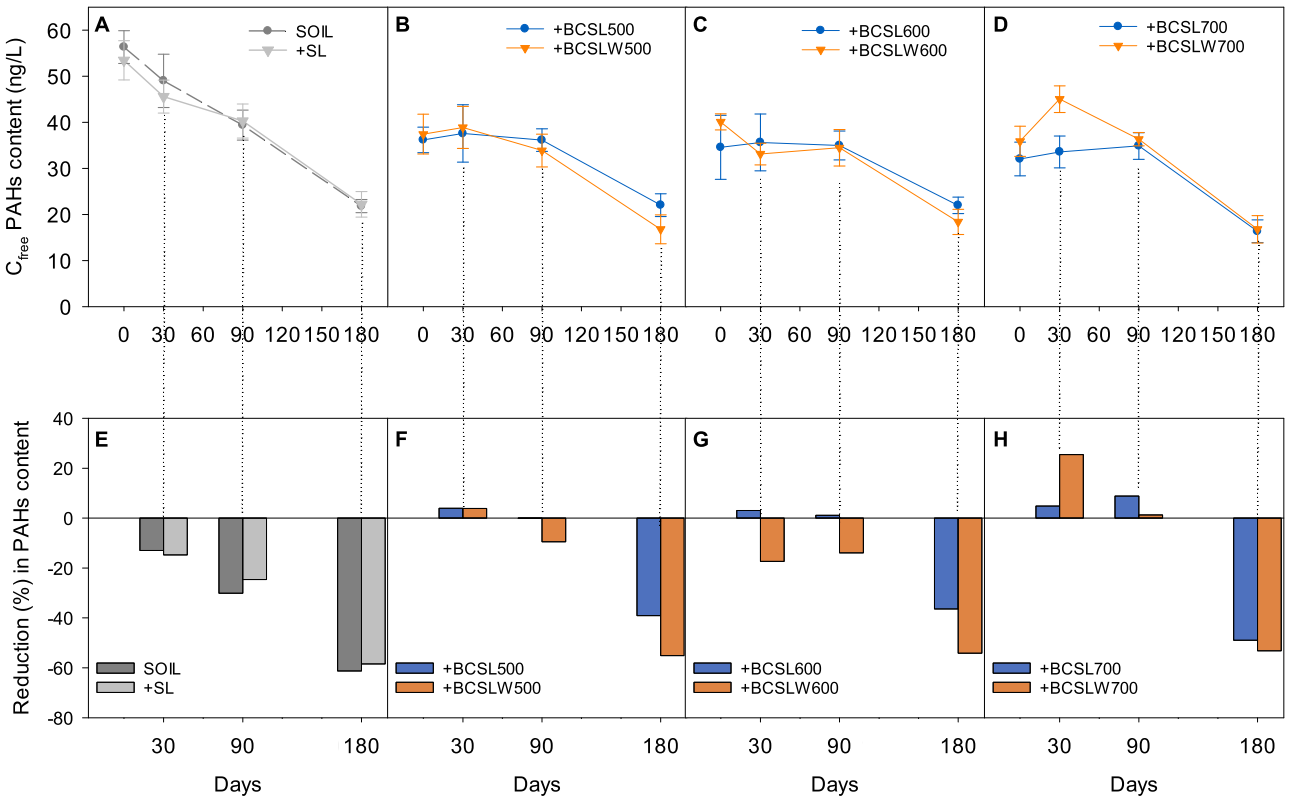

Fig. 3. Changes in freely dissolved PAHs content (Σ16 C_{free}) in soil, sewage sludge (SL-), sewage sludge-derived-biochar- (BCSL-) and sewage sludge-biomass-derived biochar- (BCSLW-) amended soil during the experiment. Values 500, 600 and 700 refer to temperature of biochar production. Biochars produced in 500 (B), 600 (C) or 700 °C (D). Bottom panel presents the reduction of 16 PAHs in particular term compare to the beginning of the experiment.

However, the content of Σ16 C_{tot} PAHs in the SL-amended soil was still higher than in the control soil by 71% (Fig. 1). After 180 days there was a further decrease in the PAH content, which was lower by 14% compared to the beginning of the experiment (Fig. 1e) and still higher by 57% than in the control soil at the corresponding assessment time point. Previous studies demonstrate that the range of PAH losses in SL-amended soils varies [39,42,43]. For example, Stanczyk-Mazanek et al. [39] observed that the content of Σ16 PAHs in SL-amended soil decreased from 27 to 45% after 3 years and depended on SL dose (from 10 to 20 t/ha). Feng et al. [42] found the level of PAHs to drop by 53% in the soil with 25% SL addition (fresh weight) after 126 days. Wołejko et al. [43] indicated that the level of PAHs decreased by only 12% during one year after SL application to soil. The extent of PAH changes in SL-amended soils is determined by many factors, but the most important are the soil and SL properties. The observed differences are probably due to the different properties of the materials used in the experiment.

After 180 days, the content of 3-, 5-, and 6-ring PAHs increased (from 13 to 33%) and 2-ring PAHs decreased (77%) in SL-amended soil with regards to the beginning of the experiment (Fig. 2b–f). The content of 4-ring PAHs after 180 days did not differ significantly ($P > 0.05$). Naphthalene (NAP), as a 2-ring compound with the lowest number of rings among the PAHs, is the most polar and volatile compound. Therefore, it can be easily leached and/or biodegraded [44]. The heavy PAHs (>4 rings) can be strongly adsorbed by soil particles and hence their persistence in the soil is>2-ring PAHs. However, the absence of degradation of 4-ring PAHs during the 180 days of the experiment is surprising and hard to explain at this moment.

Adding SL to the soil did not cause a statistically significant increase in Σ16 C_{free} PAHs (Fig. 3) as it was observed for C_{tot} (Fig. 1). Because of high organic matter content [37], SL is characterized by high affinity to PAHs [45–47], which may substantially reduce PAHs bioavailability [48]. Nonetheless, the addition of SL changed the profile of C_{free} PAHs in the soil. The content of 3-, 4-, and 5-ring PAHs was found to increase from 41 to 247% compared to the control soil (Fig. 3b–d). But the level of 2-ring PAHs, which were the dominant part of Σ16 C_{free} PAHs (Fig S3a), decreased by 13% (Fig. 3a). As a result, this was related to the balanced increase observed for the remaining PAH groups. In the soil with SL, 2-ring (85%) and 3-ring PAHs (12%) were still predominant (Fig. 3a, b). After 180 days, relative to the beginning of the experiment, an increase in 5-ring PAHs by 34%, no change in 6-ring PAHs, and a decrease in the other PAH groups (from 34 to 70%) was observed in the soil with SL addition (Fig. 2h–l).

3.2. Persistence of PAHs in SL-derived biochar-amended soil

Application of the biochars to the soil had different effects on the content of C_{tot} PAHs, depending on the biochar production temperature. Biochars produced at 500, 600 or 700 °C caused an increase of Σ16 C_{tot} PAHs to 88.0 μg/kg (±8.27 μg/kg), 106.6 μg/kg (±7.45 μg/kg) and 90.6 μg/kg (±4.65 μg/kg), respectively (Fig. 1b–d). For BCSL500 and BCSL700, the C_{tot} did not differ statistically significantly ($P > 0.05$) relative to the SL-amended soil (Fig. 1a, b, d). In BCSL600-amended soil (Fig. 1c), Σ16 C_{tot} PAHs was 23% higher than SL-amended soil, which was primarily related to the increased content of 4-, 5-, and 6-ring PAHs in this soil after adding the biochar. The Σ16 C_{tot} PAHs in biochar-amended soil (Fig. 1b, d) was higher from 117 to 163% than in the control soil (Fig. 1a–d).

After 30 days, Σ16 C_{tot} PAHs in the biochar-amended soil was lower from 19 to 41% than at the beginning of the experiment (Fig. 1f–h). In the soil amended with BCSL500 and 600, the content of Σ16 C_{tot} PAHs decreased gradually until the end of the experiment (Fig. 1b, c). Σ16 C_{tot} PAHs was however found to vary for BCSL700 (Fig. 1d). Finally, Σ16 C_{tot} PAHs also declined in this soil (Fig. 1). After 180 days the content of Σ16 C_{tot} PAHs was lower from 31 to 61% depending on biochar used than at

the beginning of the study (Fig. 2a). On the other hand, the content of Σ16 C_{tot} PAHs was higher from 27 to 41% in the BCSL500- and BCSL600-amended soil and lower by 26% than in the soil with BCSL700 relative to the control soil over the same period (Fig. 1b–d). The persistence of PAHs in the biochar-amended soil was found to be lower than in SL-amended soil. After 180 days the content of Σ16 C_{tot} PAHs decreased more, from 18 to 48%, in the soil with biochar addition than in the SL-amended soil (Fig. 2a).

When analyzing the individual PAH groups after 30 and 90 days, the content of 2–6-ring C_{tot} PAHs was found to decrease and predominantly depended on the biochar type (Fig. S2). After 180 days, in the soil with the BCSL600 and BCSL700, the content of all the PAH groups declined in the range from 27 to 74% with regards to the beginning of the study (Fig. 2). In BCSL500-amended soil, the content of the light (2- and 3-ring) PAHs decreased between 38 and 63%, whereas the content of the other groups increased from 10 to 56% compared to the beginning of the experiment.

For Σ16 C_{free} PAHs, after adding the biochar to the soil their content ranged from 32.0 to 36.2 ng/L and did not differ significantly ($P > 0.05$) between the biochars (Fig. 3b–d). The higher the biochar pyrolysis temperature, the lower the C_{free} content was determined, which is in agreement with previous works [6]. The biochar-amended soil had a lower content of Σ16 C_{free} PAHs, from 36 to 43 % and from 32 to 40 %, in comparison to the control soil and the SL-amended soil (Fig. 2), respectively. Naphthalene was the predominant compound in C_{free} and consisted (depending on biochar) from 95 to 97 %.

After 30 and 90 days, Σ16 C_{free} PAHs did not change relative to their level determined at the beginning of the experiment (Fig. 3b–d). The significant decrease of Σ16 C_{free} was indicated at 180th day of the experiment. The reduction in the content of Σ16 C_{free} PAHs regarding the beginning of the experiment ranged from 36 to 49 % (Fig. 1b–d, Fig. 3g). The highest decrease was found for BCSL700, while the lowest for the BCSL600-amended soil (Fig. 3g).

Despite that Σ16 C_{free} PAHs remained at a constant level, the individual PAHs were characterized by high dynamics of changes throughout the entire study period (Fig. S2), what was strictly dependent on biochar production conditions. Finally, after 180 days, the content of 2-, 5-, and 6-ring PAHs decreased respectively from 38 to 52%, from 51 to 86%, and from 65 to 76% in comparison with the beginning of the experiment (Fig. 2f, i, j). For BCSL500 and BCSL700, the level of 3-ring (from 59 to 190%) and 4-ring (from 35 to 94%) PAHs increased (Fig. 2g, h, r, s). A higher increase was recorded in BCSL500- than in the BCSL700-amended soil. The content of 3- and 4-ring PAHs in the soil with BCSL600 did not differ significantly between the beginning and end of the study, despite that it showed certain variations during the study period (Fig. S2).

To date, it has been shown [31,49] that the properties of biochar itself are of great importance for the persistence and bioavailability of PAHs in biochar-amended soils [50]. The biochar properties, such as porosity and specific surface area, affect decreasing or increasing the contribution of PAH adsorption through partitioning and pore-filling [51]. The chemical properties of biochar, such as the ratio of H/C (aliphaticity) and O/C (hydrophilicity), contribute to the formation (or lack of formation) of electrostatic interactions, hydrogen bonds, π-π electron-donor acceptor interactions (π-π EDA), and van der Waals interactions between the biochar and a chemical compound [51,52]. The correlations between the properties of the biochars used in the present study and the losses of Σ16 C_{tot} PAHs revealed a significant relationship ($P > 0.05$) between total pore volume (V_t) (1.000, p = 0.95) and mesopore volume (V_{meso}) (0.999, p = 0.95) (Fig. S4). The higher the porosity of the tested material, the greater the losses of PAHs were observed. We can speculate that PAH losses were related to the processes of sequestration of the studied compounds rather than to their actual losses in the investigated experimental system. High porosity promotes the adsorption of compounds, and these compounds become more difficult to extract even by strong solvents [36]. Mesopores, for which a significant relationship was also observed, could have played an important role in the strong adsorption process (Fig. S4). Based on these relationships, it can be presumed that the losses of PAHs were associated with their transition to hard or non-available forms (sequestration or bound residue) rather than with their biological, chemical, or physical degradation. A previous study also confirms the important role of mesopores in the adsorption of PAHs [47]. The additionally observed negative relationship between the losses of 5-ring C_{tot} PAHs and the H/C ratio (-1.000, p = 0.95) as well as the positive relationship with the (O + N)/C ratio (0.997, p = 0.95) (Table S3) may confirm the previous assumptions. The higher the losses, the lower the H/C value, which means greater losses with increasing aromaticity of the biochar surface. A more aromatic biochar surface always leads to greater adsorption of PAHs, which may evidence the growing intensity of the sequestration processes. When the polarity ((O + N)/C) increases, PAH losses also increase, which leads to weakened interactions of PAHs with the more polar biochar surface. This is additionally confirmed by the significant relationships ($P > 0.05$) between the losses of 6-ring C_{tot} PAHs and the content of C (−1.000, p = 0.95) and organic C (−1.000, p = 0.95) (Table S3). No significant relationships were found between the losses of C_{free} PAHs and the biochar properties (Table S4).

Alongside the biochar properties, the physico-chemical properties of soil can also affect the persistence of PAHs in soils [53,54]. The analysis of relationships between the soil properties and the PAH content, at each assessment time point, showed the DOC content is significantly correlated with the content of Σ16 C_{free} PAHs (0.732, p = 0.95) and the content of 2-ring C_{free} PAHs (0.707, p = 0.95) in the soil amended with the SL-derived biochar (Table S5). Stefaniuk et al. [40] also found a similar relationship, which results from the high affinity of PAHs to DOC [55]. The positive correlation were also observed between pH and Σ16 C_{free} PAHs (0.708, p = 0.95), 2-ring C_{free} PAHs (0.692, p = 0.95), and 5-ring C_{free} PAHs (0.684, p = 0.95). The correlations can be associated with the greater extractability of PAHs with increasing pH, especially in sandy soils [56]. A strong negative correlation was observed for conductivity (EC) in relation to total C_{tot} PAHs (−0.772, p = 0.95), total 2-ring PAHs (−0.688, p = 0.95), total 3-ring PAHs (−0.704, p = 0.95), total C_{free} PAHs (−0.807, p = 0.95), and bioavailable 2-ring PAHs (−0.803, p = 0.95). It was suggested that a salinity increase may contribute to increased adsorption of PAHs by adsorbents, which was confirmed by the present study where the significant correlations between EC and C_{free} PAHs were also observed (Table S5). The effect of salinity/conductivity is explained by the decreasing water solubility of PAHs with the increasing content of inorganic ions in soil water [53]. Increased salinity was also observed to affect the reduced activity of organisms that biodegrade PAHs [57]. A significant relationship between PAH content and soil EC was previously noted by Bengtsson and Törneman [54].

3.3. *Effect of biomass addition on PAHs persistence in SL-derived biochar-amended soil*

Adding the co-pyrolyzed (SL and biomass) biochar (BCSLW) to the soil increased the content of Σ16 PAHs to a lower extent than it was observed for SL-derived biochar (BCSL) (Fig. 1). It was related to the lower (from 7 to 52%) concentration of PAHs in BCSLW- than in the BCSL-derived biochar (Table S6). After adding BCSLW to the soil, the content of Σ16 C_{tot} PAHs ranged from 48.3 to 61.9 μg/kg (depending on the biochar production temperature) and was lower from 32 to 47 % compared to the BCSL-amended soil (Fig. 1b–d). A clear trend was observed as previously, where the content of Σ16 C_{tot} PAHs in the biochar-amended soil increases with increasing pyrolysis temperature, and correlated well with the PAH content in the biochars. The contribution of particular groups of PAHs in BCSLW-amended soil was different compare to BCSL-amended soil (Fig. S5). Generally in BCSLW-amended soil the contribution of the heavy PAHs (in total PAHs content) was lower than in BCSL-amended soil (Fig. S5).

The content of Σ16 C_{tot} PAHs in the BCSLW-amended soil declined with time, the same as it was observed for BCSL-amended soil (Fig. 1). After 180 days, Σ16 C_{tot} PAHs decreased from 14 to 24 % (depending on the biochar production temperature) in comparison with the beginning of the study (Fig. 2a). The highest losses were observed in the soil with BCSLW500, whereas the lowest ones in BCSLW600-amended soil. When comparing the persistence of Σ16 C_{tot} PAHs after 180 days between the experiments with BCSL and BCSLW, lower losses, and thus greater persistence, of Σ16 C_{tot} PAHs were found in the soil with BCSLW compared to BCSL-amended soil. Depending on the pyrolysis temperature, the losses of Σ16 C_{tot} PAHs were lower from 13 to 38% in the soil with BCSLW than in the BCSL-amended one (Fig. 2a). However, when analyzing the particular groups of C_{tot} PAHs, certain variations (dependent on the biochar production temperature) were observed (Fig. 2b–f). For 2-ring PAHs, lower losses were found for BCSLW500 and BCSLW700 (in comparison with BCSL500 and BCSL700), in the case of 3- and 4-ring PAHs for BCSLW600 and BCSLW 700 (in comparison with BCSL600 and BCSL700), while as far as 5- and 6-ring PAHs are concerned, this finding applied to all the biochars. In few cases, an opposite trend (2-ring PAHs for BCSL600) or no differences in PAH persistence were noted between BCSL and BCSLW (3- and 4-ring PAHs with regard to BC500) (Fig. 2b–f).

The presence of biomass in the SL/biomass-derived biochars increased the persistence of PAHs, which could have resulted from the greater affinity of PAHs to this biochar. Our previous study revealed [47] that the affinity of phenanthrene and pyrene to BCSL/BCSLW varies depending on the type of compound. While phenanthrene exhibited a greater affinity for BCSL than for BCSLW, a reverse tendency was observed for pyrene. This is indicated by the trend where a PAH's affinity for biochar increases with its increasing molecular mass. A similar relationship was observed in our study where the persistence of 5- and 6-ring PAHs was higher in the soil with BCSLW (for all temperatures) than in the BCSL-amended soil.

The content of Σ16 C_{free} PAHs in the soil with BCSLW ranged from 35.9 to 40.1 ng/L and, contrary to C_{tot} PAHs, was higher between 4 and 16% than in the soil with the addition of BCSL (Fig. 3). This was associated with the weaker adsorption capacity of BCSLW relative to the light (2-, 3-ring) PAHs than that of BCSL [47]. In the fraction of C_{free} PAHs, the light PAHs were predominant (Fig. S5c, d) and hence this effect was more pronounced.

For 2- and 3-ring C_{free} PAHs, the BCSLW-amended soil was characterized by their higher content compared to the soil with BCSL addition (for all the biochars) (Fig. S2). But as far as the other PAH groups are concerned, certain differences were observed depending on the type of biochar. The higher content of 4-ring PAHs was found in the soil with BCSLW600 than in that BCSL600-amended soil, whereas there were more 5-ring PAHs in the soil amended with BCSLW500 and BCSLW600 than in the soil with BCSL500 and BCSL600. The concentration of 6-ring PAHs in the soil with BCSLW (500, 600, and 700) was lower compared to BCSL.

The changes in the content of the individual groups of C_{free} PAHs in the soil with BCSLW amendment after 180 days differed significantly from the changes relating to these compounds in the BCSL-amended soil (Fig. 2h–l). When analyzing the specific PAH groups, it was found that for 2-, 3-, and 4-ring PAHs higher losses were observed for BCSLW (despite the temperature) (Fig. 2h–j), and for 5-ring PAHs for BCSLW500 and BCSLW600 only (Fig. 2K). As regards 6-ring PAHs, no statistically significant differences ($P > 0.05$) were found between the losses of these compounds in the soils amended with BCSLW and BCSL. Despite that the BCSL biochars were characterized by lower aromaticity and hydrophobicity than the BCSLW biochars (Table S1), the losses of C_{free} PAHs were greater for BCSLW. This also shows different behavior of C_{tot} and C_{free} PAHs depending on the type of feedstock from which the biochar was produced. The presence of biomass in the SL-derived biochar caused increased persistence of C_{tot} PAHs and, at the same time, better reduction of C_{free} PAHs. BCSLW bound (reduced) C_{free} PAHs and therefore they became less easily available/unavailable to microorganisms and contributed to increasing the pool of C_{tot} PAHs. BCSLW reduces C_{free} PAHs (it is more effective in immobilizing PAHs), but at the same time, this biochar limits their physical/biological degradation, making them more resistant to breakdown/degradation, and therefore their persistence increases.

Similarly, as for BCSL, statistically significant relationships between the biochar properties and the losses of C_{tot} and C_{free} PAHs were also observed for BCSLW (Table S3 and S4, Fig. S4). These relationships were sporadic and predominantly related to C_{free} PAHs, which was not previously observed for BCSL. The only significant positive correlation was found between the losses of 2-ring C_{tot} PAHs and micropore volume (V_{micro}) (0.999, $p = 0.95$). The increase in the micropore volume was accompanied by higher losses of 2-ring PAHs, which may suggest that the losses of PAHs were associated with the sequestration processes rather than the actual degradation. A previous study [58] has demonstrated that micropores can participate in the adsorption of NAP, which is due to the small size of their particle (about 0.91 nm). For C_{free} PAHs, on the other hand, positive significant relationships were noted between the losses of Σ16 C_{free} PAHs and the oxygen (O) content in the biochar (0.999, $p = 0.95$) and the (O + N)/C ratio of the biochar (0.999, $p = 0.95$). This is evidence that with the increasing polarity of the biochar surface, the PAH losses were greater. It results from weaker hydrophobic bonds. Due to the weaker binding of PAHs by the biochar surface, the possibility of their biodegradation is greater because they are more easily available to microorganisms. Furthermore, a negative correlation was found between the losses of 5-ring C_{free} PAHs and the specific surface area (S_{BET}) of the biochars (−1.000, $p = 0.95$) as well as their ash content (−1.000, $p = 0.95$) (Table S4, Fig. S4). In the case of adsorption of organic compounds by carbon materials, it is usually strongly positively correlated with the specific surface area of the biochar [59]. The negative correlation observed, in this case, may suggest a different mechanism responsible for 5-ring PAHs adsorption. The negative relationship between the losses of 5-ring PAHs and the ash content may be evidence of the blocking of pores, performing an important role in the adsorption of PAHs, and of weakened hydrophobic properties of the biochar surface [60], which impairs the sorption capacity of the biochar.

Based on the above-mentioned relationships, it can be presumed that different mechanisms are responsible for the PAHs adsorption by BCSLW than BCSL, which is confirmed by our previous study [46]. The adsorption by the BCSLW biochars is regulated by mechanisms based on hydrophobic and π-π EDA interactions. This is promoted by both the physical properties (a higher specific surface area of BCSLW in comparison with BCSL) and chemical properties (C, O, and ash content, O/C ratio value) (Table S5). As regards BCSL, on the other hand, these are processes that are related to pore filling.

Analyzing the relationships between PAH content and soil properties (Table S5), a significant correlation was found between EC and 2-ring C_{tot} PAHs (−0.720, $p = 0.95$), Σ16 C_{free} PAHs (−0.678, $p = 0.95$), 2-ring C_{free} PAHs (−0.698, $p = 0.95$), and 6-ring C_{free} PAHs (−0.671, $p = 0.95$). A statistically significant relationship of total organic carbon with 3-ring (−0.783, $p = 0.95$) and 4-ring (−0.700, $p = 0.95$) C_{free} PAHs was observed. This is associated with the strong binding of bioavailable PAHs by organic matter and due to this their bioavailability substantially decreases [61].

3.4. Sorption capacity of PAHs in BCSL- vs BCSLW-amended soil

The strength of bonds between PAHs and the soil, the SL-amended soil, or the biochar-amended soil can be estimated on the log K_{oc} basis (Fig. 4). Fig. 4 shows the log K_{oc} of NAP and PHE as an example. Throughout the entire study period, the log K_{oc} underwent the slight variations, ranging from 0.03 to 0.94 of the unit. It may indicate that the affinity of the soil/ biochar-amended soil underwent certain changes probably as a result of the changes in the matrix properties. Generally, the log K_{oc} value was lower for BCSLW than for BCSL. However, with

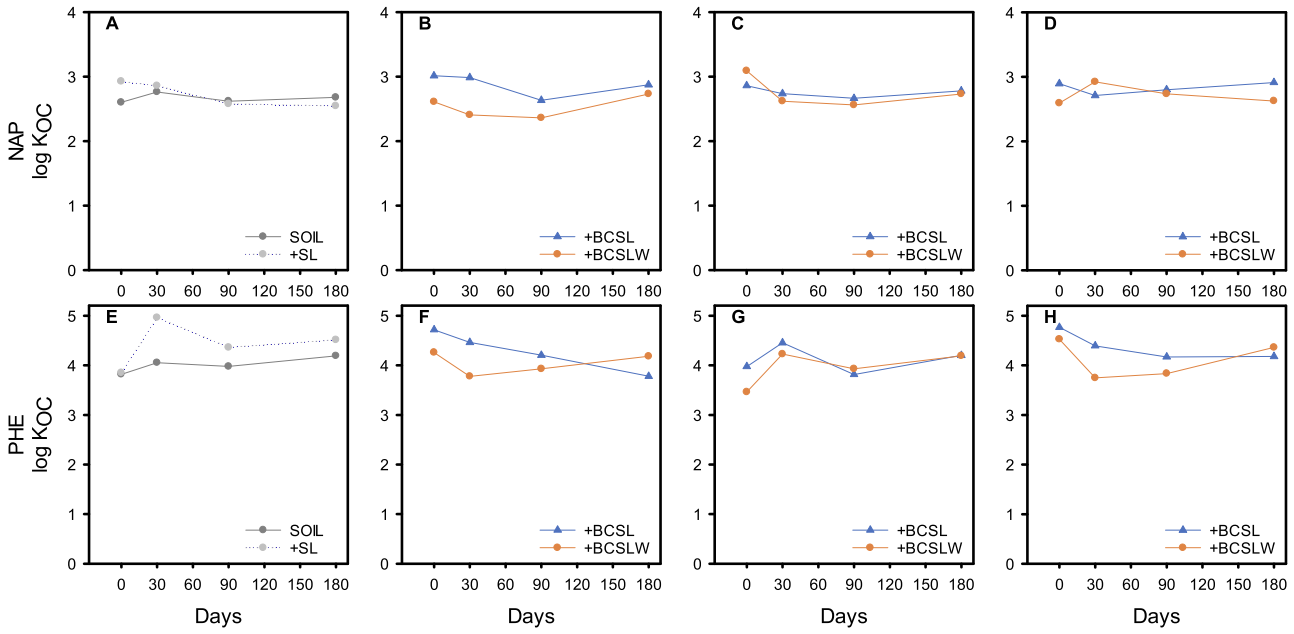

Fig. 4. Log K_{OC} values for naphtalene (NAP) and phenanthrene (PHE) measured during 180 days of the experiment in soil, soil with sewage sludge (+SL), soil with sewage sludge- derived biochar (+BCSL) or soil with sewage sludge with willow- derived biochar (+BCSLW) produced in 500 (B, F), 600 (C, G) or 700 °C (D, H).

time log K_{oc} for BCSLW became equal to or higher than for BCSL. It was especially evident for PHE at the last term (Fig. 4h). The higher values of log K_{oc} at this assessment time point can be explained by the greater persistence (C_{tot}) and higher losses (C_{free}) of PAHs in the BCSLW- than in BCSL-amended soil. This demonstrates that for NAP and PHE in BCSL-amended soil the adsorption equilibrium was reached during the duration of the experiment,. similarly to the NAP in BCSLW600 and BCSLW700-amended soil. The different trend was observed for NAP in BCSLW500- and for PHE in BCSLW600-amended soil where the adsorption equilibrium was not achieved and the adsorption may continue at the expense of a C_{free} PAHs reduction (Fig. 4).

4. Conclusions

The persistence of PAHs (determined based on C_{tot}) in the soil amended with the biochar produced from SL with biomass addition (BCSLW) was greater than in the soil amended with the biochar derived from SL alone (BCSL). This shows that the application of co-pyrolyzed (biomass/SL) biochar contributes to the higher persistence of PAHs in soil than SL-derived biochar. During the same time, however, the bioavailable fraction of PAHs (determined based on C_{free}) in the BCSLW-amended soil had higher losses than in the soil with BCSL. These observations may suggests that immediate environmental risk will be lower for BCSLW than for BCSL because fewer available PAHs will directly affect soil organisms. The greater losses of C_{free} PAHs in the soil with BCSLW than in the BCSL-amended soil, with the simultaneous lower affinity of the PAHs for BCSLW than for BCSL, may suggest that in the soil with BCSLW the losses of PAHs were more associated with the biodegradation processes than in the BCSL-amended soil in which the processes of sequestration or bound residue formation predominated. On the other hand, however, C_{free} PAHs in the BCSLW-amended soil could have enriched the pool of C_{tot} PAHs, contributing to the lower reduction of these compounds during the experiment. The differences in the mechanism of binding of the PAHs by the biochars determined their losses, as shown by the relationships between the losses of these compounds and the properties of the biochars. The different mechanism of bonding of PAHs between BCSL and BCSLW is a reason of observed differences in PAHs behavior in presence of these biochars. In the case of BCSL, the adsorption mechanism was dominated by pore filling, whereas in the case of BCSLW by adsorption based on chemical (hydrophobic and π-π EDA) interactions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cej.2021.132143.

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INTERNET SUPPORTING INFORMATION

Polycyclic aromatic hydrocarbons in co-pyrolyzed (with biomass) sewage sludge behave differently than in alone pyrolyzed sewage sludge after application to soil

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MATERIALS AND METHODS

Sewage sludge

Sewage sludge (SSL) was obtained from municipal wastewater treatment plant where SSL is stabilized biologically by aerobic fermentation and chemically by threated with lime. SSL were collected during autumn 2016 from municipal sewage treatment plant localized in Chełm (southeastern part of Poland, 510705600N, 232804000E, population of 64 000 people). Sewage treatment plant were located on agricultural areas and used mainly urban wastewater without the great impact of the industry.

Biochar preparation

Briefly, the sewage sludge (SL) before pyrolysis was grinded and sieved through a 2 mm sieve. SL was obtained from municipal wastewater treatment plant localized in Chełm (50°20′04″N 23°29′49″E) which uses mainly urban wastewater with limited influence of wastewater from industry. The willow was provided by a biomass-producing farm. Freshly cut willow was air-dried for two weeks and then cut into small pieces and sieved through 2 mm sieve. Mixtures of SL and willow (6:4 w/w) were obtained by mixing both materials in glass bottles (1000 mL) for 24 h in the dark at 10 rpm (Rotax 6.8. VELP, Italy). SL alone and SL with willow were pyrolysed in 500, 600 or 700°C, with the heating rate 10°C/ min. Temperature was held for 3 h (slow pyrolysis). During the pyrolysis the oxygen free atmosphere was maintained by constant flow of N_2. The physico-chemical properties of SL- and SL/biomass-derived biochar are presented in Table S1.

Quality assurance and quality control (QA/QC)

Preparing prior to the application, mixing matrices (biochar, sewage sludge and soil) and controlled the field experiment was by our professional partner. All samples were taken according to the PN-ISO 10381-2:2007P (ISO 10381-2:2002 - Soil quality -- Sampling -- Part 2: Guidance on sampling techniques, 2002). Chemical analyses were conducted at the University of Maria Skłodowska-Curie of Lublin (UMCS, Poland) in Department of Environmental Chemistry and Analytical Laboratory UMCS. The Analytical Laboratory UMCS is accredited by the Polish Centre for Accreditation (PCA). The procedures and methods of the chemical tests in lab were controlled according to existing standards or published papers. The QA/QC checks of the testing instruments (GC-MS, pH meter, TOC-VCSH etc.) in lab were conducted during and after installation by the supplier. To ensure quality assurance and quality control (QA/QC) analyzed blank sample, duplicate sample (n=3) and a standard reference material (PAHs - Loamy Sand, Sigma Aldrich) with each batch of samples. The testing instruments were also calibrated in lab before the chemical analysis. Blank sample values were very low or below detection limits for the corresponding method. For each PAHs, the response factor percent relative standard deviations (% RSDs) typically were 4 to 15% and always less than 24%.

Theoretical consideration of C_{free} content calculation from POM samplers

The concentration of PAH on POM passive samplers (C_{POM}) was calculated according to the equation (1):

$$C_{POM}\ (\mathrm{ng\,kg^{-1}}) = \frac{m_{PAH}\ (\mathrm{ng})}{m_{2POM}\ (\mathrm{kg})} \quad (1)$$

where m_{PAH} (ng) is the mass of PAH determined via GC-MS and m_{2POM} (kg) is the mass of two, used POM passive samplers.

Freely dissolved concentrations were calculated according to equation (2):

$$C_{free}\ (ng\ L^{-1}) = \frac{C_{POM}\ (ng\ kg^{-1}) - C_{control}\ (ng\ kg^{-1})}{K_{POM-w}\ (L\ kg^{-1})} (2)$$

where C_{free} (ng L^{-1}) is the aqueous phase concentration (the pollutant concentration that is considered bioavailable), C_{POM} (ng kg^{-1}) is the measured POM concentration in the sample and $C_{control}$ in the control and K_{POM-w} (L kg^{-1}) is the predetermined POM-water partitioning coefficient specific to each PAH compound obtained from Hawthorne et al. (2011).

Absolute recoveries from a POM spiking experiment were 64.8–92.6% for all 16 PAHs. The data was analyzed using a general linear analysis of variance (ANOVA) model. For multiple comparisons of data means, Tukey's honestly significant difference test with an α = 0.05 was performed.

Solid-water distribution coefficient calculation (K_d)

Solid-water distribution coefficient calculation (K_d) was calculated by the following equation (3):

$$K_d\ (L\ kg^{-1}) = \frac{C_{tot}\ (\mu g/kg\ )}{C_{free}\ (\mu g/\ L)} (3)$$

where C_{tot} is the PAH concentration in soil (µg/kg) and C_{free} the freely dissolved concentration in water (µg/L).

References

Hawthorne, S.B., Jonker, M.T.O., van der Heijden, S.A., Grabanski, C.B., Azzolina, N.A., Miller, D.J., 2011. Measuring Picogram per Liter Concentrations of Freely Dissolved Parent and Alkyl PAHs (PAH-34), Using Passive Sampling with Polyoxymethylene. Anal. Chem. 83, 6754–6761. doi:10.1021/ac201411v

Table S1. Biochars' main physico-chemical properties

	pH	Ash content (%)	C (%)	OC (%)	H (%)	N (%)	O (%)	O/C	H/C	(O+N)/C	S_{BET} (m^2/g)	d (nm)	V_t (m^3/g)	V_{micro} (m^3/g)	V_{meso} (m^3/g)
BCSL500N2	9.4	64.1	26.3	26.2	0.99	3.26	5.38	0.15	0.45	0.26	69.7	5.98	0.104	0.0189	0.0854
BCSL600N2	12.1	67.6	26.5	26.4	0.60	2.93	2.41	0.07	0.27	0.16	75.5	5.86	0.110	0.0211	0.0894
BCSL700N2	12.4	71.4	24.5	24.4	0.29	2.10	1.71	0.05	0.19	0.13	89.2	6.18	0.137	0.0240	0.1139
BCSLW500N2	10.8	46.4	44.6	44.2	1.66	3.33	3.93	0.07	0.44	0.13	74.6	4.66	0.087	0.0207	0.0663
BCSLW600N2	12.1	49.3	45.2	44.9	0.81	2.85	1.86	0.03	0.21	0.08	93.1	4.01	0.104	0.0351	0.0694
BCSLW700N2	12.5	50.9	46.2	46.0	0.62	2.09	0.21	0.02	0.16	0.04	104.1	4.93	0.115	0.0269	0.0881

pH- in H_2O- 1:10 (w/v)

Table S2. Chemical properties of control soil

pH	Total N content (%)	Available nutrients (mg/ 100 g of soil)			Exchangeable cations content (mg/ 100 g of soil)				Hydrolytic acidity (mmol H^+ /100 g of soil)
6.84 in H_2O	0.064	P_2O_5	K_2O	Mg	Mg	Na	K	Ca	1.25
6.80 in KCl		12.2	11.7	2.2	3.50	2.87	10.6	101	

pH in H_2O- 1:10 (m/v), pH in 1M KCl- 1:2.5 (m/v), total N – Kjeldahl method, available P and K – Egner-Riehm method, available Mg – Schachtschabel method, exchangeable cations- Kappen method, hydrolytic acidity- Kappen method

Table S3. Correlations between total PAHs content in soil with biochar addition and biochars' properties. Statistically important values indicated in red (p=0.95)

BCSL	pH	Ash content	C	OC	H	N	O	O/C	H/C	(O+N)/C	S_{BET}	d	V_t	V_{micro}	V_{meso}
Σ16 PAHs	0.708	0.947	-0.966	-0.966	-0.914	-0.994	-0.768	-0.774	-0.842	-0.794	0.993	0.851	1.000	0.963	0.999
2-ring	-0.381	0.065	-0.610	-0.610	0.025	-0.281	0.297	0.288	0.176	0.257	0.268	0.811	0.383	0.120	0.421
3-ring	0.102	0.530	-0.913	-0.913	-0.452	-0.703	-0.191	-0.200	-0.311	-0.232	0.692	0.991	0.775	0.576	0.801
4-ring	0.971	0.767	-0.279	-0.279	-0.821	-0.608	-0.946	-0.943	-0.898	-0.932	0.619	-0.006	0.519	0.730	0.483
5-ring	0.978	0.970	-0.671	-0.671	-0.988	-0.894	-0.993	-0.994	-1.000	-0.997	0.900	0.432	0.840	0.955	0.817
6-ring	0.473	0.813	-1.000	-1.000	-0.758	-0.921	-0.550	-0.558	-0.650	-0.584	0.915	0.967	0.957	0.844	0.969
BCSLW															
Σ16 PAHs	0.963	0.919	0.755	0.766	-0.976	-0.764	-0.875	-0.972	-0.977	-0.874	0.911	-0.278	0.901	0.849	0.571
2-ring	0.643	0.538	0.256	0.271	-0.684	-0.269	-0.451	-0.671	-0.687	-0.450	0.521	-0.770	0.502	0.999	0.011
3-ring	-0.455	-0.334	-0.032	-0.048	0.503	0.046	0.238	0.487	0.506	0.237	-0.316	0.894	-0.294	-0.964	0.215
4-ring	0.467	0.579	0.799	0.790	-0.418	-0.791	-0.657	-0.434	-0.414	-0.658	0.595	0.881	0.613	-0.338	0.922
5-ring	0.914	0.853	0.655	0.666	-0.935	-0.665	-0.797	-0.929	-0.936	-0.796	0.843	-0.412	0.830	0.916	0.449
6-ring	0.894	0.828	0.618	0.631	-0.917	-0.629	-0.768	-0.910	-0.919	-0.767	0.817	-0.454	0.803	0.934	0.406

pH-in H_2O; EC-electrical conductivity (mS cm^{-1}); C, OC, H, N, O-carbon, organic carbon, hydrogen, nitrogen and oxygen total content (%); O/C, H/C, (O+N)/C-molar ratios; S_{BET}-specific surface area of biochars (m^2 g^{-1}); d- mean pore size (nm); V_t- total pore volume (cm^3/g); V_{micro}- micropore volume (cm^3/g); V_{meso} – mesopores volume, V_t–V_{micro} (cm^3/g); The values are mean of three repetitions.

Table S4. Correlations between bioavailable PAHs content in soil with biochar addition and biochars' properties. Statistically important values indicated in red (p=0.95)

BCSL	pH	Ash content	C	OC	H	N	O	O/C	H/C	(O+N)/C	S_{BET}	d	V_t	V_{micro}	V_{meso}
Σ16 PAHs	0.399	0.763	-0.994	-0.994	-0.702	-0.885	-0.480	-0.488	-0.585	-0.516	0.879	0.984	0.930	0.797	0.945
2-ring	0.026	0.464	-0.879	-0.879	-0.383	-0.646	-0.115	-0.125	-0.238	-0.157	0.635	0.978	0.725	0.512	0.753
3-ring	0.921	0.655	-0.123	-0.123	-0.720	-0.474	-0.882	-0.878	-0.817	-0.862	0.486	-0.166	0.376	0.612	0.337
4-ring	0.888	0.595	-0.046	-0.046	-0.664	-0.405	-0.843	-0.838	-0.770	-0.820	0.418	-0.241	0.304	0.549	0.264
5-ring	0.580	0.880	-0.995	-0.995	-0.834	-0.962	-0.651	-0.658	-0.740	-0.682	0.958	0.927	0.986	0.905	0.992
6-ring	-0.642	-0.238	-0.343	-0.343	0.324	0.020	0.571	0.563	0.464	0.536	-0.034	0.597	0.088	-0.184	0.129
BCSLW															
Σ16 PAHs	-0.964	-0.990	-0.986	-0.988	0.948	0.988	0.999	0.953	0.946	0.999	-0.993	-0.260	-0.995	-0.453	-0.914
2-ring	-0.822	-0.740	-0.501	-0.514	0.852	0.512	0.669	0.842	0.854	0.668	-0.727	0.576	-0.711	-0.975	-0.272
3-ring	0.189	0.059	-0.247	-0.232	-0.242	0.234	0.042	-0.225	-0.246	0.043	0.039	-0.983	0.016	0.852	-0.478
4-ring	0.459	0.338	0.037	0.052	-0.507	-0.050	-0.243	-0.491	-0.510	-0.242	0.320	-0.892	0.298	0.965	-0.210
5-ring	-0.992	-1.000	-0.951	-0.955	0.984	0.955	0.994	0.987	0.983	0.994	-1.000	-0.117	-0.999	-0.578	-0.845
6-ring	0.200	0.326	0.598	0.585	-0.146	-0.587	-0.419	-0.164	-0.142	-0.420	0.345	0.978	0.366	-0.589	0.777

pH-in H_2O; EC-electrical conductivity (mS cm^{-1});C, OC, H, N, O-carbon, organic carbon, hydrogen, nitrogen and oxygen total content (%); O/C, H/C, (O+N)/C-molar ratios; S_{BET}-specific surface area of biochars (m^2 g^{-1}); d- mean pore size (nm); V_t- total pore volume (cm^3/g); V_{micro}- micropore volume (cm^3/g); V_{meso} – mesopores volume, V_t–V_{micro} (cm^3/g); The values are mean of three repetitions.

Table S5. Correlations between PAHs content in soil with biochar addition and their properties. Statistically important values indicated in red (p=0.95)

S+BCSL		pH	EC (uS/cm)	TOC (%)	DOC (mg/L)
Total	Σ16 PAHs	0.342	-0.772	0.187	0.387
	2-ring	0.202	-0.688	0.178	0.328
	3-ring	0.342	-0.704	0.401	0.305
	4-ring	0.100	-0.387	-0.122	0.131
	5-ring	0.353	-0.243	0.121	0.276
	6-ring	0.240	-0.159	0.236	0.297
Bioavailable	Σ16 PAHs	0.708	-0.807	0.299	0.732
	2-ring	0.692	-0.803	0.269	0.707
	3-ring	0.065	0.079	0.271	0.172
	4-ring	-0.185	0.086	0.182	-0.094
	5-ring	0.684	-0.494	0.241	0.525
	6-ring	-0.028	-0.611	-0.062	0.076
S+BCSLW		pH	EC (uS/cm)	TOC (%)	DOC (mg/L)
Total	Σ16 PAHs	-0.020	-0.427	-0.211	0.062
	2-ring	0.158	-0.720	-0.151	-0.239
	3-ring	0.033	-0.267	0.175	0.241
	4-ring	-0.345	0.437	-0.326	0.367
	5-ring	-0.071	0.123	-0.451	0.025
	6-ring	-0.346	0.370	-0.377	-0.018
Bioavailable	Σ16 PAHs	0.478	-0.678	-0.353	0.029
	2-ring	0.535	-0.698	-0.249	0.023
	3-ring	-0.116	-0.215	-0.783	0.053
	4-ring	-0.083	-0.070	-0.699	0.041
	5-ring	0.224	-0.337	-0.319	0.265
	6-ring	0.174	-0.671	-0.156	-0.345

Table S6. Organic-solvent extractable (C_{tot}) and freely dissolved (C_{free}) polycyclic aromatic hydrocarbons content in soil, sewage sludge (SL), biochar produced from SL (BCSL) or SL and willow (BCSLW) at different temperature (500, 600, 700°C)

Experiment	Organic-solvent extractable (C_{tot}) PAHs content (µg/kg)																
	NAP	ACY	ACE	FLU	PHE	ANT	FLT	PYR	BaA	CHR	BbF	BkF	BaP	IcdP	DahA	BEP	Σ16 PAHs
Soil	12.4 ±2.1	1.0 ±0.17	1.7 ±0.23	1.9 ±0.09	5.6 ±0.7	0.5 ±0.11	3.3 ±0.17	3.3 ±0.04	1.1 ±0.03	2.4 ±0.34	2.2 ±0.28	1.8 ±0.09	1.2 ±0.09	1.7 ±0.03	0.45 ±0.02	0.02 ±0.001	40.5 ±7.5
SL	886.3 ±95.1	34.7 ±5.12	227.7 ±31.7	369.4 ±28.5	1163.0 ±146.0	2.30 ±0.32	711.9 ±59.7	521.0 ±41.2	133.5 ±11.1	232.6 ±19.4	29.1 ±2.16	342.7 ±41.8	187.4 ±13.4	143.4 ±11.2	1148.1 ±121.2	141.3 ±12.7	6274.5 ±593.2
BCSL500	968.3 ±84.1	57.8 ± 4.12	47.3 ±3.96	161.4 ±15.1	767.1 ±59.1	33.9 ±2.96	35.2 ±4.05	29.0 ±2.53	148.5 ±15.2	10.3 ±1.09	<DL	<DL	3.95 ±0.26	<DL	<DL	<DL	2262.9 ±152.1
BCSL600	200.0 ±28.2	66.7 ± 5.71	146.9 ±12.3	140.2 ±13.7	973.6 ±82.3	58.1 ±5.42	34.5 ±3.97	27.9 ±2.23	66.9 ±5.97	5.52 ±0.71	2.08 ±0.16	4.98 ±0.51	3.93 ±0.13	<DL	<DL	<DL	1729.5 ±114.2
BCSL700	166.3 ±11.7	59.7 ± 4.36	100.2 ±9.36	154.1 ±10.9	784.2 ±65.4	28.3 ±1.98	28.2 ±1.88	28.5 ±2.44	73.9 ±7.11	8.65 ±0.37	8.72 ±0.43	1.76 ±0.12	6.50 ±0.47	<DL	<DL	<DL	1448.9 ±121.4
BCSLW500	601.5 ±58.7	25.4 ±2.37	53.5 ±4.23	82.7 ±7.12	250.7 ±21.0	10.5 ±1.09	15.7 ±1.39	26.7 ±2.19	1.97 ±0.12	5.25 ±0.41	1.75 ±0.17	0.25 ±0.01	3.05 ±0.24	<DL	<DL	<DL	1079.0 ±114.4
BCSLW600	708.4 ±61.7	25.7 ±1.99	60.0 ±5.23	68.8 ±5.78	183.7 ±20.2	18.1 ±1.36	15.7 ±1.67	18.9 ±1.74	0.97 ±0.03	3.87 ±0.29	1.90 ±0.13	0.59 ±0.02	2.55 ±0.21	<DL	<DL	<DL	1109.2 ±121.4
BCSLW700	1038.9 ±98.1	24.6 ±2.81	45.4 ±4.08	56.5 ±4.24	140.2 ±13.2	8.09 ±0.71	12.0 ±1.08	16.6 ±1.53	1.14 ±0.14	6.53 ±0.57	0.84 ±0.03	0.96 ±0.06	2.10 ±0.18	<DL	<DL	<DL	1353.9 ±98.7

	Freely dissolved (C_{free}) PAHs content (ng/L)																
	NAP	ACY	ACE	FLU	PHE	ANT	FLT	PYR	BaA	CHR	BbF	BkF	BaP	IcdP	DahA	BEP	Σ16 PAHs
Soil	52.40 ±2.7	0.24 ±0.02	1.01 ±0.17	0.73 ±0.03	1.44 ±0.014	0.16 ±0.005	0.15 ±0.007	0.17 ±0.002	0.01 ±0.001	0.02 ±0.001	0.005 ±0.001	0.005 ±0.001	0.008 ±0.001	0.002 ±0.001	0.001 ±0.001	0.003 ±0.001	56.3 ±3.58
SL	63.2 ±6.21	5.98 ±0.71	99.6 ±8.54	71.5 ±6.14	98.5 ±8.21	1.81 ±0.21	22.9 ±1.96	14.3 ±1.36	0.19 ±0.02	0.17 ±0.01	0.14 ±0.02	0.12 ±0.02	0.10 ±0.01	0.04 ±0.003	0.02 ±0.001	0.03 ±0.002	378.6 ±29.5
BCSL500	40.7 ±4.06	0.11 ±0.01	0.27 ±0.02	0.72 ±0.03	1.06 ±0.09	1.33 ±0.13	0.04 ±0.001	0.06 ±0.002	0.004 ±0.001	0.003 ±0.001	<DL	<DL	0.03 ±0.001	<DL	<DL	<DL	44.3 ±3.14
BCSL600	45.9 ±3.94	0.25 ±0.03	0.45 ±0.05	0.78 ±0.04	1.31 ±0.17	1.47 ±0.15	0.05 ±0.001	0.07 ±0.001	0.03 ±0.001	0.06 ±0.002	0.01 ±0.001	0.01 ±0.001	0.01 ±0.001	<DL	<DL	<DL	50.5 ±4.29
BCSL700	42.5 ±3.85	0.22 ±0.01	0.33 ±0.04	0.65 ±0.03	0.91 ±0.05	1.32 ±0.18	0.05 ±0.001	0.07 ±0.002	0.004 ±0.001	0.002 ±0.001	0.003 ±0.001	0.011 ±0.001	0.04 ±0.001	<DL	<DL	<DL	46.1 ±4.18

BCSLW500	26.4 ±3.06	0.17 ±0.02	0.41 ±0.03	0.72 ±0.03	1.17 ±0.18	1.65 ±0.11	0.05 ±0.002	0.06 ±0.002	0.002 ±0.001	0.003 ±0.001	0.05 ±0.002	0.05 ±0.002	0.01 ±0.001	<DL	<DL	<DL	30.8 ±3.51
BCSLW600	24.9 ±2.53	0.27 ±0.03	2.92 ±0.23	0.76 ±0.04	0.30 ±0.21	0.98 ±0.10	0.04 ±0.002	0.06 ±0.002	<DL	<DL	0.01 ±0.003	0.03 ±0.001	0.08 ±0.003	<DL	<DL	<DL	30.4 ±3.63
BCSLW700	27.2 ±3.10	0.32 ±0.02	2.02 ±0.30	0.99 ±0.03	1.47 ±0.13	2.01 ±0.16	0.06 ±0.002	0.08 ±0.003	<DL	<DL	0.01 ±0.003	0.02 ±0.001	0.01 ±0.004	<DL	<DL	<DL	34.2 ±4.09

DL-detection limit.

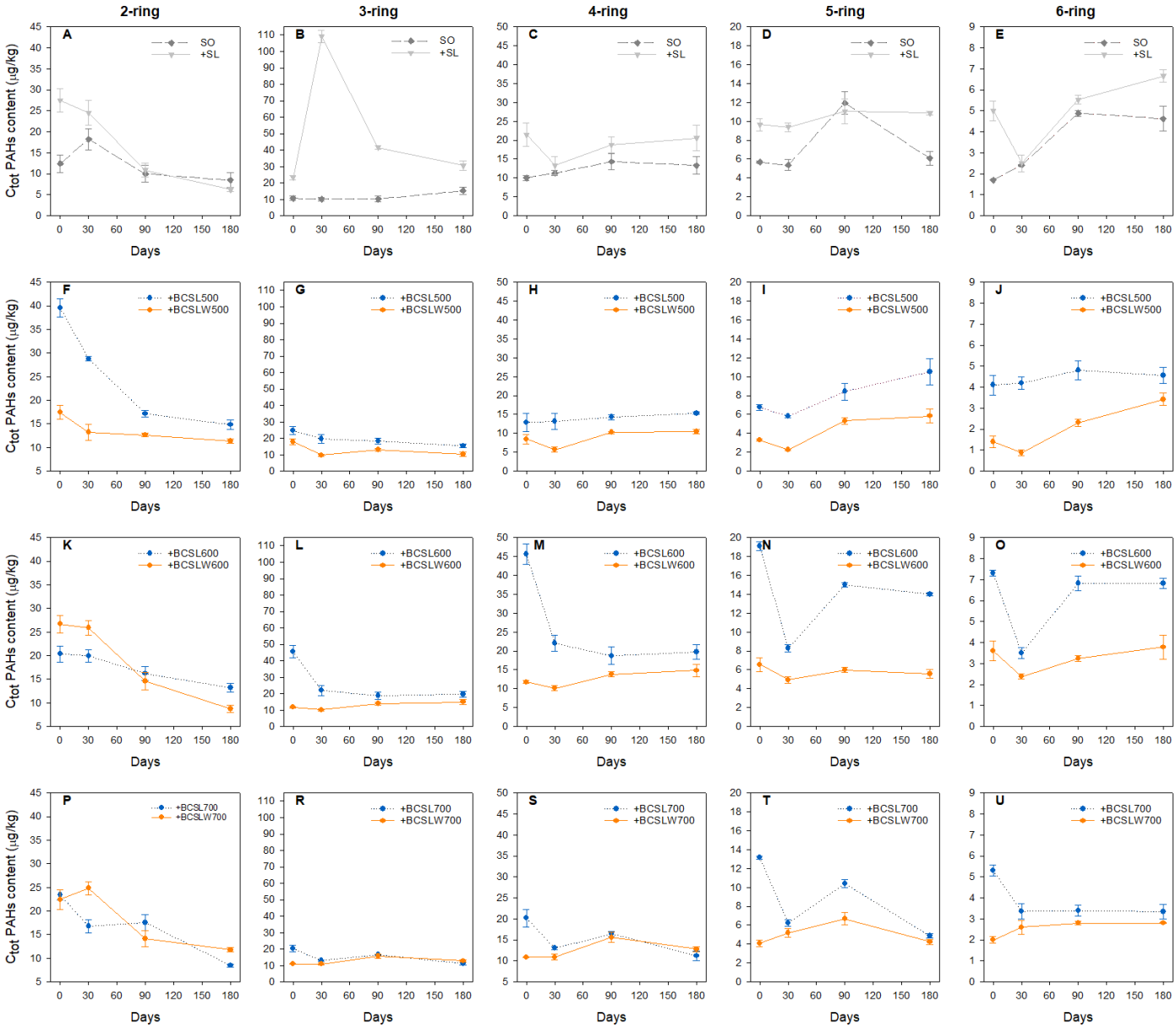

Fig. S1. Changes in C_{tot} PAHs (2-6-ring C_{tot}) in soil (SO), soil with sewage sludge addition (+SL), soil with SL- derived biochar (+BCSL) or soil with SL with willow- derived biochar (+BCSLW) during 180 days of experiment.

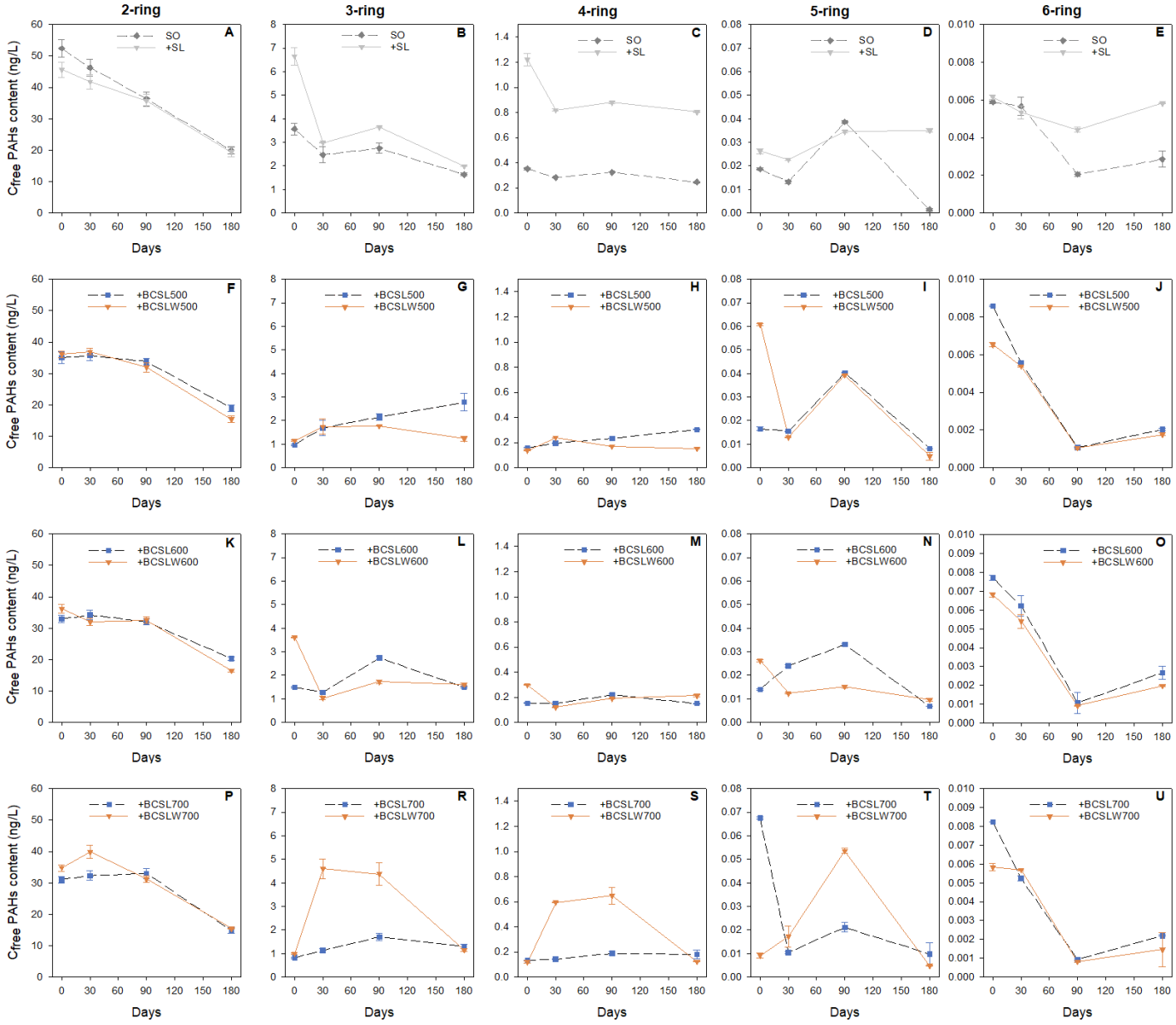

Fig. S2. Changes in C_{free} PAHs (2-6-ring C_{free}) in soil (SO), soil with sewage sludge addition (+SL), soil with SL-derived biochar (+BCSL) or soil with SL with willow- derived biochar (+BCSLW) during 180 days of experiment.

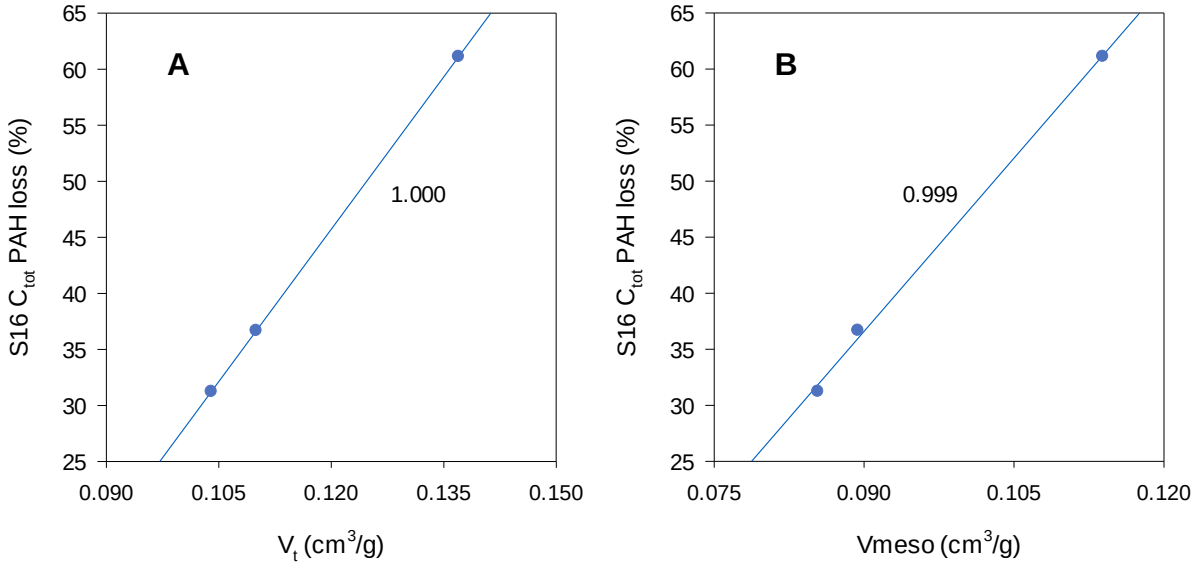

Fig. S3. Correlation between SL- derived biochar properties and PAH losses.

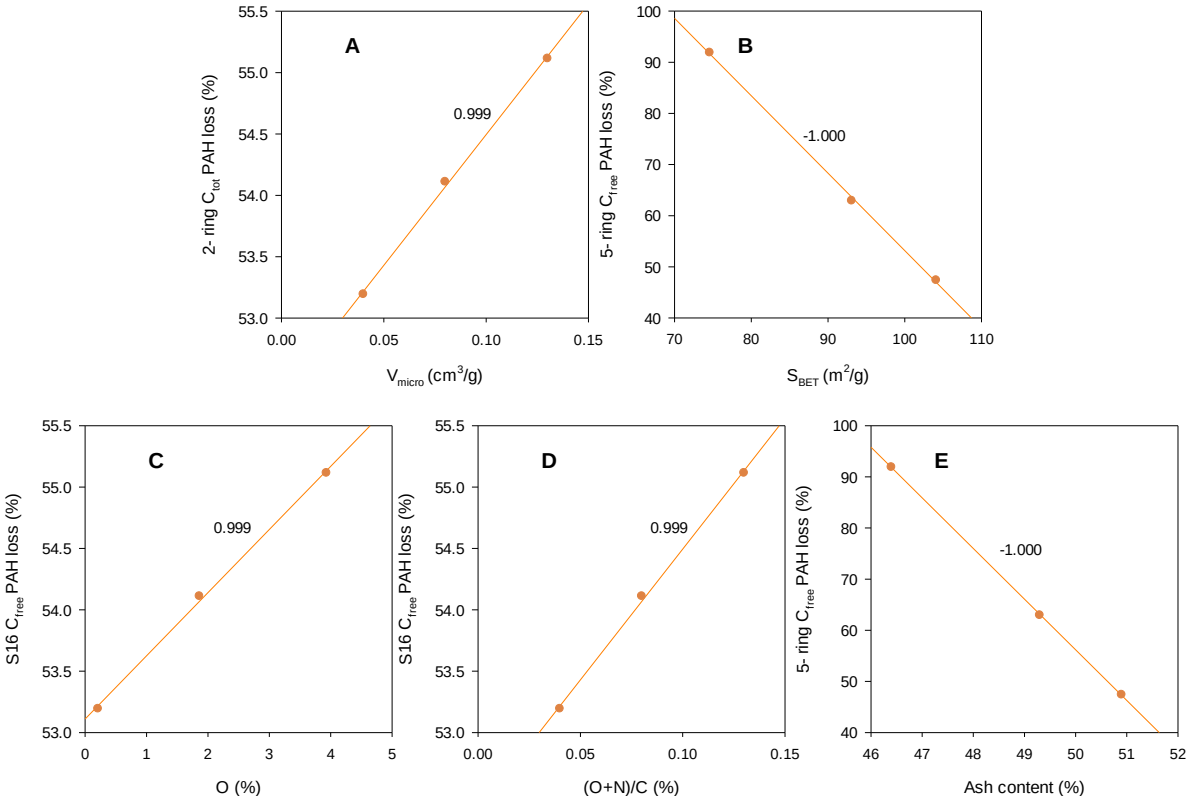

Fig. S4. Correlation between SL and willow- derived biochar properties and PAH losses.

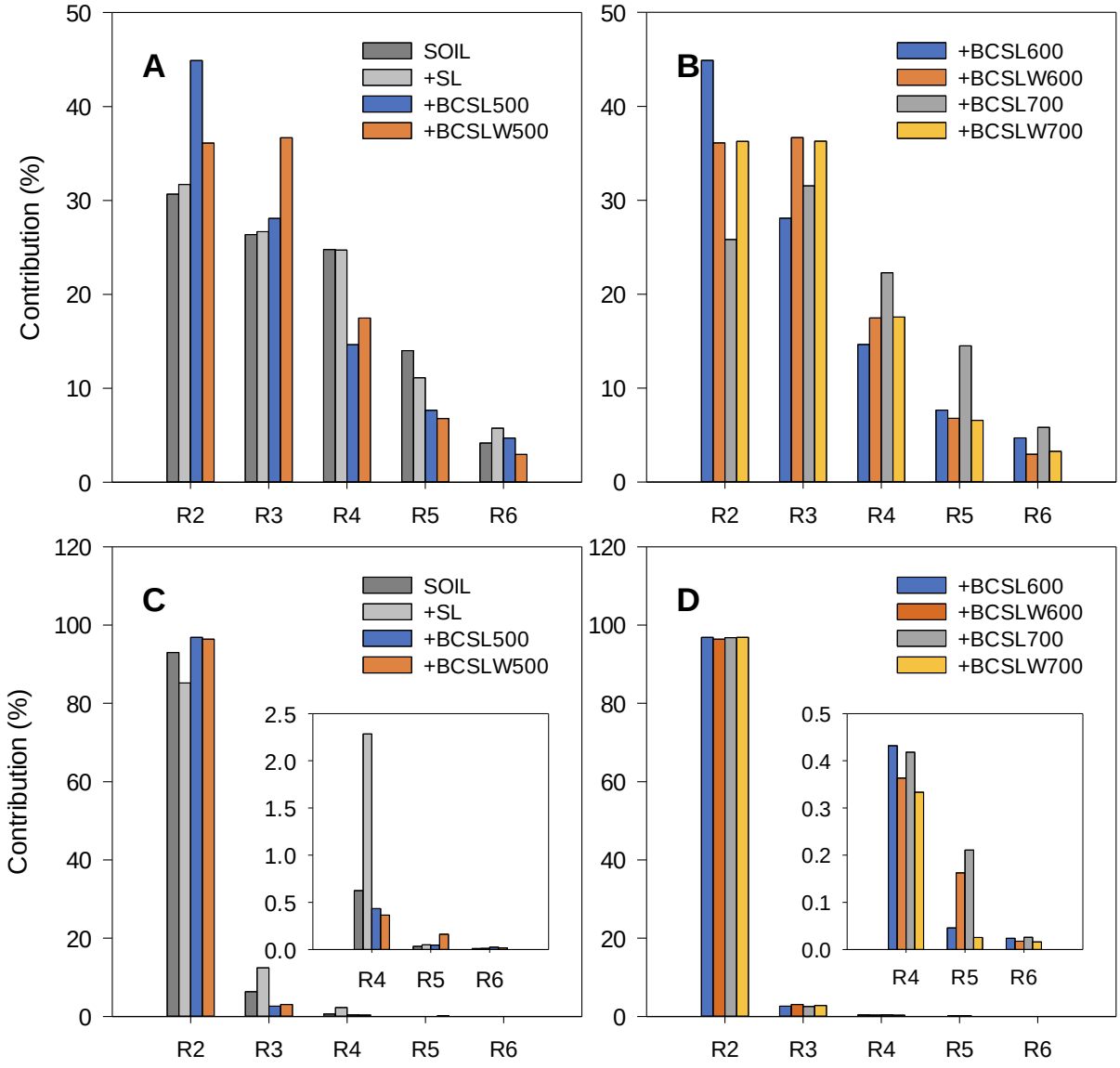

Fig. S5. Organic solvent extractable (C_{tot}) and freely dissolved (C_{free}) PAHs group contribution (%) in soil, soil with sewage sludge (+SL), with SL-derived biochar (+BCSL) or SLW- derived biochar (+BCSLW) pyrolyzed in 500, 600 or 700^{o}C at the beginning of the experiment. R2- 2 ring PAHs, R3- 3-ring PAHs etc.

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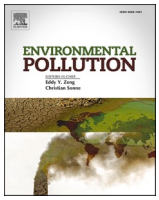

Ecotoxicity of sewage sludge- or sewage sludge/willow-derived biochar-amended soil☆

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ABSTRACT

Co-pyrolysis of sewage sludge (SL) with plant biomass gains attention as a way to minimize SL-derived biochar drawbacks, such as high amount of toxic substances, low specific surface area and carbon content. The toxicity of soil amended with SL- (BCSL) or SL/biomass (BCSLW)-derived biochar was evaluated in long-term pot experiment (180 days). The results were compared to SL-amended soil. Biochars produced at 500, 600, or 700 °C were added to the soil (podzolic loamy sand) at a 2% (w/w) dose. Samples were collected at four different time points (at the beginning, after 30, 90 and 180 days) to assess the potential toxicity of SL-, BCSL- or BCSLW-amended soil. The bacteria *Aliivibrio fischeri* (luminescence inhibition – Microtox), the plant *Lepidium sativum* (root growth and germination inhibition test – Phytotoxkit F), and the invertebrate *Folsomia candida* (mortality and reproduction inhibition test – Collembolan test) were used as the test organisms. Depending on the organism tested and the sample collection time point variable results were observed. In general, SL-amended soil was more toxic than soil with biochars. The leachates from BCSLW-amended soil were more toxic to *A. fischeri* than leachate from BCSL-amended soil. A different tendency was observed in the case of phytotoxicity. Leachate from BCSL-amended soil was more toxic to *L. sativum* compared to BCSLW-amended soil. The effect of biochars on *F. candida* was very diversified, which did not allow a clear trend to be observed. The toxic effect of SL-, BCSL- or BCSW-amended soil to particular organisms was observed in different time, point's periods, which may suggest the different factors affecting this toxicity.

1. Introduction

The generation of sewage sludge (SL) is from 100 up to 350 thousands of tons per year in particular European countries (Grobelak et al., 2019) or even up to 44 million tons per year in China (Guo et al., 2020). SL consists mainly of organic matter and elements such as phosphorous and nitrogen, which are crucial from an agricultural point of view (Agrafioti et al., 2013; Raheem et al., 2018). Unfortunately, the SL may contain toxic substances, (heavy metals (HM), perfluorinated compounds, pharmaceuticals, and other personal care products as well as polycyclic aromatic hydrocarbons (PAHs). Other problematic components of SL are pathogens and the eggs of parasites (Agrafioti et al., 2013; Fijalkowski et al., 2017). For the reuse of SL, for example as a fertilizer, it is necessary to put it through a conversion process, which will reduce the amount of chemical and biological hazardous factors (Frišták et al., 2018; Raheem et al., 2018). One way of reducing the risk behind reusing SL is pyrolysis (Cieślik et al., 2015), that is, the thermochemical conversion of organic matter under low-oxygen conditions at high temperature (Godlewska et al., 2021; Roy and Dias, 2017). SL-derived biochar is usually safer than raw SL (Chagas et al., 2021; Chen et al., 2020; Gopinath et al., 2021; Singh et al., 2020; Zielińska et al., 2015), though biochars still can have higher HM and PAHs levels compared to biochars from plant material (Kong et al., 2019; Xing et al., 2019; Zielińska et al., 2015). To minimize the potential negative aspects connected with SL-derived biochar, while at the same time keeping its environmentally valuable components, it was proposed to co-pyrolyse SL and biomass (Huang et al., 2017; Jin et al., 2017; Kończak et al., 2019a; Wang et al., 2018, 2019). By using such a conversion process, potentially negative outcomes related to SL-derived biochar (by reducing the bioavailability of metals and PAHs) are eliminated (Jin

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et al., 2017; Kończak et al., 2019a; Kończak and Oleszczuk, 2020) and improvement of some of the physico-chemical properties is also observed (Huang et al., 2017; Jin et al., 2017; Kończak et al., 2019b). It should also be stressed that toxic substances in biochar are not the only possible source of its toxicity toward organisms (Godlewska et al., 2021). The observed toxic effect on different organisms very often results from the physico-chemical properties of biochar, which indirectly determine the properties of the environment/soil (higher pH, salinity, availability of nutrients), in which those organisms are functioning (Godlewska et al., 2021; Lehmann et al., 2011; Prodana et al., 2019; Ren et al., 2018). Expanding the scope of physico-chemical and contaminants analysis by adding ecotoxicological tests makes it more appropriate and actual to determine the potential risk. Additionally, the ecotoxicological analysis takes into account possible interactions between the contaminants in the direct and indirect context of physico-chemical soil factors. SL-derived biochars added to soil showed an adverse effect on microorganisms, plants, and invertebrates (Kong et al., 2019; Mierzwa-Hersztek et al., 2018; Simón et al., 2018). However SL/B-derived biochar was observed to have diversified toxic effects in comparison to SL-derived biochar (Gondek et al., 2014; Kończak et al., 2020). Biochar produced from SL with the addition of willow (Kończak et al., 2020) was less toxic for *L. sativum, F. candida*, and *A. fischeri* compared to biochar produced from SL alone. It has been shown (Gondek et al., 2014) that biochar produced from SL with the addition of rape straw, wheat straw, sawdust or the bark of coniferous trees was more toxic for *A. fischeri*, but less toxic for *L. sativum* compared to biochar produced from SL only. So far, research has only been into a direct comparison of biochars produced from SL and a mixture of SL and biomass (Gondek et al., 2014; Kończak et al., 2020), overlooking the soil effect. The effect of soil on the possible toxicity of biochar is extremely crucial because it can significantly change (increase or reduce) its toxicity compared to that measured directly (without the soil). In particular, there is a lack of studies into the long-term use of co-pyrolyzed biochar. Moreover, determination of the effect of biochar added to soil on organisms is essential because of the common usage of biochar as a soil additive. Biochar produced from SL and biomass could be less toxic for organisms compared to biochar produced just from SL (Kończak et al., 2020), which would influence the higher safety of using it in the environment. This hypothesis needs validation in long-term systems where the toxicity of the biochar is determined in soil.

The aim of the study was to determine the toxicity of soil with the addition of biochar produced from biomass and SL as a method for eliminating a potential undesirable impact of SL-derived biochar on the environment. The influence was evaluated for organisms representing various trophic groups (plants, bacteria, and arthropods) in a pot experiment during a 180-day incubation of biochar with soil. In addition, the influence of the pyrolysis temperature on the toxic effect was determined. Both the solid phase and leachates from soil were evaluated. The study was based on two hypotheses that (1) biochar-amended soil will be less toxic than SL-amended soil because biochar's higher affinity to potential toxic elements and compounds (2) soil amended with co-pyrolyzed biochar will be longer less toxic than soil amended with SL-derived biochar to all of the tested organisms due to higher carbon content and stability of co-pyrolyzed biochar.

2. Materials and methods

2.1. Biochars and soil

Detailed information about feedstock properties, biochar production conditions, and the properties of the biochar produced depending on temperature was presented in our previous publications (Kończak et al., 2019a, 2019b). The physico-chemical properties of SL- and SL/biomass-derived biochar are presented in Table S1.

The soil was obtained from the Bezek Experimental Station Poland, 51°12′06′′N 23°16′06′′E) (Poland). The soil was classified as podzolic soil lying on marl substrate with the granulometric composition of loamy sand (according to World Reference Base for Soil Resources) situated in a warm temperate climate zone. The physico-chemical properties of soil are presented in Table S2.

2.2. Pot experiment

The experiment was carried out in plastic containers (20 L). Dry SL-(BCSL) or SL/biomass-derived biochars (BCSLW) were added to soil at the amount of 2% w/w (dry weight) and mixed to prepare a homogeneous mixture. The mixture was placed in the containers and the amount of water was set to 60% of soil water holding capacity. Soil moisture was maintained by weighing the pots periodically and adding necessary deionized water to bring the soils to 60% water holding capacity. The experiment was carried out at a constant temperature (23 ± 2 °C) and the dark. Samples (about 50 g) for the analyses were collected at the beginning of the experiment (0) and after 30, 90, and 180 days from the beginning of the experiment. Samples were then air-dried for 24 h and stored in the fridge.

2.3. Ecotoxicological tests

Liquid phase tests were performed on bacteria and plants. Elutriates were obtained according to the EN 12457-2 protocol (EN 12457-2, 2002). The samples were mixed with deionized water in a single-stage batch-test performed at a liquid-to-solid (L/S) ratio of 100 g/L for 24 h. The glass bottles were shaken in a roller-rotating device at 10 rpm. The extracts were filtered by filter with a porosity of 0.45 μm. The Microtox® Toxicity Test was used to evaluate the inhibition of the luminescence in the marine bacteria *A. fischeri* according to the test protocol (Johnson, 2005). The tests were carried out using a Microtox M500 analyzer. The light output of the luminescent bacteria from soils' leachates was compared with the light output of a blank control sample. The test on plants (*Lepidium sativum*) was performed according to OECD procedure (OECD, 1984).

Solid-phase tests were carried out using plants (*L. sativum*) and springtails (*Folsomia candida*). To evaluate the effect of samples on plants, Phytotoxkit F test was used ISO guideline 18763 (ISO, 2016). The mortality and reproduction of *F. candida* were monitored. The ISO guideline 11267 for testing chemical effects on the reproduction of springtails was followed (ISO, 1999). The bioassays were performed in three replicates. Detailed information about all tests is presented in supporting information.

3. Results

3.1. Leachates toxicity from control and SL-amended soil

Leachates from the control soil were toxic to *A. fischeri* inhibiting the luminescence by 42% (Fig. 1A). This is a typical phenomenon often observed for natural soils resulting from specific soil parameters, and also with low contaminant content. The *A. fischeri* test is very sensitive (Doherty, 2001), which is why even relatively low concentrations of toxic substances can show a toxic matrix effect. During the next sampling time periods, the toxicity varied (Fig. 1B and C) and eventually reached the 9% level after 180 days (Fig. 1D). The soil leachates from control also inhibited the growth of *L. sativum* roots at the 15% level (at the beginning) (Fig. 2), increasing the negative effect to 45% and 53% on the 30th and 90th days of the experiment respectively. By the final time point, the toxicity of control soil decreased insignificantly ($p > 0.05$), though still at a high 45% level.

Adding SL to soil decreased its toxicity only toward *A. fischeri* (Fig. 1A) at the beginning of the study. After 180 days (Fig. 1D), however, leachates from SL-amended soil inhibited the luminescence of *A. fischeri* 73% more than the control soil. Adding SL to soil also had a negative impact on *L. sativum* (Fig. 2A). Leachates from SL-amended soil

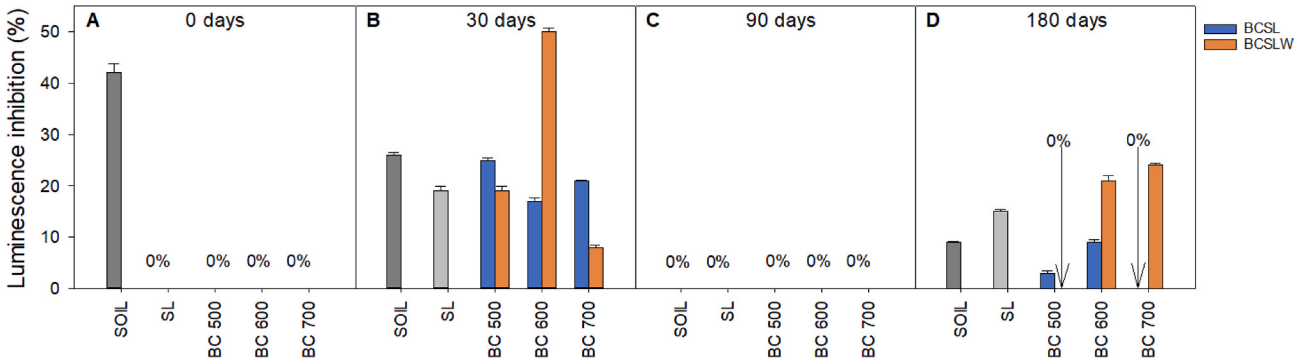

Fig. 1. *Allivibrio fischeri* luminescence inhibition (%) in Microtox® test.

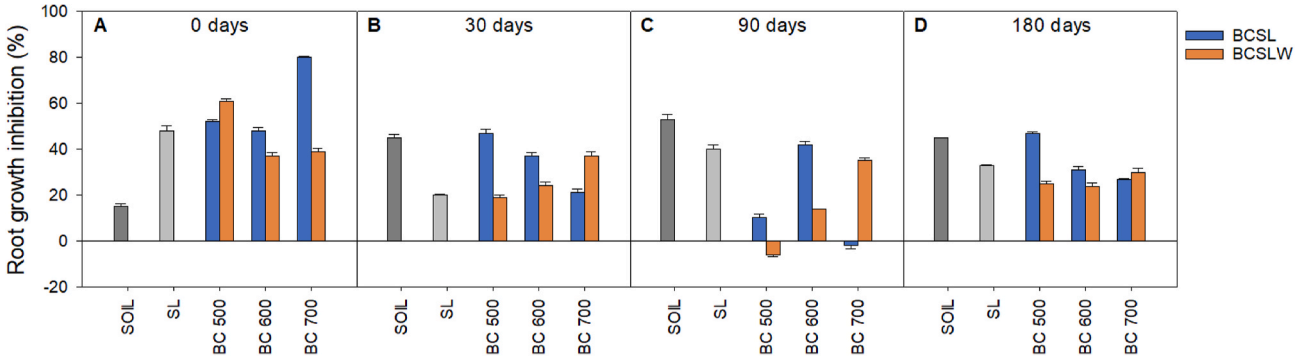

Fig. 2. *Lepidium sativum* root growth inhibition (positive values) or stimulation (negative values) (%) in liquid phase test after 0 days (A), 30 days (B), 90 days (C) and 180 days (D) of the experiment.

inhibited root growth 2-times more than leachates from the control soil. During the subsequent time periods, the toxicity of leachates from SL-amended soil decreased and the root growth inhibition of *L. sativum* was from 25 to 55% lower than for leachates from the control soil (Fig. 2B–D).

3.2. Solid-phase toxicity of SL-amended soil

Adding SL to soil caused inhibition of root growth in solid-phase tests (Phytotoxkit F) (Fig. 3A), which could also be observed for leachates (Fig. 2A) at the beginning of the study. In contrast to the leachates, however, over time the toxic effect was enhanced in SL-amended soil (Fig. 3B and C), thus in the final time period (Fig. 3D) toxicity reached a level of 77%. The toxicity of the SL-amended soil was higher than in the control soil by 77% (Fig. 3). The differences in the toxicity observed between leachates, and in the solid phase especially in the final stage of experiment may suggest that the possible toxic factor (that was dissolved in water) degraded over time or was strongly bound to a solid phase what resulted in decreasing the toxicity of the leachates. This could be antibiotics, commonly occurring in sewage sludge (Buta et al., 2021), whose toxicity toward the plants was confirmed in previous studies (Carballo et al., 2021; Pollock et al., 1983; Timmerer et al., 2020). However, increasing the solid-phase toxicity over time indicates that in sewage sludge occur other substances insoluble in water but present in solid matrix that were released over time and adversely affect plants. The release of these substances may occur during the mineralization of sewage sludge's organic matter (Luthy et al., 1997).

In the contrast, the harmful influence of SL on *F. candida* mortality was not observed at the beginning of the study (Fig. 4A) and a stimulating effect on *F. candida* was noted (Fig. 5A). Nevertheless, after 30 days, SL caused a significant ($P > 0.05$) increase in the *F. candida* mortality at the level of 93% (Fig. 4B). Over the following time periods, the mortality of *F. candida* gradually decreased and in the last stage of the experiment reached 44% (Fig. 4D). While at the beginning of the

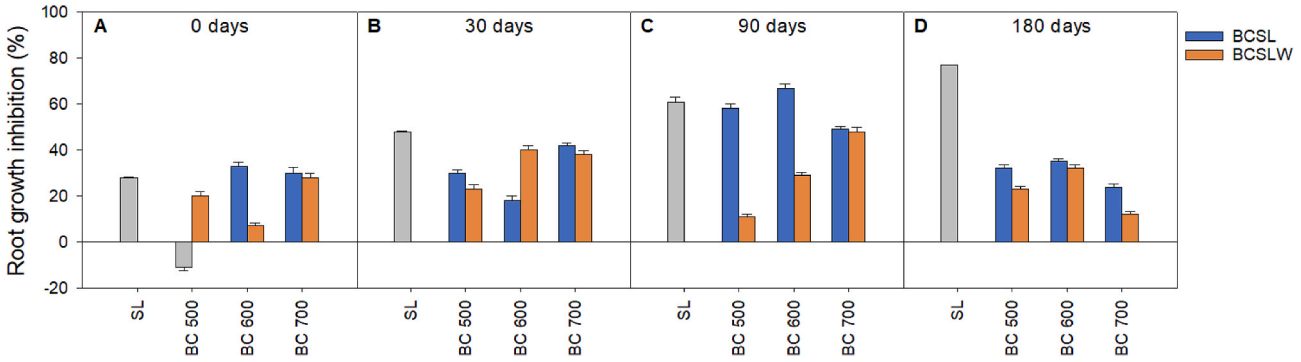

Fig. 3. *Lepidium sativum* root growth inhibition (positive values) or stimulation (negative values) (%) in solid phase test during the experiment.

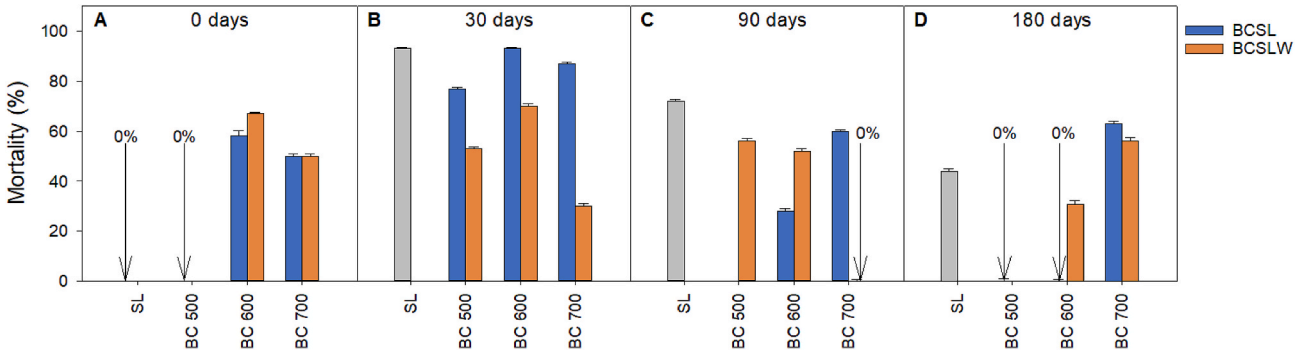

Fig. 4. *Folsomia candida* mortality (%).

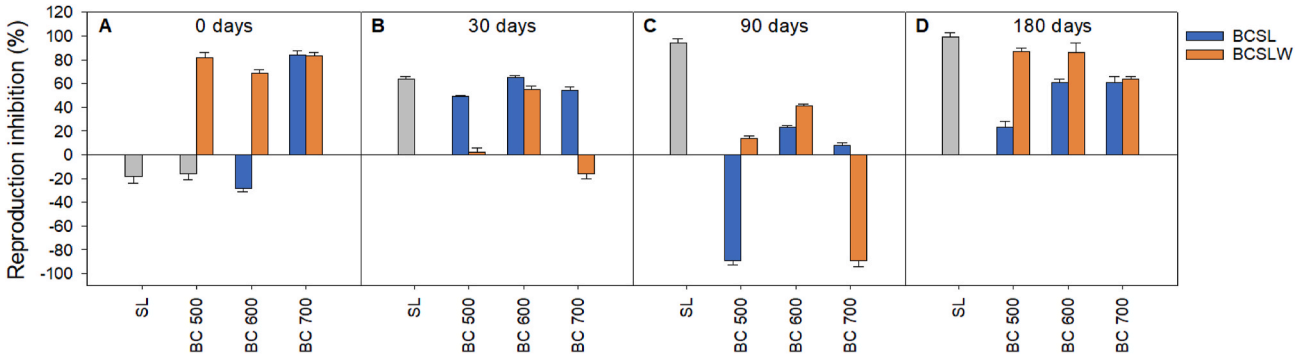

Fig. 5. *Folsomia candida* reproduction inhibition (positive change) or stimulation (negative change) (%).

study stimulation of the reproduction of *F. candida* was observed, in the following months gradual reproduction inhibition was observed, which reached the 99% at the end of the study (Fig. 5D).

3.3. Toxicity of leachates from BCSL-amended soil

Adding SL-derived biochars produced at 500, 600, and 700 °C to the soil entirely reduced its toxicity toward *A. fischeri* (Fig. 1A). After 30 days of the experiment an increase in luminescence inhibition was observed, depending on the biochar pyrolysis temperature and ranged from 17 to 25% (Fig. 1B). In the following stages of the experiment, the inhibition was not observed (after 90 days) or was at a very low level (<10% after 180 days) (Fig. 1C and D).

Leachates from the biochar-amended soil were, however, toxic toward *L. sativum*, inhibiting root growth from 48 to 80% depending on the pyrolysis temperature (Fig. 2A). After 30 days, the inhibition of root growth decreased compared to the beginning of the study by 10%, 24%, and 74% for soils with BCSL500, BCSL600, and BCSL700, respectively. During the following period (90 days), the toxicity of leachates toward *L. sativum* root growth was differentiated depending on the biochar (Fig. 2C). After 180 days, SL-derived biochars inhibited root growth by 27–47%, which was not significantly different ($p > 0.05$) with SL-amended soil. It was also observed that with the increasing temperature of biochar production, a reduced harmful effect on *L. sativum* was noted (Fig. 2D), which was also observed on the 30th day of the experiment (Fig. 2B).

3.4. Solid-phase toxicity of BCSL-amended soil

The addition of SL-derived biochar to the soil had also a varied effect on the root growth of *L. sativum* in the solid-phase tests (Fig. 3). Adding BCSL600 and 700 to soil inhibited root growth at a level close to observed to SL-amended soil (Fig. 3A). However, the BCSL500-amended soil stimulated the root growth of *L. sativum*. After 30 days of experiment, all BCSLs inhibited root growth in the range from 18 to 42% (Fig. 3B). Those values were lower than for SL-amended soil. In the following period (after 90 days), the results were not significantly different ($p > 0.05$) between BCSL- and SL-amended soil. Only in the soil with BCSL700 significantly lower values ($p \leq 0.05$) of root growth inhibition were noted compared to SL-amended soil (Fig. 3C). After 180 days of the experiment, the toxic effect toward *L. sativum* was not different between the biochars. However, in soil with BCSL a lower inhibition of *L. sativum* root growth, from 55 to 68%, was observed compared to SL-amended soil (Fig. 4D).

The addition of BCSL500 did not cause an adverse impact on *F. candida* mortality (similarly to the SL-amended soil), while biochars BCSL600 and 700 caused mortality at levels of 58 and 50%, respectively (Fig. 4A). After 30 days, all the BCSLs caused mortality from 77 to 93% (Fig. 4B), similarly to SL-amended soil. During the following time periods (Fig. 4C and D), the mortality of *F. candida* in BCSL-amended soil decreased and after 180 days reached a stimulating level for BC500 and BC600 (Fig. 4D). For BCSL700, the mortality was still high (63%) and was higher by 43% compared to SL-amended soil.

The impact of biochars on *F. candida* reproduction was also varied depending on the biochar producing temperature (Fig. 5). At the beginning of the experiment, BCSL500 and 600 stimulated reproduction similarly as it was observed for SL-amended soil. However, BCSL700 inhibited the reproduction of *F. candida* at an 84% level (Fig. 5A). After 30 days, all of the BCSLs inhibited reproduction from 49 to 65% (Fig. 5B), which was close to the SL-amended soil. After 90 days, a significant ($p \leq 0.05$) decrease of a harmful effect of all of the biochars on *F. candida* reproduction was noted (Fig. 5C), afterwards, at the end of the experiment, a harmful impact of BCSL on reproduction occurred again for all BCSLs. Reproduction inhibition on the 180th day ranged from 23% to 61% and was still significantly ($p \leq 0.05$) lower (from 38 to 77%) compared to SL-amended soil (Fig. 5D).

3.5. Toxicity of leachates from BCSLW-amended soil

Similar to SL and BCSL, the negative effect of BCSLW-amended soil toward A. *fischeri* was not observed at the beginning of the study (Fig. 1A). After 30 days, an adverse effect occurred and, depending on the biochar production temperature, luminescence inhibition ranged from 8 to 50%. In the case of BCSLW700 only the values were significantly ($p \leq 0.05$) lower compared to SL- or BCSL700-amended soil. A significantly ($p \leq 0.05$) higher value (compared to BCSL600) was also noted for BCSLW600, which inhibited luminescence at a 50% level. In other experimental variants, the level of luminescence inhibition did not significantly ($p \leq 0.05$) differ from both SL- and BCLS-amended soils. After 90 days, the adverse effect of leachates from BCSLW-amended soil was not observed (similarly to other variants). After 180 days, luminescence inhibition in soil with BCSLW600 and 700 was observed, which was not significantly ($p \leq 0.05$) different from SL-amended soil and was significantly ($P > 0.05$) higher compared to soil with BCSLs (Fig. 1D). BCSLW500-amended soil did not show only the toxic properties toward *A. fischeri* (Fig. 1D).

Adding BCSLW inhibited *L. sativum* root growth from 37 to 61% (Fig. 2A). However, the value was higher to BCSLW500 only compared to SL- and BCSL-amended soil (18 and 28%, respectively), while for BCSLW600 and 700 it was significantly ($p \leq 0.05$) lower (from 22 to 51%). After 30 days, the adverse effect of BCSLW towards *L. sativum* was reduced from 4 to 70% depending of temperature of biochar production (Fig. 2B). During this period, a clear correlation between the toxic effect and the pyrolysis temperature was observed. In the following period, a further decrease of the harmful impact of BCSLW on *L. sativum* root growth was observed (Fig. 2C). After 180 days, the inhibition of the root growth ranged from 24 to 30% and was significantly ($p \leq 0.05$) lower compared to SL-amended soil. There was no difference in *L. sativum* growth inhibition between BCSL600/700- and BCSLW600/700-amended soil and it was lower by 46% for BCSL500-compared to BCSLW500-amended soil (Fig. 2D).

3.6. Solid-phase toxicity of BCSLW-amended soil

In the solid-phase test with *L. sativum*, similarly to previous tests, a significant disparity depending on pyrolysis temperature was observed. Adding BCSLW to the soil caused root growth inhibition in the range of 7–28%. The lowest harmful effect was noted for BCSLW600, for which the toxicity was also lower compared to SL- and BCSL600-amended soil (Fig. 3A). The influence of BCSLW700 on *L. sativum* did not differ significantly ($p \leq 0.05$) compared to SL- and BCSL700-amended soil, while the toxicity in BCSLW500-amended soil was significantly ($p \leq 0.05$) lower than in SL- and higher than in BCSL500-amended soil. After 30 days, an increase of the toxicity toward *L. sativum* for all BCSLWs occurred, leading to root growth inhibition from 23 to 40%. However, those values were lower than values observed in the SL-amended soil and, in most cases, were not different to the values for BCSL-amended soil (with the exception of BCSL/W600) (Fig. 3B). After 90 days, the toxicity decreased for BCSLW500 and 600 and also did not change for BCSLW700. The relationship between the effect and the pyrolysis temperature trend was observed (Fig. 3C). During the last experimental stage (Fig. 3D), the toxicity increased for BCSLW500, did not change for BCSLW600 and decreased for BCSLW700. The toxicity of the BCSLW-amended soils was lower compared to SL-amended soil and also to BCSL500-and BCSL700-amended soil. Significant ($p \leq 0.05$) differences between BCSL and BCSLW biochars produced at 600 °C were not found (Fig. 3).

The influence of BCSLW toward *F. candida* mortality varied and, as previously, determined by pyrolysis temperature. BCSLW500 did not cause any mortality effect toward *F. candida*, similarly to SL- and BCSL500-amended soil (Fig. 4A). After adding BCSLW600 and 700, the mortality *F. candida* increased significantly ($p \leq 0.05$) compared to SL-amended soil (67 and 50%, respectively), and did not differ significantly ($p > 0.05$) than in BCSL600/700-amended soil. After 30 days, an increase in mortality in the experiment with BCSLW500, and a decrease in the experiment with BCSLW700 was also observed (Fig. 4B). The mortality of *F. candida* after 30 days was lower compared to soil with SL and BCSL. On the 90th day of the experiment, *F. candida* mortality did not change (BCSLW500) or was only slightly higher (BCSLW600) compared to earlier experiment stage. However, a significant ($P > 0.05$) decrease in mortality after 90 days for BCSLW700-amended soil was noted (Fig. 4C). After 180 days, another change occurred that caused a significant ($p \leq 0.05$) decrease in mortality in the BCSLW500/600-amended soil (below the level observed for SL-amended soil) and a significant ($p \leq 0.05$) increase in BCSLW700-amended soil (Fig. 4D).

Adding BCSLW caused reproduction inhibition of *F. candida* in the range from 69 to 83% (Fig. 5A). Those values did not differ only for the BCSL and BCSLW produced at 700 °C. For the remaining two biochars (BCSLW500 and 600), the values were significantly ($p \leq 0.05$) higher than in the SL- and BCSL-amended soil. After 30 and 90 days, the inhibition of reproduction after adding BCSLW significantly ($p \leq 0.05$) decreased compared to the beginning of the study (Fig. 5B and C). Even a stimulating effect of BCSLW700 on reproduction of *F. candida* was noted. The last stage of experiment (Fig. 5D) showed, however, an adverse influence of BCSLW on reproduction, which was comparable to the value observed for the SL-amended soil (for all temperatures) and in the BCSL600-and BCSL700-amended soils.

4. Discussion

The initial absence of an effect in BCSL/BCSLW-amended soil on the toxicity toward *A. fischeri* is comparable with other studies where poultry litter-derived (Mierzwa-Hersztek et al., 2016) and wheat straw- and Miscanthus-derived biochars (Mierzwa-Hersztek et al., 2017) were used. However, in mentioned studies the direct toxicity of the biochar was determined, disregarding the effects of soil and the long-term changes that biochar can undergo. It is well known that, over time, biochar undergoes aging processes (Wang et al., 2020), which determine its properties and interactions with contaminants (Campos et al., 2021; Siatecka and Oleszczuk, 2022). The increase of toxicity toward *A. fischeri* probably resulted from the release of substances weakly bonded with the biochar, which were degraded or leached during incubation, which influenced the decreasing toxicity. On the other hand, an increase of toxicity observed in the last period resulted from a slow release of toxic substances, bonded stronger to the biochar. After 180 days, it can be observed that the luminescence inhibition increased along with the increasing DOC content in the soil leachate ($r^2 = 0.848$, $p \leq 0.05$) (Table S4; Fig. S1), which very often adsorb PAHs (Yang et al., 2014) and other toxic contaminants (Krop et al., 2001). The toxic effect after 180 days of the experiment was clearer for BCSLW than for BCSL, which suggests that the adsorption of contaminants responsible for the toxic effect toward *A. fischeri* was stronger for the BCSL than BCSLW.

The phytotoxic effect of leachates from BCSL and BCSLW-amended soil was observed from the beginning of the experiment, which could be connected to phytotoxic substances presented in biochars as has been previously confirmed (Hilber et al., 2017). However, the toxic effect of leachates from BCSL/BCSLW-amended soil at the beginning of study decreased gradually with time. Nevertheless, it is important to emphasize that on the 90th day of the experiment, the toxic effect was the lowest, after which an increase in toxicity occurred. A similar effect was not observed for the solid-phase test with *L. sativum*. Evaluation of the leachates toxicity allows to determine the direct toxicity of biochar and the potential impact of leachates containing varied biochar constituents. The solid-phase test, on the other hand, is more suitable for approximation of real environmental conditions, although the effect of biochar on plants may vary depending on the type of soil (Brtnicky et al., 2021). In Figure S2, the ratio between leachates and solid-phase test results for *L. sativum* is presented. Values > 1 suggest higher toxicity of the liquid

phase compared to the solid phase. The toxic effect varied and was more dependent on the sampling period than on the kind of biochar (Fig. S2). In the first and last stage of the experiment (for most of the samples), the toxic effect was stronger for the liquid-phase compared to the solid-phase. However, on the 30th and 90th days of the experiment the solid phase was more toxic than leachates (Fig. S2). It was also found a significant correlation between Σ16 C_{tot} PAHs ($r^2 = 0.819$, $p \leq 0.05$) and 4-6-ring C_{tot} PAHs, and the solid phase toxicity toward *L. sativum* on the 90th day of the experiment (Table S5). The lack of a similar correlation at the beginning of the study for leachates may indicate that substances dissolved in water mainly cause the toxic effect in the early stage after biochar adding to soil. These substances are degraded or leachated during the first 30 days of the experiment causing a decrease of toxic effect of leachates in the next two stages of the experiment (after 30 and 90-days). After 180 days, the new toxic substances are probably released as a consequence of decreasing of adsorption capacity of the biochar (as a result of aging) (Siatecka and Oleszczuk, 2022; Wang et al., 2020), which again lead to adversely affect of BCSL/BCSLW on *L. sativum* in the liquid-phase test. In the last stage of the experiment, significant ($p \leq 0.05$) correlations between C_{tot} (3-, 4- and 6-ring) and C_{free} (4-6-ring) and the toxicity of the solid-phase were confirmed. A statistically significant correlation ($p \leq 0.05$) was also observed between the phytotoxicity of leachates (*L. sativum*) from specific experimental variants and the content of 3-ring C_{free} PAHs ($r^2 = 0.884$, $p \leq 0.001$) (Table S5). Previous studies carried out by Rogovska et al. (2012) suggested also that PAHs in biochars may be harmful to plants. Apart from the toxic effect caused by contaminants presented in biochar, an adverse effect could be a result of disturbed nutrient cycling in presence of biochar (Niu et al., 2014). It can be confirmed by the negative correlation observed between the solid phase test with *L. sativum* and the content of available K ($r^2 = -0.773$, $p \leq 0.05$) and available Mg ($r^2 = -0.809$, $p \leq 0.05$) on the 90- and 180-day of the experiment, respectively (Table S4).

It is difficult to relate the obtained results to the data of other authors due to the different characteristics of the feedstock used for the production of biochar and the pyrolysis conditions. For instance, biochar produced from SL at 300 °C increased the biomass of *Poa pratensis* L. from 40 to 250% during the 200-day experiment (Mierzwa-Hersztek et al., 2018). The corn yield increased also (from 40 to 50%) after adding biochars from SL to soils at the dose 0.8% (Faria et al., 2018; Figueiredo et al., 2019). A harmful effect of biochar addition was observed by Gascó et al. (2016). SL-derived biochar produced at 600 °C and added to soil at a dose of 8% decreased the lettuce stem length by 32% (sandy loam soil) or decreased lettuce root dry weight by 75% (sandy soil) [56]. There is a lack of studies on the effect of co-pyrolyzed SL-derived biochar.

While in the case of plants there is a broad spectrum of data on the effect of biochar, especially produced from biomass (Godlewska et al., 2021), for *F. candida* data is scarce (Godlewska et al., 2021). The toxic effect of biochar on this group of organisms may result from the increased pH and salinity of the soil after adding biochar but also from harmful substances such as heavy metals and PAHs which can be toxic to *F. candida* (Conti et al., 2018; Mierzwa-Hersztek et al., 2018; Stefaniuk et al., 2016). In the present study, the mortality of *F. candida* decreased over time to reach the lowest values for most of the variants (apart from BCSL/W700) in the last stage of the experiment (Fig. 4D). In the case of reproduction inhibition, a similar trend to the solid-phase test with *L. sativum* was observed, in which decreasing toxicity occurred until the 90th day of the experiment, after which an increase in toxicity was observed again. This was, as was previously explained for *L. sativum*, associated with the release a new contaminants from biochar as a result of biochar aging. Recent studies (Bielská et al., 2018; Domene et al., 2015; Gruss et al., 2019; Maaβ et al., 2019; Stefaniuk et al., 2016) concerning the toxicity of biochar produced from various feedstock toward *F. candida* show a differentiated effect. Collembolan mortality or reproduction was generally unaffected by biochar (Conti et al., 2018; Domene, 2016; Domene et al., 2015; Hale et al., 2013), but when inhibited, it was mostly influenced by feedstock and dose (Conti et al., 2018; Domene et al., 2015; Gruss et al., 2019; Raclavská et al., 2018). However, the studies concerned biochar produced from biomass and not from sewage sludge, which is very often burdened with a high load of contaminants (Zielińska and Oleszczuk, 2016). As a possible reason of the toxicity of biochars toward *F. candida* (apart from contaminants) soluble Na and NH_4 (Domene et al., 2015), high pH and salinity (Conti et al., 2018) are often indicated.

The varied influence of biochars on the toxic effect toward all of the studied organisms depends on the pyrolysis temperature, feedstock type and the sampling period may suggest that the toxic effect every time is caused by different substances specific for each biochar, whose ecotoxicological activity changes over time. According to the studies (Gul et al., 2015; Shi et al., 2019; Tomczyk et al., 2020), the higher temperature of pyrolysis, the higher pH of the biochar, which was connected to increasing ash content and oxygen functional groups. In addition, aging of biochar in the soil causes decreasing of biochar's pH value (Gul et al., 2015). Salinity also increases with increasing pyrolysis temperature, due to weight loss during the process (Tomczyk et al., 2020). The calculated correlation coefficients between the soil properties and the individual test endpoints in different periods are presented in Fig. 6. In the first two periods (0 d/30 d), sporadic correlations were noted, while in the following two periods (90 d/180 d), a clear trend was observed, suggesting a significant influence of PAHs and some soil properties (Mg, K_2O, EC, CEC) on *L. sativum* for the liquid-phase test. The adverse influence of EC and CEC on plants was previously observed and could be connected with excess of Na after adding biochar to soil (Kronzucker et al., 2013). This assumption confirms our previous hypothesis that the toxic effect of biochar is determined by various factors that change over time. The negative coefficients between K_2O (on 90th day) and Mg (on 180th day) could suggest a deficiency of those elements as a result of their decreased bioavailability in presence of biochar.

One of the objectives of the study was to evaluate the effect of adding biomass (willow) to sewage sludge as a way to decrease the potential adverse impact on the environment. Because the toxicity was very varied depending on the biochar producing temperature, sampling period and feedstock, analysis of the changes in the ecotoxicity of BCSL compared to BCSLW was conducted, which is presented as a heatmap (Fig. 7). When the toxicity of BCSLW < BCSL, the value of 1 was selected (green), while for BCSLW > BCSL the selected value is −1 (red). The lack of significant differences ($p \leq 0.05$) between BCSLW and BCSL was selected as 0 (yellow). It is evident that the effect of the addition of biomass to the sewage sludge was mainly determined by test type and less by the period of sampling and the biochar producing temperature (Fig. 7). In the Microtox® test for most of the cases, a significant ($p \leq 0.05$) influence of adding biomass on the biochar's toxicity was not observed. Significant ($p \leq 0.05$) differences were noted only in the second and the last period of the study. An apparent positive effect for most biochars and periods was observed for the solid-phase test with *L. sativum* and to a slighter extent for the liquid phase, especially on the 90th and 180th days of the study. In the tests with *F. candida*, for mortality a positive influence of adding biomass at the beginning of the study (0 and 30 days) was noted, then after 90 and 180 days, an apparent harmful effect of biomass for biochars produced at 500 and 600 °C was emphasized. However, the most differentiated effect was observed toward *F. candida* reproduction. Only in three cases did the influence of adding biomass positively affect *F. candida* reproduction, while for the other biochars and periods significant ($p \leq 0.05$) differences between BCSL and BCSLW were not observed, or adding biomass had an adverse influence (Fig. 7).

5. Conclusions

In the long-term perspective and for most tests, the addition of sewage sludge to the soil was more toxic compared to adding biochars. The toxic effects of BCSL or BCSLW-amended soil are diversified depending on the type of tested organism. In most cases, sewage sludge-

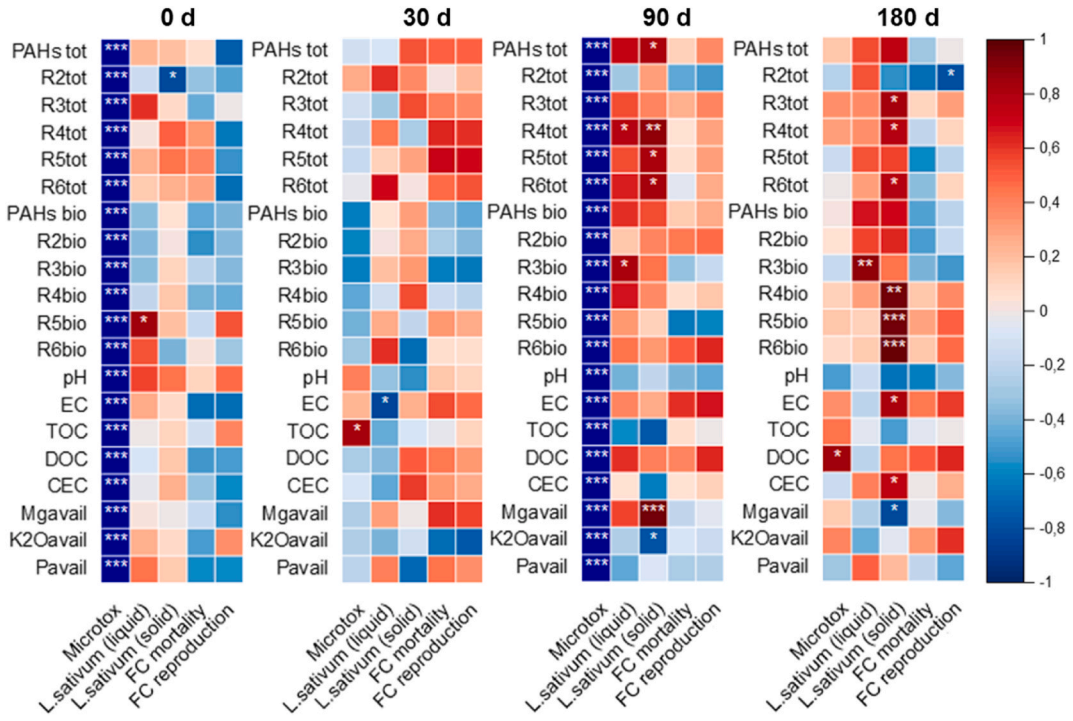

Fig. 6. Correlation plot presenting the values of Pearson correlation factor between chemical and ecotoxicological endpoints. The significant correlations were indicated by * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

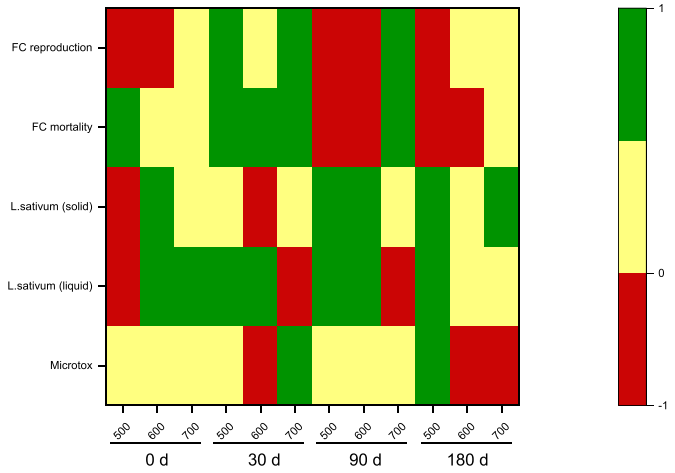

Fig. 7. Heatmap with relative changes in ecotoxicity of BCSL compared to BCSLW. The statistically significant increase, no change, decrease of toxicity ($p < 0.05$) was marked as red, yellow, green, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

derived biochar was more toxic than co-pyrolyzed (sewage sludge/willow) biochar. The influence of pyrolysis temperature on biochar toxicity was observed, however, this varied depending on the tested organism. It also has to be emphasized that the toxic effect was shown in different study periods which can suggest a varied influence of different factors affecting toxicity. Therefore, long-term studies are necessary to determine the possible potential risk.

Credit author statement

Paulina Godlewska: Conceptualization, Methodology, Investigation, Writing – original draft; Izabela Jośko: Methodology, Patryk Oleszczuk conceptualization, review and editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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INTERNET SUPPORTING INFORMATION

Ecotoxicity of sewage sludge- or sewage sludge/willow- derived biochar-amended soil

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MATERIALS AND METHODS

Sewage sludge

Sewage sludge (SL) was obtained from municipal wastewater treatment plant where SL is stabilized biologically by aerobic fermentation and chemically by threated with lime. SSL were collected during autumn 2016 from municipal sewage treatment plant localized in Chełm (southeastern part of Poland, 510705600N, 232804000E, population of 64 000 people). Sewage treatment plant were located on agricultural areas and used mainly urban wastewater without the great impact of the industry.

Biochar preparation

Briefly, the sewage sludge (SL) before pyrolysis was grinded and sieved through a 2 mm sieve. SL was obtained from municipal wastewater treatment plant localized in Chełm (50°20′04″N 23°29′49″E) which uses mainly urban wastewater with limited influence of wastewater from industry. The willow was provided by a biomass-producing farm. Freshly cut willow was air-dried for two weeks and then cut into small pieces and sieved through 2 mm sieve. Mixtures of SL and willow (6:4 w/w) were obtained by mixing both materials in glass bottles (1000 mL) for 24 h in the dark at 10 rpm (Rotax 6.8. VELP, Italy). SL alone and SL with willow were pyrolysed in 500, 600 or 700°C, with the heating rate 10°C/ min. Temperature was held for 3 h (slow pyrolysis). During the pyrolysis the oxygen free atmosphere was maintained by constant flow of N_2. The physico-chemical properties of SL- and SL/biomass-derived biochar are presented in Table S1.

Statistical analysis

Mean values were taken from a triplicate data set. The differences between ecotoxicological endpoints in particular terms (and between terms) and variants were evaluated using a one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. Repeated measures analysis of variance (ANOVA) was performed to investigate whether sampling time had an effect on the tests' results, as the within subject factor. Pearson's correlation coefficient test was performed to investigate the relations between physico-chemical properties and tests' results with Statistica 5.0. Significance was set at $*p \leq 0.05$.

Table S1. Biochars' main physico-chemical properties

	pH	Ash content (%)	C (%)	OC (%)	H (%)	N (%)	O (%)	O/C	H/C	(O+N)/C	S_{BET} (m^2/g)	d (nm)	V_t (m^3/g)	V_{micro} (m^3/g)	V_{meso} (m^3/g)
BCSL500	9.4	64.1	26.3	26.2	0.99	3.26	5.38	0.15	0.45	0.26	69.7	5.98	0.104	0.0189	0.0854
BCSL600	12.1	67.6	26.5	26.4	0.60	2.93	2.41	0.07	0.27	0.16	75.5	5.86	0.110	0.0211	0.0894
BCSL700	12.4	71.4	24.5	24.4	0.29	2.10	1.71	0.05	0.19	0.13	89.2	6.18	0.137	0.0240	0.1139
BCSLW500	10.8	46.4	44.6	44.2	1.66	3.33	3.93	0.07	0.44	0.13	74.6	4.66	0.087	0.0207	0.0663
BCSLW600	12.1	49.3	45.2	44.9	0.81	2.85	1.86	0.03	0.21	0.08	93.1	4.01	0.104	0.0351	0.0694
BCSLW700	12.5	50.9	46.2	46.0	0.62	2.09	0.21	0.02	0.16	0.04	104.1	4.93	0.115	0.0269	0.0881

pH- in H_2O- 1:10 (w/v)

Table S2. Chemical properties of control soil

pH	Total N content (%)	Available nutrients (mg/ 100 g of soil)			Exchangeable cations content (mg/ 100 g of soil)				Hydrolytic acidity (mmol H^+ /100 g of soil)
6.84 in H_2O	0.064	P_2O_5	K_2O	Mg	Mg	Na	K	Ca	1.25
6.80 in KCl		12.2	11.7	2.2	3.50	2.87	10.6	101	

pH in H_2O- 1:10 (m/v), pH in 1M KCl- 1:2.5 (m/v), total N – Kjeldahl method, available P and K – Egner-Riehm method, available Mg – Schachtschabel method, exchangeable cations- Kappen method, hydrolytic acidity- Kappen method

Table S3. RMANOVA results to evaluate effect of sampling time on ecotoxicological tests' results.

Source of variation		F ratio	*p* value
Microtox		12.57	0.000
L. sativum	Water	3.87	0.024
	Solid	6.84	0.003
F. candida	Mortality	8.44	0.001
	Reproduction	4.64	0.014

Table S4. Correlations between ecotoxicological tests results of the experiment and total and chemical properties of soil with SL- or SL and willow- derived biochar addition. Statistically important coefficients were marked with *red (p≤ 0.05) or **green (p ≤0.0.01) or ***violet (p≤0.001).

							Available		
		pH	EC	TOC	DOC	CEC	Mg	K	P
0 days									
V. fischeri	Microtox test	0	0	0	0	0	0	0	0
L. sativum	Liquid	0.574	0.277	-0.010	-0.082	-0.030	0.033	0.245	0.444
	Solid	0.442	0.086	0.101	0.168	0.241	-0.016	0.080	0.143
F. candida	Mortality	0.105	-0.661	-0.100	-0.513	-0.325	-0.165	-0.481	-0.574
	Reproduction	0.478	-0.664	0.388	-0.491	-0.575	-0.559	0.342	-0.564
30 days									
V. fischeri	Microtox test	0.406	0.231	*0.833	-0.265	-0.099	-0.257	-0.251	-0.218
L. sativum	Liquid	-0.340	-0.829	-0.423	-0.375	-0.445	0.307	-0.382	0.419
	Solid	-0.549	0.256	-0.096	0.515	0.597	-0.017	-0.122	-0.691
F. candida	Mortality	0.163	0.557	-0.032	0.424	0.325	0.602	-0.672	0.458
	Reproduction	0.119	0.473	0.117	0.327	0.268	0.563	-0.754	0.352
90 days									
V. fischeri	Microtox test	0	0	0	0	0	0	0	0
L. sativum	Liquid	-0.417	0.396	-0.573	0.620	0.046	0.578	-0.257	-0.445
	Solid	-0.182	0.268	-0.744	0.413	-0.607	***0.953	*-0.773	-0.072
F. candida	Mortality	-0.392	0.604	0.074	0.394	0.057	-0.191	-0.097	-0.271
	Reproduction	-0.443	0.674	0.000	0.627	0.138	-0.050	-0.141	-0.249
180 days									
V. fischeri	Microtox test	-0.483	0.346	0.444	*0.848	-0.154	0.147	0.394	-0.316
L. sativum	Liquid	-0.156	-0.203	-0.055	-0.209	0.402	-0.242	-0.440	0.492
	Solid	-0.656	0.816	-0.491	0.441	*0.758	*-0.809	-0.050	0.205
F. candida	Mortality	-0.608	0.427	-0.042	0.500	-0.010	-0.033	0.334	-0.190
	Reproduction	-0.361	0.593	-0.002	0.625	0.258	-0.366	0.616	-0.458

Table S5. Correlations between ecotoxicological tests results of the experiment and total and bioavailable PAHs content in soil with SL, SL- derived biochar and SL/willow- derived biochar addition. Statistically important coefficients were marked with *red red ($p \leq 0.05$) or **green ($p \leq 0.0.01$) or ***violet ($p \leq 0.001$).

		Total PAHs						Bioavailable PAHs					
		Σ16	R2	R3	R4	R5	R6	Σ16	R2	R3	R4	R5	R6
0 days													
V. fischeri	Microtox test	0	0	0	0	0	0	0	0	0	0	0	0
L.	Liquid	0.236	-0.154	0.606	0.025	0.240	0.146	-0.357	-0.364	-0.356	-0.191	*0.854	0.521
sativum	Solid	0.195	*-0.801	0.096	0.486	0.448	0.253	0.042	0.000	0.102	0.169	0.197	-0.394
F.	Mortality	0.079	-0.335	-0.422	0.332	0.391	0.288	-0.451	-0.549	-0.214	-0.403	-0.161	0.024
candida	Reproduction	-0.731	-0.468	-0.011	-0.621	-0.521	-0.672	-0.382	-0.374	-0.364	-0.439	0.534	-0.311
30 days													
V. fischeri	Microtox test	-0.107	0.276	-0.127	-0.193	-0.161	-0.040	-0.614	-0.597	-0.610	-0.459	-0.418	-0.314
L.	Liquid	-0.087	0.609	-0.284	0.435	0.123	0.697	0.044	0.003	0.191	-0.136	0.277	0.620
sativum	Solid	0.522	0.368	0.558	-0.275	0.291	0.010	0.301	0.262	0.323	0.541	-0.186	-0.680
F.	Mortality	0.494	0.001	0.416	0.622	0.704	0.463	-0.362	-0.273	-0.612	-0.152	0.329	0.067
candida	Reproduction	0.480	0.217	0.368	0.608	0.689	0.526	-0.443	-0.373	-0.632	-0.207	0.268	0.064
90 days													
V. fischeri	Microtox test	0	0	0	0	0	0	0	0	0	0	0	0
L.	Liquid	0.726	-0.294	0.560	0.761	0.544	0.650	0.601	0.161	0.806	0.667	0.322	0.445
sativum	Solid	*0.819	0.302	0.398	*0.890	*0.808	*0.821	0.553	0.397	0.458	0.376	0.133	0.328
F.	Mortality	0.135	-0.448	0.258	0.052	0.070	-0.055	0.143	0.439	-0.338	0.068	-0.629	0.512
candida	Reproduction	0.368	-0.510	0.390	0.293	0.309	0.280	0.270	0.479	-0.166	0.175	-0.600	0.623
180 days													
V. fischeri	Microtox test	0.177	-0.230	0.355	0.308	-0.151	-0.011	0.018	0.053	-0.172	0.122	0.168	0.099
L.	Liquid	0.558	0.531	0.377	0.345	0.527	0.309	0.674	0.571	**0.884	0.305	0.133	0.150
sativum	Solid	0.753	-0.547	*0.833	*0.779	0.570	*0.778	0.692	0.633	0.441	**0.950	***0.951	***0.974
F.	Mortality	-0.303	-0.661	0.118	-0.184	-0.568	-0.341	-0.465	-0.497	-0.380	0.170	0.296	0.162

candida	Reproduction	-0.011	-0.786	0.306	0.118	-0.208	0.115	-0.212	-0.174	-0.511	0.376	0.494	0.468

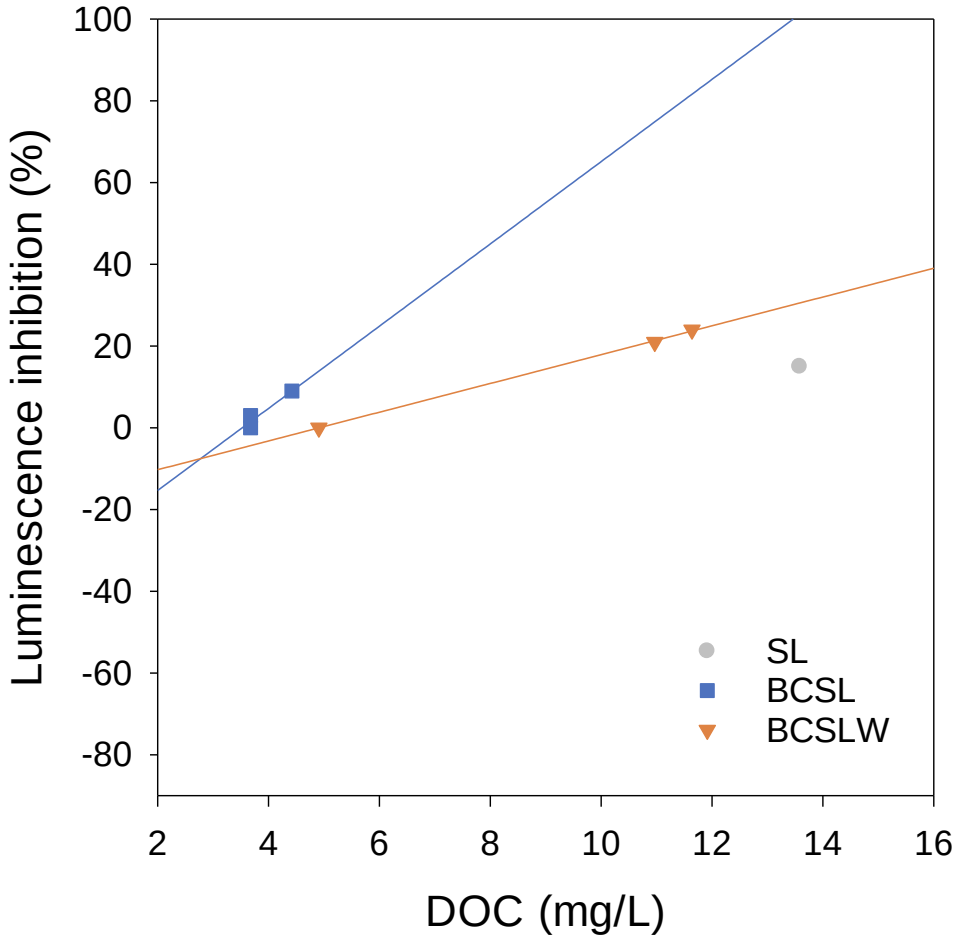

Fig. S1. Correlation between DOC content and luminescence inhibition of *A. fischeri* after 180 days of the experiment.

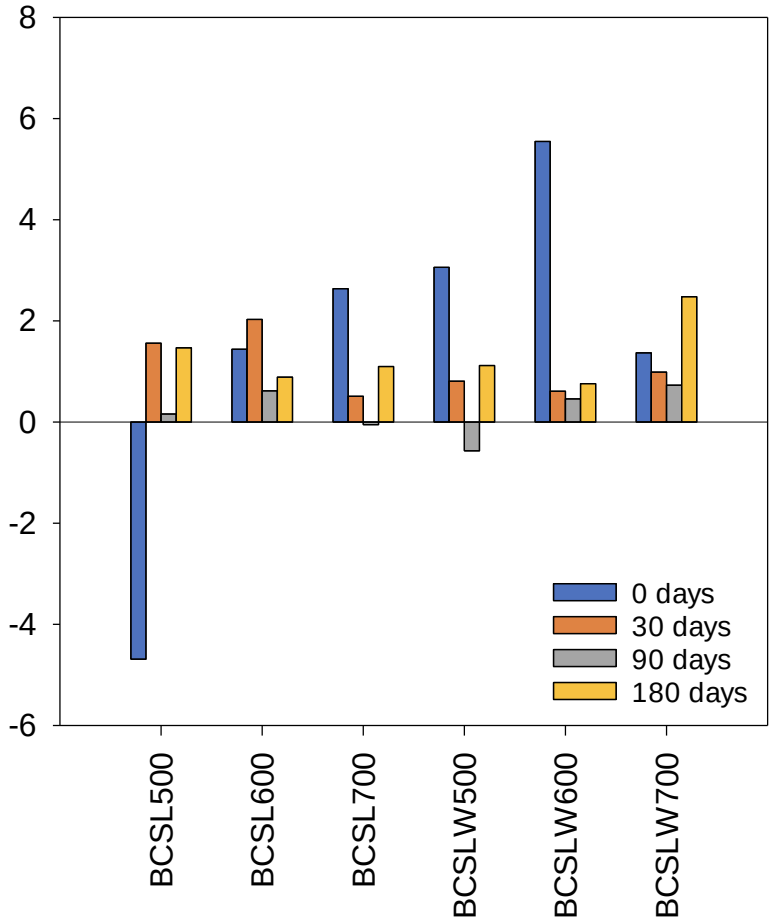

Fig. S2. Comparison between leachates and solid phase *L. sativum* test toxicity. Values over 1 indicate greater toxicity of leachates.

D7

P. Godlewska, P. Oleszczuk,

Effect of carrier gas during sewage sludge or sewage sludge-willow co-pyrolysis on the persistence and bioavailability of polycyclic aromatic hydrocarbons in biochar-amended soil,

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Effect of carrier gas during pyrolysis on the persistence and bioavailability of polycyclic aromatic hydrocarbons in biochar-amended soil☆

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Abstract: In this study the persistence (based on extractable, C_{tot}) and bioavailability (based on freely dissolved content, C_{free}) of polycyclic aromatic hydrocarbons (PAHs) in biochar-amended soil was investigated. Biochar produced at 500 or 700 °C from sewage sludge (BC) or sewage sludge and willow (W) mixture (BCW) in an atmosphere of nitrogen (N_2) or carbon dioxide (CO_2) was evaluated. The biochars were applied to the real soil (podzolic loamy sand) at a dose of 2% (w/w). The content of C_{tot} and C_{free} PAHs was monitored for 180 days. The biochar production conditions determined the C_{tot} and C_{free} PAHs in the soil. A change of carrier gas from N_2 to CO_2 caused an increase in C_{tot} PAH losses in the soil from 19 to 75% for the biochar produced from SL and from 49 to 206% for the co-pyrolyzed biochar. As regards C_{free} PAHs, the change from N_2 to CO_2 increased the losses of C_{free} PAHs only for the biochar derived from SL at a temperature of 500 °C (by 21%). In the soil with the other biochars (produced at 700 °C from SL as well as at 500 and 700 °C from SL/W), the C_{free} increased from 17 to 26% compared to the same biochars produced in an atmosphere of N_2.

1. Introduction

Sewage sludge (SL) is a biowaste that is produced in wastewater treatment plants in substantial amounts all over the world (*EC report*, 2017; Guo et al., 2020). The most favorite method of its management is to use it as fertilizer in agriculture and forestry (*EC report*, 2017; Stańczyk-Mazanek et al., 2019) due to high content of many valuable nutrients (phosphorus, nitrogen), but also of organic matter and macro- and micronutrients (Wołejko et al., 2018). Nonetheless, SL also contains undesired biological material (parasite eggs and weed seeds) (Cieślik et al., 2015) and toxic substances, such as heavy metals, personal care products (including pharmaceuticals) and polycyclic aromatic hydrocarbons (PAHs) (Wołejko et al., 2018; Stańczyk-Mazanek et al., 2019; Pulkrabová et al., 2019; Lin et al., 2020). Most of these contaminants are mutagenic and toxic and may accumulate in soil affecting harmfully different soil organisms (Wang et al., 2017; Bandowe et al., 2021; Alengebawy et al., 2021; Gworek et al., 2021). Thermal conversion of SL through pyrolysis is one of the proposed methods to reduce the disadvantages of this biowaste (Zhang et al., 2017; Raheem et al., 2018). Pyrolysis is a process of thermochemical decomposition of organic matter at a temperature from 300 to 900 °C with limited oxygen (Raheem et al., 2018). Biochar, which primarily consists of carbon, while in the case of SL-derived biochar it also has a substantial proportion of minerals, is a solid product of pyrolysis (Agrafioti et al., 2013; Chanaka Ukalska-Jaruga et al., 2020; Chen et al., 2020; Xing et al., 2021). The properties of biochar can be influenced through changes in pyrolysis temperature (Ahmad et al., 2012; Zielińska and Oleszczuk, 2016; Guo et al., 2021), but also in the feedstock (mixture of feedstocks) (Antonângelo et al., 2019; Igalavithana et al., 2019; Rajapaksha et al., 2019) or carrier gas used (Azuara et al., 2017; Igalavithana et al., 2019; Kończak et al., 2019a, 2019b; Guo et al., 2021). It has been showed (Madej et al., 2016; Kończak et al., 2019a) that depending on different pyrolysis conditions, the PAHs content in biochar may also change.

PAHs are one of the groups of substances that are termed as persistent organic pollutants (POPs) (Pulkrabová et al., 2019; Stańczyk-Mazanek et al., 2019) which are characterized by high toxicity (cancerogenicity, mutagenicity, and teratogenicity), persistence, and ability to accumulate in soil (Wang et al., 2017; Pulkrabová et al., 2019; Stańczyk-Mazanek et al., 2019). In spite of modification in pyrolysis parameters, PAHs are still present in biochar (Hale et al., 2012; Zielińska and Oleszczuk, 2015, 2016; Wang et al., 2017). However, the literature lacks information on the persistence of PAHs, in particular their

☆ This paper has been recommended for acceptance by.

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bioavailability, produced under different pyrolysis conditions, especially after application of this type of biochars to soil. Our previous study (Godlewska and Oleszczuk, 2022) demonstrated that SL-derived biochar co-pyrolyzed with lignocellulosic biomass (willow) increased the persistence of PAHs in the soil by reducing their bioavailability.

Another method to manipulate the biochar properties by changing pyrolysis conditions is to use different carrier gases. Research shows that a change of carrier gas from N_2 (frequently used during pyrolysis) to CO_2 reduces the PAH content in biochar (Zhou et al., 2014; Kończak et al., 2019a; He et al., 2020). It has also been observed that biochar produced in an atmosphere of CO_2 has higher affinity for PAHs (Kończak et al., 2019a) than biochar obtained in an N_2 atmosphere. Strong affinity leads to sequestration of PAHs, thus reducing their bioavailability and potential toxicity to organisms (Oleszczuk, 2007; Chen and Yuan, 2011). The increased specific surface area and porosity, which characterize biochar produced in an atmosphere of CO_2, may also contribute to greater adsorption/sequestration of PAHs by biochar in soil, thereby affecting their persistence and bioavailability (Godlewska et al., 2019). Pores filling or π-π interaction between surface of the biochar and PAHs molecule are proposed as the main mechanisms responsible for PAHs bonding by biochars (Ahmad et al., 2014b).

Apart from the direct effect of biochar on PAH persistence and bioavailability, attention should also be paid to indirect factors. Incorporation of biochar into soil can change the environment properties (pH, salinity, water content or microbiological activity) and these factors indirectly affect not only the persistence and bioavailability of PAHs, but also the degree of transformation of these compounds (Ahmad et al., 2014a; Godlewska et al., 2021). Also, we should not forget about changes that the biochar surface undergoes during incubation with soil (aging) (Sigmund et al., 2017; de la Rosa et al., 2018; Siatecka and Oleszczuk, 2022), which may in time reduce the biochar's capacity to adsorb PAHs.

According to our best knowledge, there are no studies investigating the effect of carrier gas on the properties and content of contaminants in biochar-amended soil. Due to the many advantages of application of SL-derived biochar as soil amendment, it is important to study the behavior of PAHs in soil amended with biochar produced in a CO_2 atmosphere. The aim of this study was to determine the total (C_{tot}) and bioavailable (C_{free}) PAH content in soil amended with SL-derived biochar produced in an atmosphere of N_2 or CO_2 and amended with biochar produced from SL and willow (W) in an atmosphere of N_2 or CO_2 in the long term experiment. The effect of pyrolysis temperature (500 or 700 °C) on the above-mentioned parameters was also studied.

2. Materials and methods

2.1. Biochars and soil

Pyrolysis conditions were described elsewhere (Kończak et al., 2019a, 2019b). Briefly, the sewage sludge (SL) was grinded and sieved through a 2 mm sieve. SL was provided by municipal wastewater treatment plant localized in Chełm (50°20′04″N 23°29′49″E), which uses mainly municipal wastewater with limited influence of wastewater from industry. The willow was provided by a biomass-producing farm. Freshly cut willow was air-dried for two weeks and then cut into small pieces and sieved through 2 mm sieve. Mixtures of SL and willow (6:4 w/w) were obtained by mixing both materials in glass bottles (1000 mL) for 24 h in the dark at 10 rpm (Rotax 6.8. VELP, Italy). SL alone and SL with willow were pyrolyzed at 500 or 700 °C, with the heating rate 10 °C/min. Temperature was held for 3 h (slow pyrolysis). During the pyrolysis the oxygen free atmosphere was maintained by constant flow of N_2 or CO_2. The physico-chemical properties of SL- and SL/biomass-derived biochar are presented in Table S1. Soil for experiment was provided by the Bezek Experimental Station (51°12'06″N 23°16′06″E), which is localized in southeastern part of Poland. The soil can be classified as a loamy sand on the basis of it textural composition. The physico-chemical soil properties are showed in Table S2.

2.2. Pot experiment

Dry biochars produced from sewage sludge (BC) or mixture of sewage sludge and willow (BCW) at N_2 or CO_2 atmosphere where mixed thoroughly with soil at the dose of 2% (w/w) in 20L plastic containers. Podzolic loamy sand with moderate pH (Table S2) was used as a soil in this experiment. This type of soil is very common in Poland and usually the best effect of biochar application is achieved for this type of soil. Soil water holding capacity (WHC) at 60% was maintained with deionized water. Soil moisture was kept on constant level by weighing the pots and the water losses were refilled to keep the constant mass of the pot. The pot experiment was conducted in controlled conditions (23 ± 2 °C, the dark). In particular periods of time (after 0, 30, 90 and 180 days), about 50 g of the soil, BC- and BCW-amended soil was sampled, air-dried and stored at −20 °C before analysis.

2.3. Organic solvent extractable content (C_{tot}) of PAHs

To determine the organic solvent extractable content of PAH, the harsh extraction method was used. Samples obtained from the pot experiment (10 g) were extracted by n-hexane (99% analytical purity, POCH, Poland) using the Soxhlet apparatus for 24 h. Deuterated Σ9 PAHs (PAH-MIX 9 deutered, 100 μg/mL in cyclohexane, 98.1–99.7% analytical purity, Dr Ehrenstorfer LGC Labor GmbH, Germany) were added before extraction to the samples and used as an internal standard (IS). After extraction, extract was evaporated to volume of 1 mL using RVC 2–25 CD plus equipment (Martin Christ, Germany). A qualitative and quantitative analysis of Σ16 PAHs was carried out using Gas Chromatograph (Thermo Scientific Trace 1300) equipped with Mass Spectrometer (Thermo Scientific ISQ LT). A Rxi®-5 ms capillary column (30 m × 0.25 mm, ID x 0.25 μm film thickness) from Restek (USA) was used.

2.4. Freely dissolved content (C_{free}) of PAHs

Determination of the bioavailable PAHs was based on the polyoxymethylene (POM) method described previously by Cornelissen et al. (2008). Briefly, the dry samples (1 g) were added to glass flasks (50 mL) with the solution of sodium azide (200 mg L^{-1}) in Millipore water (40 mL) (to prevent microorganisms activity), strips of POM (55 μm thick, 0.3 g for all batches) and tightly closed with glass lid. After 28 days, POMs were gently removed from the solution, cleaned with Millipore water and dry using a tissue. Cleaned POMs were extracted using 20 mL of acetone:heptane mixture (20:80) for 48 h. The volume of the solvent was reduced to 1 mL and the content of PAHs was determined using previously mentioned method (Cornelissen et al., 2008; Zielińska and Oleszczuk, 2015).

2.5. Data analysis

All results regarding of PAHs content are mean value of triplicate data set. The differences between organic solvent extractable (C_{tot}) or freely dissolved (C_{free}) PAHs between particular experimental variants were determined using a one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. The correlations between the selected properties of BC- or BCW-amended soil and C_{tot} and C_{free} PAHs were determined using Pearson's correlation analysis with Statistica 6.0.

2.6. Quality assurance and quality control (QA/QC)

All samples were taken according to the PN-ISO 10381–2:2007P (ISO 10381–2:2002 - Soil quality – Sampling – Part 2: Guidance on sampling techniques, 2002). The procedures and methods of the chemical tests in lab were controlled according to existing standards or published papers. The QA/QC checks of the testing instruments (GC-MS, pH meter, TOC-

VCSH etc.) in lab were conducted during and after installation by the supplier. To ensure quality assurance and quality control (QA/QC) analyzed blank sample, duplicate sample (n = 3) and a standard reference material (PAHs - Loamy Sand, Sigma Aldrich) with each batch of samples. The testing instruments were also calibrated in lab before the chemical analysis. Blank sample values were very low or below detection limits for the corresponding method. For each PAHs, the response factor percent relative standard deviations (% RSDs) typically were 4–15% and always less than 24%.

3. Results and discussion

3.1. Persistence of PAHs in sewage sludge-derived biochar-amended soil

The addition to the soil of the SL-derived biochars produced in N_2, depending on the pyrolysis temperature, had a significant effect on the content of Σ16 C_{tot} PAHs (Fig. 1). After adding the biochar produced at 500 and 700 °C, the Σ16 C_{tot} PAHs content was respectively 88.0 μg/kg (±8.27 μg/kg) and 90.6 μg/kg (±4.65 μg/kg), being higher by 117 and 124% than in the control soil.

After 30 days of the experiment, Σ16 C_{tot} PAHs in the biochar-amended soil decreased by 19% (BC500–N2) and 41% (BC700–N2) relative to the beginning of the experiment. At the successive time points, however, a significantly lower content of Σ16 C_{tot} PAHs, compared to the 30th day was only found in the 180th day of the experiment (Fig. 1). The content of Σ16 C_{tot} PAHs was lower by 31% (BC500–N2) and 61% (BC700–N2) than at the beginning of the study as well as it was higher by 27% in the soil with BC500–N2 and lower by 26% in the soil with BC700–N2 compared to the control soil in the same last experimental stage.

Regarding to individual PAH groups, during the first 30 days, a significant decrease in the content of 2-, 3- and 5-ring PAHs was observed (Figs. S1A and B) in the experiment with BC500–N2, respectively by 27, 21, and 14%, as well as in all the groups for BC700–N2, with the decrease ranging from 28 to 53% (Fig. S1). The content of the other PAH groups in the soil with BC500–N2 did not change significantly ($P > 0.05$) (Figs. S1C and E). After 90 days, the 2-3-ring PAH content was observed to further decrease, whereas the 4-6-ring PAH increased from 11 to 25% comparing to the beginning of the study in the experiment with BC500–N2. On the other hand, in the experiment with BC700–N2 the content of all groups decreased from 19 to 61%. After 180 days, the content of 2- and 3-ring PAHs in the soil with BC500–N2 only and of all PAH groups in the soil with BC700–N2 was lower than at the beginning of the experiment (Fig. S1). In the experiment with BC500–N2, the content of the other PAH groups after 180 days was higher from 10 to 56% in comparison to the beginning of the study ($P \leq 0.05$).

3.2. Freely dissolved PAHs content in sewage sludge-derived biochar-amended soil

After biochar application to the soil, the content of Σ16 C_{free} PAHs was 32.0 (BC700–N2) and 36.2 ng/L (BC500–N2) and it did not differ significantly ($P > 0.05$) between the biochars (Fig. 2). The addition of biochar to the soil caused a significant reduction ($P \leq 0.05$) in Σ16 C_{free} PAHs in it by 36% for BC500–N2 and by 43% for BC700–N2.

During the first 90 days of the experiment, the Σ16 C_{free} PAH content remained on constant level. It was only on the 180th day of the study that a significant ($P \leq 0.05$) decrease in the content Σ16 C_{free} PAHs was found - by 39% (BC500–N2) and 49% (BC700–N2) relative to the beginning of the study (Fig. 2).

Despite that the content of Σ16 C_{free} PAHs remained at a constant level throughout most of the experiment, the individual PAHs were characterized by high dynamics of changes which were dependent on the biochar production temperature. An increase in 3- and 4-ring PAH content and a decrease in 5- and 6-ring PAHs (except for BC500–N2) were generally observed (Fig. S2). Ultimately, after 180 days the content of 2-, 5- and 6-ring PAHs was lower, compared to the beginning of the experiment, by respectively 46, 51 and 76% for BC500–N2 and by 52, 86 and 73% for BC700–N2 ($P \leq 0.05$). An increase was however observed in the level of 3-ring (BC500–N2 – 190% and BC700–N2 - 59%) and 4-ring PAHs (BC500–N2 – 94% and BC700–N2 - 35%).

3.3. Influence of CO2 on persistence of PAHs (Ctot)

Adding the biochar produced in an atmosphere of CO_2 to the soil caused a lower increase in C_{tot} content than that found for the biochar produced in N_2, which was associated with the lower total PAHs content in this biochar (Table S3). The content of Σ16 C_{tot} PAHs in the soil with BC500–CO2 and BC700–CO2 was lower respectively by 20 and 19% than in the soil with BC500–N2 and BC700–N2 ($P \leq 0.05$).

After 30 days of the experiment, the Σ16 C_{tot} content did not change in the soil with BC500–CO_2, while in the soil with BC700–CO2 it decreased by 42% compared to the beginning of the study ($P \leq 0.05$). This difference may indicat the different trends in PAH behavior for the biochars produced at 500 °C in an atmosphere of N_2 and CO_2. In spite of their initial constant level in the soil with BC500–CO2, ultimately a significantly lower (by 34%) content of Σ16 C_{tot} PAHs was found after 180 days than in the soil with BC500–N2 (Fig. 1). In the soil with BC700–CO2, after 90 and 180 days the Σ16 C_{tot} PAH content did not differ significantly ($P > 0.05$) with BC700–N2 and it was lower by 49% compared to the beginning of the study (Fig. 1). It may show that the behavior of PAHs in the biochars produced at the lower temperature (500 °C) is affected by the carrier gas, whereas for the biochars produced at a temperature of 700 °C the changes in PAHs are similar regardless of

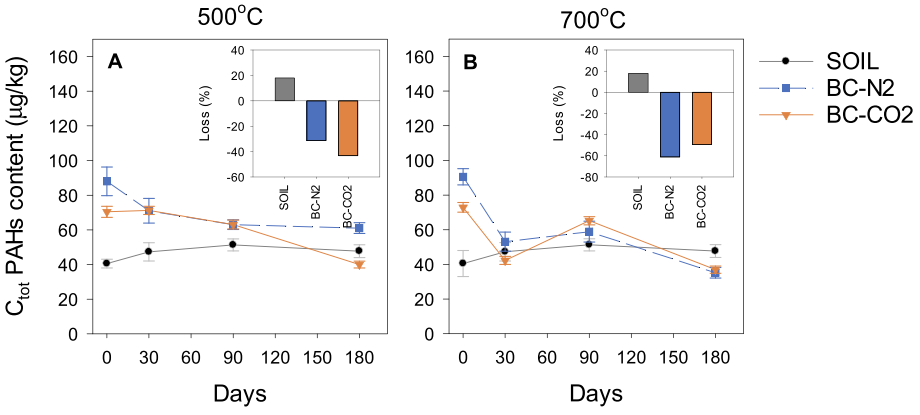

Fig. 1. Changes in total PAHs content (Σ16 C_{tot}) in soil (SOIL), soil with SL-derived biochar in N_2 atmosphere (BC–N2) or soil with SL-derived biochar in CO_2 atmosphere (BC–CO2) during 180 days of experiment. Biochars produced in 500 (A) or 700 °C (B).

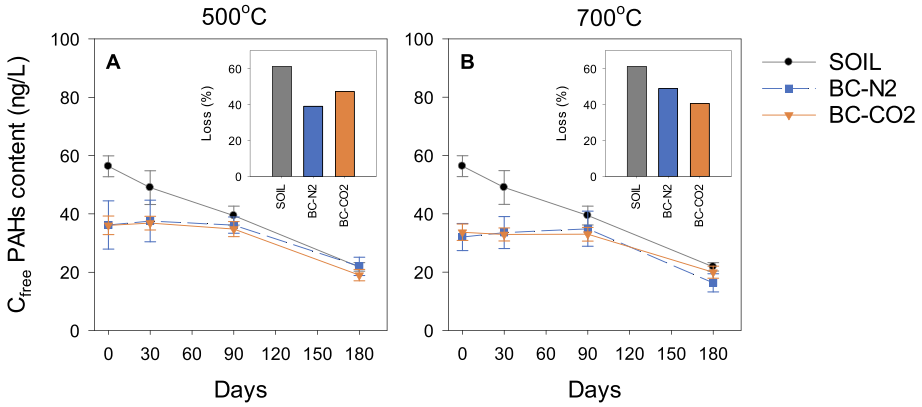

Fig. 2. Changes in water dissolved PAHs content (Σ16 C_{free}) in soil (SOIL), soil with SL-derived biochar in N_2 atmosphere (BC–N2) or soil with SL-derived biochar in CO_2 atmosphere (BC–CO2) during 180 days of experiment. Biochars produced in 500 (A) or 700 °C (B).

the carrier gas used during pyrolysis.

When analyzing the individual PAHs (Fig. S1), it was found that after 30 days after BC500–CO2 application to soil only the naphthalene content decreased (by 18%), while the content of 3- and 4-ring PAHs increased by 52 and 24%, respectively ($P \leq 0.05$). During the period of 30 days, the 5- and 6-ring PAH content did not change relative to the beginning of the experiment. After 90 days a further decrease in the 2-ring PAHs content, no change for 3-, 4- and 5-ring PAHs, and a 62% increase 6-ring PAHs were observed. Finally, after 180 days the 2- and 3-ring PAH content was lower by 73 and 22%, the 6-ring PAH content was higher by 35%, whereas the content of 4- and 5-ring PAHs did not change in comparison with the beginning of the study (Fig. S1). In the case of the biochar BC700–CO2, the 2-ring PAH content increased by 22%, whereas the content of the 3-6-ring PAH groups significantly ($P \leq 0.05$) decreased (from 19 to 73%). At the successive time points, for all PAH groups their content was observed to decrease, reaching a level lower by 34–62% after 180 days compared to the beginning of the study.

Apart from few divergence, the direction of changes in the individual C_{tot} PAH groups did not differ significantly ($P > 0.05$), regardless of the biochar production conditions (N_2 or CO_2) (Fig. S1). This was particularly seen for the biochars produced at 500 °C where, apart from the differences in the content of the individual PAHs (mainly at the beginning of the study due to the different PAH content in the initial biochar), the trend was similar between the biochars. While for the total PAHs their behavior was similar, for 2- and 3-ring PAHs clear differences were observed between the biochars produced in N_2 and CO_2 (Fig. S1).

3.4. Effect of CO_2 on freely dissolved PAHs (C_{free})

Fig. 2 illustrates the change in the content of Σ16 C_{free} PAHs in the soil with the BCs obtained in a CO_2 atmosphere. The addition of the biochar produced in CO_2 and the subsequent changes did not differ significantly from the C_{free} content observed for the biochars produced in N_2 (Fig. 2). The absence of differences resulted from the same behavior of 2-ring PAHs, dominant in the group of C_{free} PAHs (Fig. S2A). The addition of BC500–CO2 and BC700–CO2 increased the 3- and 4-ring PAH content more than application of the same biochars but produced in an atmosphere of N_2 (BC500–N2, BC700–N2) (Figs. S2B and C). The analysis of the other PAH groups revealed significant differences ($P \leq 0.05$) between both the content and behavior of the individual PAHs in the soils amended with the biochars obtained in CO_2 and N_2. It was, however, observed that the differences between the biochars produced in CO_2 and N_2 were greater for the biochars produced at 500 °C relative to 700 °C. It was also found that the behavior of the individual groups of C_{free} PAHs did not differ significantly ($P > 0.05$) between the biochars produced at 500 and 700 °C in an atmosphere of CO_2 (Fig. S2).

3.5. Persistence of PAHs in co-pyrolyzed biochar-amended soil

Application of the biochars produced from the mixture of SL and willow in an N_2 atmosphere into the soil increased the content of Σ16 C_{tot} PAHs in it by 19 and 53% in the soil with BCW500–N2 and BCW700–N2, respectively (Fig. 3). After 30 days the Σ16 C_{tot} PAH content in the soil with BCW500–N2 decreased by 34% ($P \leq 0.05$), whereas it did not change in the soil with BCW700–N2 ($P > 0.05$). At the successive time points, for both biochars a significantly lower content ($P \leq 0.05$) was found relative to the 30th day only on the 180th day of the study (Fig. 3). Ultimately, after 180 days the content of Σ16 C_{tot} PAHs was lower by 14% (BCW500–N2) and 23% (BCW700–N2) in comparison to the beginning of the study. Compared to the control soil, the Σ16 C_{tot} PAH content was lower by 13% in the experiment with BCW500–N2 and did not differ significantly in the experiment with BCW700–N2.

Regarding to the content of the individual PAH groups, it was found that during the first 30 days the content all PAH groups decreased in the soil with BCW500–N2 (from 24 to 45%) (Figs. S3A and B). In the experiment with BCW700–N2, only the 3-ring PAH content decreased (by 18%), whereas the content of 2-, 5- and 6-ring PAHs increased in the range from 11 to 28% (Figs. S3C and E). The 4-ring PAH content, on the other hand, did not change. After 90 days from biochar incorporation, in the experiment with BCW500–N2 the content of 2 and 3-ring PAHs was observed to further decrease (by 27 and 27%), while the 4-6-ring PAH content increased (from 21 to 62%) in comparison with the beginning of the study. After 180 days only the content of 2- and 3-ring PAHs was lower than at the beginning of the study (Fig. S3), whereas the content of most of the other PAH groups was higher from 18% to 139% relative to the beginning of the experiment. An exception were 5-ring PAHs in the experiment with BCW700–N2, whose content did not differ significantly between the beginning and end of the study, in spite of significant variations during the experiment.

3.6. Freely dissolved PAHs content in co-pyrolyzed biochar-amended soil

Immediately after application of the mixture of sewage sludge and willow-derived biochar into the soil, the content of Σ16 C_{free} PAHs did not differ significantly ($P > 0.05$) between the biochars produced at the different temperatures (Fig. 4). However, the addition of biochar to the soil caused a decrease in Σ16 C_{free} PAHs from 34% to 36% ($P \leq 0.05$).

During the first 90 days of the experiment, the content of Σ16 C_{free} PAHs remained at a constant level in the experiment with both BCW500–N2 and BCW700–N2. It was only on the 180th day of the experiment that there was a significant decrease in the content of Σ16 C_{free} PAHs, which was 55% (BCW500–N2) and 53% (BCW700–N2) compared to the beginning of the study (Fig. 4).

Despite that the level of Σ16 C_{free} PAHs remained constant

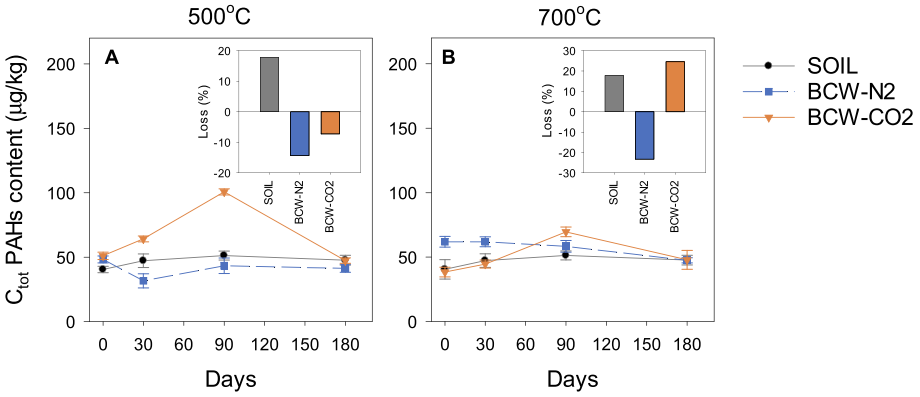

Fig. 3. Changes in total PAHs content (Σ16 C_{tot}) in soil (SOIL), soil with SL with willow-derived biochar in N_2 atmosphere (BCW–N2) or soil with SL with willow-derived biochar in CO_2 atmosphere (BCW–CO2) during 180 days of experiment. Biochars produced in 500 (A, C) or 700 °C (B, D).

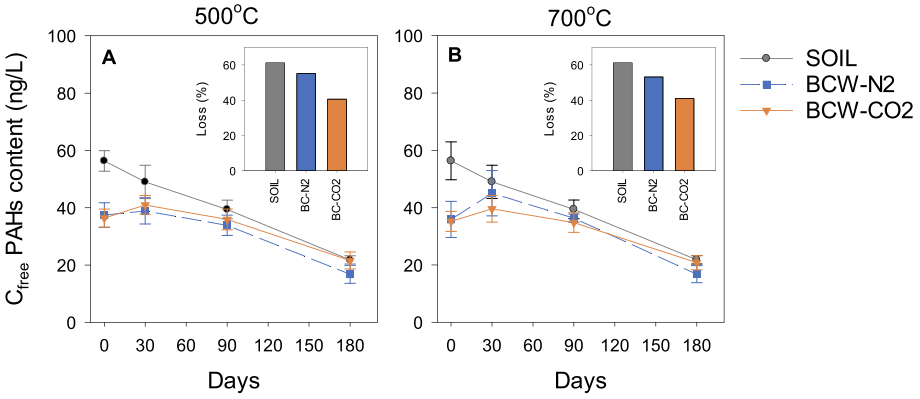

Fig. 4. Changes in water dissolved PAHs content (Σ16 C_{free}) in soil (SOIL)), soil with SL with willow-derived biochar in N_2 atmosphere (BCW–N2) or soil with SL with willow-derived biochar in CO_2 atmosphere (BCW–CO2) during 180 days of experiment. Biochars produced in 500 (A, C) or 700 °C (B, D).

throughout most of the experiment, the individual PAHs were characterized by high dynamics of changes. Generally, the 3- and 4-ring PAH content increased, while the 6-ring PAH content decreased (Fig. S4). The 2-ring PAH content remained unchanged during the first 90 days of the experiment, while the 5-ring PAH content varied significantly. Ultimately, after 180 days of the study the content of 2-, 5- and 6-ring C_{free} PAHs was significantly lower compared to the beginning of the experiment, respectively by 57, 92 and 73% for BCW500–N2 as well as by 55, 47 and 75% for BCW700–N2. However, an increase in the level of 3-ring (BCW700–N2 by 15%) and 4-ring PAHs (BCW500–N2 by 12%) was observed. The 3-ring PAH content in the soil with BCW500–N2 and the 4-ring PAH content in the soil with BCW700–N2 did not change compared to the beginning of the study.

3.7. *Influence of CO_2 on persistence of PAHs in co-pyrolyzed biochar*

The behavior of Σ16 C_{tot} PAHs in the soil amended with the co-pyrolyzed biochar produced in CO_2 was completely different than that observed for the co-pyrolyzed biochar obtained in N_2 (Fig. 3). Adding the biochars produced in CO_2 to the soil did not affect the content of Σ16 C_{tot} PAHs in it ($P > 0.05$).

After 30 days the content of Σ16 C_{tot} PAHs in the soil with the biochars produced in an atmosphere of CO_2 increased by 26% (BCW500–CO2) and by 16% (BCW700–CO2) compared to the beginning of the study ($P \leq 0.05$). At the successive time points, the PAH content continued to increase (by 97% for BCW500–CO2 and by 81% for BCW700–CO2 relative to the beginning of the study). It was only between the 90th and 180th day of the experiment that there was an abrupt decrease to the level observed at the beginning of the study. The content of Σ16 C_{tot} PAHs did not differ between the biochars produced in N_2 and CO_2 only at the beginning and end of the study. At the other time points, statistically significant differences ($P \leq 0.05$) were found, which may indicate different behavior of PAHs depending on gas conditions during pyrolysis.

When analyzing the content of the individual PAH groups (Fig. S3), during the first 30 days the 2-, 5- and 6-ring PAH content was found to increase from 44 to 66% in the experiment with BCW500–CO2, while the 3-5-ring PAH content increased from 10 to 62% in the experiment with BCW700–CO2. In the case of the other PAH groups, the changes were not statistically significant (Fig. S3) ($P > 0.05$). During the next 90 days, the 2-ring PAH content decreased (BCW500–CO2), while that of the other groups (3-6-ring ones) increased (BCW500–CO2, BCW700–CO2). After 180 days significant differences were observed between the biochars produced at 500 and 700 °C. In the case of BCW500–CO2, the 2- and 3-ring PAH content fell by 31 and 40%, respectively, whereas the content of 4-6-ring PAHs increased from 27 to 97%. In the case of BCW700–CO2, only the 2-ring PAH content decreased by 42%. The 3-6-ring PAH content increased from 131 to 546%.

3.8. *Freely dissolved PAHs in co-pyrolyzed biochar-amended soil*

At the beginning of the study, the content of Σ16 C_{free} PAHs was between 35.2 (BCW700–CO2) and 36.4 ng/L (BCW500–CO2) and did not differ significantly ($P > 0.05$) in comparison with the soil with the biochars obtained in N_2, being lower by respectively 35 and 38% than in the control soil ($P \leq 0.05$) (Fig. 4). 2-ring PAHs were predominant in the soil, which had also been observed for the previous treatments and

which was due to the dominant proportion of PAHs in the C_{free} fraction.

After 30 days of the experiment, no significant changes ($P > 0.05$) were observed in the content of Σ16 C_{free} PAHs, but at the successive time points there was a significant fall ($P \leq 0.05$). After 180 days the Σ16 C_{free} PAH content was lower by 41% (in the case of both biochars) than at the beginning of the study. The soil amended with BCW500–CO2 and BCW700–CO2 had a 28% and 24% higher content of Σ16 C_{free} PAHs, respectively, after 180 days compared to their counterparts obtained in an N_2 atmosphere.

The behavior of the individual PAH groups was dependent on the number of rings. In the case of 2-ring PAHs, during the first 90 days no changes were noted, but during the period between the 90th and 180th day of the study there was an abrupt fall (Figs. S4A and F). The 2-ring PAH content was lower than at the beginning of the study by 42% (both for BCW500–CO2 and BCW700–CO2). No significant differences were found between the biochars produced in N_2 and CO_2. The behavior of 3- and 4-ring PAHs was similar between BCW500–CO2 and BCW700–CO2 as well as statistically significantly different ($P \leq 0.05$), but only in the case of BCW500–N2 (for most of the time points). After 180 days the 3-ring PAH content did not differ statistically significantly relative to the beginning of the study ($P > 0.05$), whereas that of 4-ring PAHs was lower than at the beginning of the study by 25% (BCW500–CO2) and 26% (BCW700–CO2). During the first 30 days, the content of C_{free} 5- and 6-ring PAHs did not change significantly, either. In the case of 5-ring PAHs, there was a significant increase in their content in the period between the 30th and 90th day of the study, but on the 180th day a fall was recorded to the level from the beginning of the experiment (Figs. S4D and I). For most of the time points, no significant differences ($P > 0.05$) in the content of these PAHs were observed between the biochars produced in CO_2 and N_2. The 6-ring PAH content, however, decreased significantly ($P \leq 0.05$) and was lower by 72% (BCW500–CO2) and 82% (BCW700–CO2) at the last time point than at the beginning of the study (Figs. S4E and J).

4. Discussion

Changes in PAH content in biochar-amended soil vary and are predominantly dependent on the type of feedstock and pyrolysis conditions, such as temperature, pressure or carrier gas (Ahmad et al., 2014a).

The existing studies regarding the persistence of PAHs in biochar-amended soils are mainly focused on C_{tot} PAHs and biomass-derived biochars. For example, Maienza et al. (2017) and Rombolà et al. (2019) studied the persistence of PAHs in soil with the addition of biochar produced from orchard pruning waste at 500 °C. These authors observed an increase in PAH content after adding biochar to the soil, but in the successive years the PAH content decreased by 40–42% (Maienza et al., 2017; Rombolà et al., 2019). According to these authors, the decrease in PAH content in the biochar-amended soil was an effect of overlapping processes, such as leaching, photodegradation, biodegradation, bioaccumulation, and volatilization. de Resende et al. (2018) investigated the effect of biochar addition on C_{tot} PAH content in two different soils (Ferralsol, located at different sites) and using two biochars (savannah wood biochar produced at 350 °C and eucalyptus biochar produced at 450 °C). In this case, the PAH content in the savannah wood biochar-amended soil was found to be higher by 43% and 30% after 3 and 5 years of the experiment, respectively. It was only after 6 years that the authors found the PAH content in the biochar-amended soil to become equal to the PAH content in the control soil (de Resende et al., 2018). The results found in this study demonstrate that C_{tot} PAH content may decrease from 43 to 49% over a period of 6 months, which is significantly determined by biochar production conditions.

The change in the content of both C_{tot} PAHs and C_{free} PAHs clearly differed for the biochars tested and it was significantly dependent on the stage of the experiment. Such behavior of PAHs hinders precise determination of the effect of the carrier gas used during pyrolysis on the persistence and bioavailability of PAHs in biochar-amended soil.

Fig. 5 shows the difference in the change in PAH content during the 180 days of the experiment in the soil amended with the biochar produced in N_2 relative to CO_2. For SL-derived biochar, the change of carrier gas from N_2 to CO_2 reduced the persistence of PAHs by 38% after adding BC500–CO_2 to the soil, while it increased the persistence of PAHs by 19% in the soil with BC700–CO2 (Fig. 5A). This indicates that depending on the pyrolysis temperature, the effect of carrier gas on PAH persistence varies, which may be due to the different properties of the biochars produced.

Fig. 6 presents a heatmap that illustrates the differences in the persistence and bioavailability of PAHs during the 180 days of the experiment between the treatments with the biochars produced in N_2 and CO_2. For SL-derived biochars, the persistence of Σ16 C_{tot} PAHs was higher when BC500–N2 and BC700–CO2 were added to the soil (Fig. 6A). The differences between these two temperature treatments were associated with the persistence of 3- and 4-ring PAHs. For 2- and 3-ring PAHs, the persistence was higher when the biochars produced in CO_2 were added to the soil. This was the same for 6-ring PAHs and BC500–CO2. As far as the other groups are concerned, there were no statistically significant differences ($P > 0.05$). Σ16 C_{tot} PAHs in the soil amended with the co-pyrolyzed biochars were more persistent when CO_2 was the carrier gas in comparison with N_2. This was most visible for BCW700–CO2 (Fig. 6B); the persistence of all groups, apart from 2-ring PAHs, was higher when CO_2 was the gas used. In the case of 2-ring PAHs, there were no significant differences ($P > 0.05$). As regards BCW500, the change of gas to CO_2 was favorable only for 2-ring PAHs, no differences were observed for 3- and 4-ring PAHs ($P > 0.05$), while BCW500–N2 was more favorable for the persistence of 5- and 6-ring PAHs.

To determine the biochar properties that could have had a potential impact on the persistence of PAHs in the soil, a Pearson's correlation analysis was performed (Table S4). The physico-chemical properties of biochars, such as porosity or chemical composition, have a significant effect on the sorption of organic compounds (Quilliam et al., 2013; Ahmad et al., 2014b; Mohan et al., 2014). The statistical analysis of the relationship between the properties of the biochars and PAH losses after 180 days revealed a statistically significant correlation between the average pore size (d) and the loss of Σ16 C_{tot} PAHs (0.982, $p = 0.95$). The higher the pore size of the tested material, the greater the losses of Σ16 C_{tot} PAHs were observed. This may suggest that PAH losses were related to the processes of sequestration of the studied compounds rather than to their actual losses in the investigated experimental system. High porosity promotes the adsorption of contaminants, due to which they become more difficult to extract even if strong extractants are used (Ukalska-Jaruga et al., 2020). A negative correlation was observed between the specific surface area (S_{BET}) of BC-CO2 and the losses of 5-ring (−0.961, $p = 0.95$) and 6-ring (−0.966, $p = 0.95$) C_{tot} PAHs as well as between the micropore volume (V_{micro}) and the losses of 5-ring (−0.986, $p = 0.95$) and 6-ring (−0.988, $p = 0.95$) C_{tot} PAHs. The higher the microporosity and S_{BET} of the tested material, the lesser the losses of high molecular weight PAHs were observed. This could mean that heavy PAHs were retained on the biochar surface but they were not strongly sequestrated, which would prevent extraction of PAHs by the solvent used. A relationship between the organic carbon (OC) content and the loss of Σ16 C_{tot} PAHs was also observed (−0.957, $p = 0.95$). In this case, the reduced losses are explained by PAH sequestration.

In the case of the biochars produced in N_2, on the other hand, statistically significant positive correlations were observed between the average pore size (d) and the loss of 2-ring C_{tot} PAHs (0.968, $p = 0.95$), between the total pore volume (V_t) and the loss of 5-ring C_{tot} PAHs (0.981, $p = 0.95$) as well as between the mesopore volume (V_{meso}) and the loss of Σ16 C_{tot} PAHs (0.956, $p = 0.95$) and 5-ring C_{tot} PAHs (0.951, $p = 0.95$). The biochars obtained in N_2 had larger sized pores than the biochars produced in CO_2 (Table S2). This may suggest that their accessibility for PAH molecules was greater. This caused higher losses of PAHs, being a result of sequestration-related processes. Adsorption in

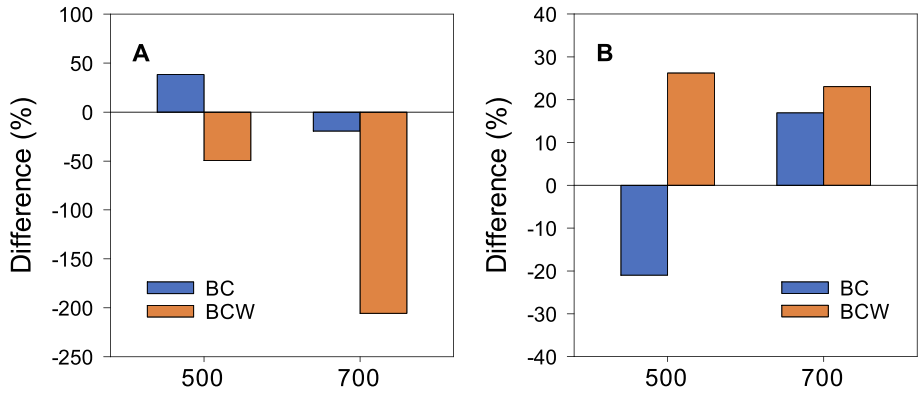

Fig. 5. Difference in Σ16 C_{tot} (A) and Σ16 C_{free} (B) PAHs loss between biochars produced in N_2 or CO_2. The negative value stands for higher persistence (A) or greater loss (B) in soil amended with biochar produced in CO_2, while positive value stands for higher persistence (A) or loss (B) in soil amended with biochar produced in N_2.

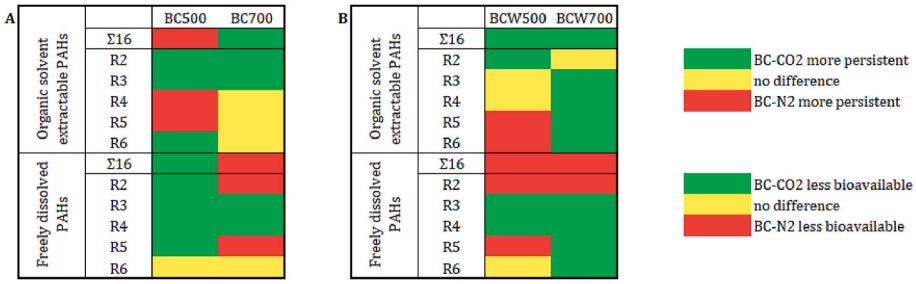

Fig. 6. Heatmap with relative changes in persistence or bioavailability of PAHs in soil with BC (A) or BCW (B) produced in N_2 compared to CO_2.

pores is a very frequent mechanism leading to the retention of PAHs in biochar (Wang et al., 2016; Hu et al., 2018; Jin et al., 2018; Ambaye et al., 2021).

On the other hand, different results were observed for C_{free} PAHs. The change of N_2 to CO_2 increased the losses of C_{free} PAHs for BC500 (by 21%), whereas for BC700 the losses were observed to decrease by 17% (Fig. 5). The reduction in the Σ16 C_{free} PAH content was higher (that is, more favorable) for BC500–CO2 and BC700–N2 (Fig. 6). The more favorable effect of N_2 when the biochar produced at 700 °C was added to the soil is associated with the content of 2- and 5-ring C_{free} PAHs. The content of 6-ring C_{free} PAHs changed similarly regardless of the carrier gas. The changes in the other groups were more favorable when the biochar was produced in CO_2. In the case of BCW, the reduction in Σ16 C_{free} PAHs was more distinct when N_2 was the carrier gas. The content of the 2-ring PAH group, dominant in the composition of Σ16 C_{free}, decreased to a greater extent when BCW-N2 was added to the soil compared to BCW-CO2. In the case of BCW500, the change in 5-ring C_{free} PAHs was more favorable when N_2 was the carrier gas, while the change in 6-ring PAHs was comparable for both carrier gases. The change in the other C_{free} PAH groups when BCW was added to the soil was more favorable when CO_2 was the carrier gas. Based on the analysis of the correlations between the properties of the biochars produced in CO_2 and the loss of PAHs (Table S6), a relationship was observed between S_{BET} and the loss of 6-ring C_{free} PAHs (0.963, $p = 0.95$), the average pore size and the loss of 3-ring C_{free} PAHs (0.952, $p = 0.95$) and the loss of 4-ring C_{free} PAHs (−0.970, $p = 0.95$) as well as between the micropore volume (V_{micro}) and the loss of 6-ring C_{free} PAHs (0.977, $p = 0.95$). Relationships with the chemical properties of the biochars were also observed, i.e. between C content and the loss of 3-ring C_{free} PAHs (−0.965, $p = 0.95$) and the loss of 4-ring C_{free} PAHs (0.988, $p = 0.95$) as well as between OC content and the loss of 3-ring C_{free} PAHs (−0.966, $p = 0.95$) and the loss of 4-ring C_{free} PAHs (0.988, $p = 0.95$). These correlations indicate the adsorption of 3- and 6-ring PAHs in biochar pores, but in the case of 4-ring PAHs the adsorption was a result of hydrophobic interactions. No relationships were found between the biochar properties and the loss of C_{free} PAHs in the case of the biochars produced in N_2.

The content of C_{tot} and C_{free} Σ16 PAHs at the individual evaluation time points was also analyzed (Fig. S5). C_{tot} content greatly varied depending on the time point, but in most cases (in particular during the first 30 days) a higher PAH content was observed in the soil with BC/W–N2 than for BC/W–CO2. At the third evaluation time point, however, an opposite trend was observed which indicated a higher content of C_{tot} Σ16 PAHs in the soil with CO_2 than in that with N_2. Nonetheless, at the last time point no significant differences were observed in the content of C_{tot} Σ16 PAHs in the soil amended with BC/W–N2 and BC/W–CO2, except for BC500. No significant differences were found in the content of C_{free} Σ16 PAHs in the soil with BC/W–N2 and BC/W–CO2, either.

5. Conclusions

The change of gas from N_2 to CO_2 significantly increased the loss of organic solvent-extractable (C_{tot}) PAHs in the soil with biochar addition. The changes in the physico-chemical properties of the biochars, such as specific surface area (S_{BET}) and porosity (d, V_{tot}, V_{macro}, V_{micro}), were responsible for the greater persistence; however, this was not mainly due to higher adsorption but an increase in the susceptibility of PAHs to biodegradation.

The bioavailability of PAHs decreased as result of the change of gas only for the biochar produced at 500 °C from sewage sludge alone (BC500–CO2). In the other cases, the bioavailability of PAHs increased. As far as BC500–CO2 is concerned, PAHs could have had greater access to organic carbon, due to which the adsorption occurred and the bioavailability of 3- and 4-ring PAHs decreased.

Credit author statement

Paulina Godlewska Conceptualization, Methodology, Investigation, Writing - original draft; Patryk Oleszczuk conceptualization, review and

editing, supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2022.120145.

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INTERNET SUPPORTING INFORMATION

Effect of carrier gas change during sewage sludge or sewage sludge and willow pyrolysis on ecotoxicity of biochar-amended soil

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MATERIALS AND METHODS

Sewage sludge

Sewage sludge (SL) was obtained from municipal wastewater treatment plant where SL is stabilized biologically by aerobic fermentation and chemically by threated with lime. SL were collected during autumn 2016 from municipal sewage treatment plant localized in Chełm (southeastern part of Poland, 510705600N, 232804000E, population of 64 000 people). Sewage treatment plant were located on agricultural areas and used mainly urban wastewater without the great impact of the industry.

Biochar preparation

Briefly, the sewage sludge (SL) before pyrolysis was grinded and sieved through a 2 mm sieve. SL was obtained from municipal wastewater treatment plant localized in Chełm (50°20′04″N 23°29′49″E) which uses mainly urban wastewater with limited influence of wastewater from industry. The willow was provided by a biomass-producing farm. Freshly cut willow was air-dried for two weeks and then cut into small pieces and sieved through 2 mm sieve. Mixtures of SL and willow (6:4 w/w) were obtained by mixing both materials in glass bottles (1000 mL) for 24 h in the dark at 10 rpm (Rotax 6.8. VELP, Italy). SL alone and SL with willow were pyrolysed in 500, 600 or 700°C, with the heating rate 10°C/ min. Temperature was held for 3 h (slow pyrolysis). During the pyrolysis the oxygen free atmosphere was maintained by constant flow of N_2. The physico-chemical properties of SL- and SL/biomass-derived biochar are presented in Table S1.

Statistical analysis

Mean values were taken from a triplicate data set. The differences between ecotoxicological endpoints in particular terms (and between terms) and variants were evaluated using a one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. Repeated measures analysis of variance (ANOVA) was performed to investigate whether sampling time had an effect on the tests' results, as the within subject factor (Table S6). Pearson's correlation coefficient test was performed to investigate the relations between physico-chemical properties and tests' results with Statistica 5.0. Significance was set at $*P \geq 0.05$.

Table S1. The physico-chemical properties of biochars used in the experiment.

	pH	Ash content (%)	C (%)	OC (%)	H (%)	N (%)	O (%)	O/C	H/C	(O+N)/C	S_{BET} (m^2/g)	d (nm)	V_t (m^3/g)	V_{micro} (m^3/g)	V_{meso} (m^3/g)
BC500-N2	9.4	64.1	26.3	26.2	0.99	3.26	5.38	0.15	0.45	0.26	69.7	5.98	0.104	0.0189	0.0854
BC700-N2	12.4	71.4	24.5	24.4	0.29	2.10	1.71	0.05	0.19	0.13	89.2	6.18	0.137	0.0240	0.1139
BCW500-N2	10.8	46.4	44.6	44.2	1.66	3.33	3.93	0.07	0.44	0.13	74.6	4.66	0.087	0.0207	0.0663
BCW700-N2	12.5	50.9	46.2	46.0	0.62	2.09	0.21	0.02	0.16	0.04	104.1	4.93	0.115	0.0269	0.0881
BC500-CO2	9.2	59.2	25.1	24.5	0.68	3.17	11.8	0.35	0.32	0.46	71.1	5.59	0.099	0.0198	0.0798
BC700-CO2	9.8	69.7	22.7	22.1	0.16	1.86	2.64	0.1	0.09	0.17	83.5	5.86	0.122	0.0206	0.1018
BCW500-CO2	9.3	43.3	44.7	44.2	1.58	3.35	7.08	0.12	0.42	0.18	88.7	4.14	0.091	0.0262	0.0656
BCW700-CO2	9.8	48.6	47.7	47.2	0.59	2.49	0.69	0.01	0.15	0.06	152.5	3.61	0.137	0.0468	0.0908

pH- in H_2O- 1:10 (w/v)

Table S2. The water-extractable metal content (mg/L) of biochar and biochar's feedstock used in the experiment.

	Zn	Cu	Cr	Ni	Cd	Pb	Co	Mn	Fe	Ba	B	Al
SL	10.97 ±0.51	0.73 ±0.02	0.12 ±0.02	2.39 ±0.3	0.26 ±0.002	0.31 ±0.01	0.48 ±0.02	8.47 ±0.52	22.3 ±1.90	1.02 ±0.20	22.3 ±1.30	1196 ±59.3
W	5.24 ±0.22	0.27 ±0.03	0.04 ±0.001	10.97 ±0.51	0.23 ±0.01	0.10 ±0.02	0.01 ±0.002	1.96 ±0.20	3.35 ±0.41	0.41 ±0.02	0.41 ±0.01	134 ±12.3
BC500-N2	6.36 ±0.83	0.30 ±0.02	0.01 ±0.001	0.03 ±0.001	0.05 ±0.002	0.01 ±0.001	0.03 ±0.001	0.32 ±0.02	0.23 ±0.01	0.37 ±0.02	19.8 ±1.70	765 ±65.3
BC700-N2	4.97 ±0.61	0.28 ±0.05	0.01 ±0.001	0.03 ±0.005	0.07 ±0.002	0.01 ±0.001	0.02 ±0.001	0.15 ±0.01	0.21 ±0.01	0.87 ±0.01	3.75 ±0.50	106 ±9.34
BCW500-N2	3.12 ±0.31	0.11 ±0.01	0.02 ±0.002	0.02 ±0.002	< DL	< DL	0.02 ±0.001	0.21 ±0.02	0.11 ±0.02	0.30 ±0.01	12.5 ±0.92	777 ±59.3
BCW700-N2	0.30 ±0.01	0.24 ±0.01	0.01 ±0.001	0.01 ±0.001	< DL	< DL	0.01 ±0.001	0.01 ±0.001	0.01 ±0.002	0.75 ±0.05	2.12 ±0.62	102 ±19.8
BC500-CO2	4.61 ±0.21	0.22 ±0.01	0.01 ±0.001	0.06 ±0.002	< DL	< DL	< DL	0.07 ±0.002	0.18 ±0.03	0.42 ±0.02	7.83 ±0.51	2183 ±98.7
BC700-CO2	0.95 ±0.02	0.01 ±0.002	0.02 ±0.002	0.02 ±0.001	< DL	< DL	< DL	0.02 ±0.002	0.03 ±0.001	0.73 ±0.06	4.59 ±1.30	1183 ±91.3
BCW500-CO2	2.01 ±0.33	0.10 ±0.01	0.01 ±0.001	0.01 ±0.002	< DL	< DL	< DL	0.08 ±0.001	0.01 ±0.001	0.17 ±0.06	5.6 ±1.61	4274 ±259
BCW700-CO2	0.59 ±0.02	0.03 ±0.001	0.01 ±0.003	0.01 ±0.003	< DL	< DL	< DL	0.01 ±0.002	0.01 ±0.001	0.22 ±0.04	4.91 ±1.21	1189 ±98.3

Table S3. Chemical properties of control soil.

pH	Total N content (%)	Available nutrients (mg/ 100 g of soil)			Exchangeable cations content (mg/ 100 g of soil)				Hydrolytic acidity (mmol H^+ /100 g of soil)
6.84 in H_2O	0.064	P_2O_5	K_2O	Mg	Mg	Na	K	Ca	1.25
6.80 in KCl		12.2	11.7	2.2	3.50	2.87	10.6	101	

pH in H_2O- 1:10 (m/v), pH in 1M KCl- 1:2.5 (m/v), total N – Kjeldahl method, available P and K – Egner-Riehm method, available Mg – Schachtschabel method, exchangeable cations- Kappen method, hydrolytic acidity- Kappen method

Table S4. Chemical properties of soil with biochar pyrolyzed in N_2.

	pH	EC	TOC	DOC	CEC	Available		
						Mg	K	P
BC500-N2								
0 days	6.9	130.6	1.1	10.9	11.0	4.9	12.7	16.4
30 days	6.9	131.6	0.8	7.9	11.0	4.2	12.3	20.0
90 days	7.1	155.5	1.2	11.3	10.3	3.9	13.0	31.0
180 days	6.6	188.8	1.0	3.7	10.4	2.4	12.4	30.3
BC700-N2								
0 days	7.2	111.1	1.0	10.1	11.0	3.9	11.9	14.7
30 days	7.0	182.7	1.0	9.1	11.2	3.2	12.1	14.2
90 days	7.2	196.9	0.8	8.6	8.9	3.9	12.3	25.7
180 days	6.5	292.0	0.7	3.7	9.6	2.5	12.7	25.6
BCW500-N2								
0 days	6.9	91.6	1.2	9.1	9.4	2.1	15.4	15.0
30 days	7.1	161.5	1.4	8.4	11.4	2.9	17.2	17.9
90 days	7.0	174.4	1.7	13.0	11.7	2.6	16.4	27.0
180 days	6.9	177.5	1.4	4.9	10.4	2.0	14.3	20.0
BCW700-N2								
0 days	7.1	87.5	1.6	10.1	10.9	4.3	14.2	13.0
30 days	6.7	136.1	0.7	8.9	11.4	3.5	15.4	14.1
90 days	6.9	169.9	0.8	10.5	10.8	3.7	14.6	21.1
180 days	6.1	210.8	1.8	11.6	9.6	2.7	14.1	17.4

pH- in water 1:10 (w/v), EC (µS/cm), TOC (%), DOC (mL/g), CEC (mmol/kg), available P (mg P_2O_5/ 100 g of soil) and K (mg K_2O/ 100 g of soil) – Egner-Riehm method, available Mg (mg Mg/ 100 g of soil) – Schachtschabel method

Table S5. Chemical properties of soil with biochar pyrolyzed in CO_2.

	pH	EC	TOC	DOC	CEC	Available		
						Mg	K	P
BC500-CO2								
0 days	6.8	169.8	0.9	12.0	11.5	2.6	12.4	18.4
30 days	7.0	196.4	0.6	8.4	9.9	0.1	11.1	26.3
90 days	7.0	92.1	1.0	8.6	11.0	3.8	11.8	13.0
180 days	7.4	92.6	1.2	12.2	10.9	0.1	11.1	27.9
BC700-CO2								
0 days	7.0	189.8	1.0	12.6	11.9	2.7	11.8	20.2
30 days	6.3	316.0	1.0	15.7	10.1	2.5	11.7	28.2
90 days	7.2	146.7	1.8	14.1	11.6	3.3	11.3	13.9
180 days	6.3	111.8	0.8	11.7	9.1	2.5	13.4	28.7
BCW500-CO2								
0 days	7.0	124.5	1.2	8.7	11.7	3.0	11.4	14.8
30 days	7.0	130.3	1.5	18.4	11.6	2.1	15.3	24.5
90 days	7.3	104.5	1.3	10.3	10.8	0.1	15.4	25.0
180 days	6.1	180.4	2.2	11.6	9.3	2.7	14.5	26.4
BCW700-CO2								
0 days	7.1	66.3	1.1	9.1	10.6	3.4	12.3	12.4
30 days	7.4	68.5	0.9	9.4	10.3	2.5	12.3	15.7
90 days	7.8	72.8	0.8	9.9	8.0	0.1	12.4	22.7
180 days	6.1	116.7	1.2	11.8	9.1	2.2	11.8	16.3

Table S6. RMANOVA results.

Source of variation		d.f.	F ratio	p value
A. fischeri	Microtox	3	10.33	0.000
L. sativum	Water	3	3.32	0.037
	Solid	3	2.12	0.013
F. candida	Mortality	3	2.88	0.060
	Reproduction	3	4.70	0.012

Table S7. *Aliivibrio fischeri* luminescence inhibition test (Microtox) results from multiple studies with biochars produced from sewage sludge or co-pyrolysed sewage sludge with plant biomass.

Biochar	Pyrolysis temperature (°C)	Experiment	Soil type	Luminescence inhibition	Reference
Sewage sludge	500	Lab Biochar-amended soil leachate (1:10)	Podzolic loamy sand	15%	[1]
	600			18%	
	700			<10%	
Sewage sludge (1)	300	Lab Biochar-amended soil leachate (1:4)	Sandy acid soil	30% (dose 0.5%), 50% (dose 1.0%) and 42% (dose 2.0%); control soil inhibited luminescence at 49%	[2]
Sewage sludge (2)	300			49% (dose 0.5%), 49% (dose 1.0%) and 47% (dose 2.0%); control soil inhibited luminescence at 49%	
Sewage sludge (3)	300			39% (dose 0.5%), 38% (dose 1.0%) and 39% (dose 2.0%); control soil inhibited luminescence at 49%	
Sewage sludge (A)	200	Biochar's leachate (1:100)	Without soil	22%	[3]
Sewage sludge (A) + rape straw	200			34%	
Sewage sludge (A) + wheat straw	200			28%	
Sewage sludge (A) + sawdust	200			25%	
Sewage sludge (A) + bark	200			17%	
Sewage sludge (B)	200			<10%	
Sewage sludge (B) + rape straw	200			25%	
Sewage sludge (B) + wheat straw	200			23%	
Sewage sludge (B) + sawdust	200			23%	
Sewage sludge (B) + bark	200			13%	
	300		Without soil	Stimulation of luminescence 26%	[4]

Sewage sludge + wheat straw	600	Biochar's leachate (1:20)		Stimulation of luminescence 25%	
Sewage sludge + sawdust	300			Stimulation of luminescence 25%	
	600			Stimulation of luminescence 26%	
Sewage sludge + bark	300			Stimulation of luminescence 18%	
	600			Stimulation of luminescence 23%	
Sewage sludge	500, 600 or 700	Biochar's leachate (1:10)	Without soil	0-94%, toxicity increased with increasing temperature	[5]
Sewage sludge + willow (8:2)	500, 600 or 700			0-91%, toxicity increased with increasing temperature	
Sewage sludge + willow (6:4)	500, 600 or 700			0-92%, toxicity increased with increasing temperature	
Sewage sludge	300	Biochar's leachate	Without soil	48%	[6]
	500			<10%	
	700			<10%	
	900			<10%	

Table S8. *Foslomia candida* mortality and reproduction inhibition test results from multiple studies with biochars produced from sewage sludge or co-pyrolysed sewage sludge with plant biomass.

Biochar	Pyrolysis	Experiment	Soil type	Effect	Reference
Sewage sludge	500ºC	Lab Dose: 1% (30 t/ha) at the beginning	Loamy sand	Mortality 20-90% Reproduction inhibition 100%	[7]
		Lab Dose: 1% (30 t/ha) after 6 months		Mortality decreased 10% Reproduction stimulation 140-180%	
Sewage sludge	500, 600 or 700ºC	Lab Dose: 1% (30 t/ha)	Loamy sand	Reproduction inhibition 30-80%, toxicity increased with increasing temperature	[8]
Sewage sludge + willow (8:2)	500, 600 or 700ºC			Reproduction stimulation 5-20% (500, 600 ºC) or inhibition 60% (700ºC)	
Sewage sludge + willow (6:4)	500, 600 or 700ºC			Reproduction stimulation 18-30% (500, 600 ºC) or inhibition 50% (700ºC)	
Sewage sludge	550	Dose: 0.5, 1.3, 3.2, 8, 20, and 50%,	Hypercalcic Calcisol	Reproduction stimulation at dose 1.3% and 3.2% or inhibition at 50% dose	[9]
Sewage sludge	500	Dose: 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 20.0%	ND	Reproduction inhibition 18-60%, toxicity increased with increasing dose	[10]

Table S9. Germination and root growth inhibition test results on plants from multiple studies with biochars produced from sewage sludge or co-pyrolysed sewage sludge with plant biomass.

Biochar	Pyrolysis	Experiment	Soil type	Plant	Effect	Reference
Sewage sludge	200ºC Residence time: 30 min	Biochar's leachate (1:100)	Without soil	*L. sativum*	Germination inhibition 0-25% Root growth inhibition 24-38%	[11]
Sewage sludge + rape straw					Germination inhibition 15-20% Root growth inhibition 9-34%	
Sewage sludge + wheat straw					Germination inhibition 5% Root growth inhibition 32-34%	

Sewage sludge + sawdust					Germination inhibition 0-5% Root growth inhibition 34-37%	
Sewage sludge + bark					Germination inhibition 10-20% Root growth inhibition 15-34%	
Sewage sludge	500ºC	Lab Dose: 1% (30 t/ha)	Loamy sand	*L. sativum*	Germination inhibition 0-20% Root growth inhibition 0-10% or stimulation 60%	[7]
Sewage sludge KN	500ºC	Lab Dose: 1% (30 t/ha) aqueous extracts	Loamy sand	*L. sativum*	Germination inhibition 0-30% Root growth inhibition 0-5% or stimulation 10%	
Sewage sludge	500, 600 or 700ºC	Biochar's leachate (1:100)	Without soil	*L. sativum*	Root growth stimulation 55% or inhibition 39-57%, toxicity increased with increasing temperature	[8]
Sewage sludge + willow (8:2)	500, 600 or 700ºC				Root growth stimulation 10-98% or 5% inhibition, toxicity increased with increasing temperature	
Sewage sludge + willow (6:4)	500, 600 or 700ºC				Root growth stimulation 105% or inhibition 5-15%, toxicity increased with increasing temperature	
Sewage sludge	500, 600 or 700ºC	Dose:1%	ND	*L. sativum*	Root growth inhibition 6-8%	
Sewage sludge + willow (8:2)	500, 600 or 700ºC				Root growth stimulation <5%	
Sewage sludge + willow (6:4)	500, 600 or 700ºC				Root growth stimulation <5%	
Paper sludge+ wheat husks	Temp. 500ºC Residence time: 20 min	Lab Dose: 8% w/w Temp. 28ºC Duration: 1 month	Haplic Cambisols ST- sandy loam SA- sandy	Lettuce (*Lactuca sativa*) Lentil (*Lens culinaris*)	Lettuce: ST- 33% lower stem length. SA- no statistical difference Lentil: ST- 14% lower aerial biomass, 38% lower root dry weight. SA- higher aerial biomass, stem length and root dry weight 15-38%	[12]
Sewage sludge					Lettuce: ST- 32% lower lower stem length, 75% lower root dry weight. SA- 34% higher stem length	

					Lentil: ST- no statistical difference. SA- 21% lower stem length	
Sewage sludge (from three different treatment plants)	Temp. 300ºC Residence time: 15 min	Lab Dose: 0.5 or 1 or 2% w/w Duration: 200 days	Loamy sand	*Poa pratensis* L.	1) From 60% to over 2.5- fold higher biomass depending on biochar dose. The higher dose, the higher biomass. 2) 80% higher biomass 3) 40-80% higher biomass	[13]
Sewage sludge	Temp. 300ºC Residence time: 30 min	Field Dose: 15 t/ ha Duration: 2 years	Red Yellow Latosol (Typic Haplustox)	Corn (hybrid LG6030)	42% higher grain yield	[14]
Sewage sludge	Temp. 300 or 500ºC Residence time: 30 min	Field Dose: 15 t/ ha Duration: 1 year	Clayey Oxisol (Typic Haplustox)	Corn	45% higher grain yield	[15]
Sewage sludge	Temp. 850ºC Residence time: 4 h	Field 20, 40 and 60 t/ha	Loamy sand	peanut (cv. No.16 Huayu)	the addition of biochar increased crop yield by 35-60%, the highest increase was noted for 40 t/ha addition	[16]
Sewage sludge	300, 500 or 700ºC	Lab aqueous extracts	Without soil	*Triticum aestivum* L.	No changes for seed germination Root growth inhibition- 6-18% Toxicity increased with increasing temperature	[17]
Sewage sludge	300, 500, 700 or 900ºC	Lab Dose: 5%	Sand	*Triticum* spp.	No changes for seed germination No changes for root length	[6]
Sewage sludge (different kinds: KN, KZ, CM, and SI)	500, 600 or 700ºC	Lab Dose: 1%	Standard soil (OECD)	*L. sativum*	KN, KZ, CM: 500ºC- stimulation of root growth (<10%), 600ºC and 700ºC- inhibition of root growth (<20%) SI: Stimulation of root growth (<20%)	[18]
Sewage sludge (different kinds: SS1, SS2, and SS3)	300ºC Residence time: 15 min	Lab aqueous extracts	Without soil	*L. sativum*	Inhibition 25% or stimulation 6% of root growth, depending of the sludge kind	[19]

Sewage sludge	550°C Residence time: 15 min	Dose: 0.4, 0.9, 2.1, 4.9, 11.3 and 26 %, 10.6 t/ha to 676 t/ha	Fluventic Haploxerept, sandy loam agricultural soil	*Lactuca sativa* and *Lolium perenne*	No toxic effect, EC10 >26% (>676 t/ha) for aboveground or belowground biomass	[20]

Table S10. Correlations between ecotoxicological tests results of the experiment and chemical properties of soil with SL- derived biochar addition. Statistically important coefficients were marked with *red (p=0.95) or **green (p=0.99) or ***violet (p=0.999).

		pH	EC	TOC	DOC	CEC	Available		
							Mg	K	P
0 days									
A. fischeri	Microtox test	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
L. sativum	Liquid	0.923	-0.760	-0.088	-0.799	-0.600	0.306	-0.466	-0.784
	Solid	0.497	-0.189	-0.790	-0.245	-0.090	-0.451	-0.523	-0.291
F. candida	Mortality	0.906	-0.727	-0.171	-0.769	-0.570	0.236	-0.483	-0.761
	Reproduction	0.287	0.432	-0.663	0.375	0.559	-0.858	-0.782	0.354
30 days									
A. fischeri	Microtox test	0.086	-0.540	0.321	-0.274	0.810	0.944	*0.952	-0.589
L. sativum	Liquid	-0.879	0.515	0.513	0.727	-0.063	0.505	0.355	0.408
	Solid	0.737	-0.784	0.102	-0.723	0.882	0.478	0.617	*-0.980
F. candida	Mortality	0.546	-0.732	0.266	-0.589	*0.973	0.714	0.819	*-0.965
	Reproduction	0.870	-0.610	-0.844	-0.810	-0.163	-0.590	-0.472	-0.140
90 days									
A. fischeri	Microtox test	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
L. sativum	Liquid	0.401	-0.076	*0.980	0.925	0.728	*-0.958	-0.684	-0.487
	Solid	0.105	0.576	-0.625	-0.432	-0.810	0.876	*0.960	*0.957
F. candida	Mortality	0.317	0.026	0.085	-0.081	0.040	-0.499	-0.804	-0.688
	Reproduction	-0.422	-0.588	-0.197	-0.414	0.248	-0.230	-0.678	-0.833
180 days									
A. fischeri	Microtox test	0.671	-0.898	0.608	0.942	0.385	-0.811	-0.503	0.129
L. sativum	Liquid	-0.218	0.199	0.232	-0.631	0.270	0.360	0.059	0.708
	Solid	-0.760	0.664	-0.496	-0.842	-0.386	0.875	0.607	0.235
F. candida	Mortality	-0.160	-0.096	-0.532	0.536	-0.609	-0.003	0.321	-0.650
	Reproduction	0.322	0.699	-0.065	-0.531	0.290	-0.178	-0.443	-0.716

Table S11. Correlations between ecotoxicological tests results of the experiment and total and bioavailable PAHs content in soil with SL- derived biochar addition. Statistically important coefficients were marked with *red (p=0.95) or **green (p=0.99) or ***violet (p=0.999).

		Total PAHs						Bioavailable PAHs					
		Σ16	R2	R3	R4	R5	R6	Σ16	R2	R3	R4	R5	R6
0 days													
A. fischeri	Microtox test	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
L. sativum	Liquid	0.599	-0.138	0.754	*0.972	***0.999	0.889	*-0.982	-0.876	-0.552	-0.715	**0.997	0.552
	Solid	-0.218	-0.812	-0.007	0.563	0.662	0.346	-0.708	-0.856	0.229	-0.015	0.671	-0.202
F. candida	Mortality	0.529	-0.220	0.697	*0.959	**0.997	0.863	*-0.988	-0.907	-0.482	-0.660	**0.996	0.491
	Reproduction	-0.448	-0.652	-0.433	0.260	0.254	-0.236	-0.207	-0.345	0.333	0.119	0.282	-0.692
30 days													
A. fischeri	Microtox test	0.405	0.548	0.592	-0.205	-0.734	-0.579	0.353	0.366	-0.110	0.508	-0.702	0.908
L. sativum	Liquid	-0.402	-0.239	-0.357	-0.841	-0.754	-0.081	-0.660	-0.665	-0.046	0.877	-0.651	0.014
	Solid	0.474	0.465	0.648	0.361	-0.137	-0.669	0.728	0.755	-0.349	-0.414	-0.236	0.845
F. candida	Mortality	0.428	0.474	0.637	0.140	-0.404	-0.766	0.619	0.647	-0.381	-0.154	-0.476	*0.965
	Reproduction	0.666	0.516	0.544	*0.988	0.875	0.448	0.762	0.750	0.495	-0.587	0.847	-0.145
90 days													
A. fischeri	Microtox test	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
L. sativum	Liquid	-0.858	0.341	-0.429	-0.766	-0.763	-0.688	-0.645	-0.317	-0.897	-0.850	-0.313	0.591
	Solid	0.377	0.334	0.714	0.118	0.109	0.164	*0.968	0.888	0.691	0.887	0.847	*-0.969
F. candida	Mortality	-0.323	-0.069	*-0.987	0.025	0.052	-0.322	-0.797	-0.696	-0.620	-0.734	-0.867	0.488
	Reproduction	0.302	-0.737	-0.688	0.610	0.626	0.385	-0.721	-0.918	-0.108	-0.412	-0.921	0.690
180 days													
A. fischeri	Microtox test	0.444	-0.086	0.759	-0.411	-0.512	-0.689	0.844	0.882	-0.200	-0.631	0.774	*-0.963
L. sativum	Liquid	0.391	0.771	-0.163	*0.966	*0.973	0.669	-0.167	-0.268	0.921	**0.992	-0.397	0.465
	Solid	-0.335	0.190	-0.773	0.687	0.765	0.898	-0.798	-0.856	0.404	0.806	-0.873	0.945
F. candida	Mortality	-0.678	*-0.953	-0.195	-0.843	-0.828	-0.341	-0.158	-0.056	*-0.984	-0.889	0.034	-0.178
	Reproduction	0.010	0.156	0.131	-0.379	-0.320	-0.427	-0.056	-0.047	-0.110	-0.072	0.261	0.135

Table S12. Correlations between ecotoxicological tests results of the experiment and chemical properties of soil with SLW- derived biochar addition. Statistically important coefficients were marked with *red (p=0.95) or **green (p=0.99) or ***violet (p=0.999).

		pH	EC	TOC	DOC	CEC	Available		
							Mg	K	P
0 days									
A. fischeri	Microtox test	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
L. sativum	Liquid	-0.872	0.647	-0.232	-0.384	-0.349	-0.764	0.412	*0.968
	Solid	0.085	-0.004	0.739	0.708	-0.394	0.130	0.837	0.166
F. candida	Mortality	0.715	-0.138	**0.999	0.947	0.197	0.790	0.321	-0.408
	Reproduction	-0.393	0.731	0.378	0.126	0.041	-0.182	0.367	0.723
30 days									
A. fischeri	Microtox test	0.561	-0.084	0.860	0.721	0.081	*-0.963	-0.033	0.874
L. sativum	Liquid	-0.709	0.313	0.094	0.677	0.688	0.013	0.235	0.456
	Solid	-0.899	0.784	0.230	0.388	0.949	0.290	0.726	0.341
F. candida	Mortality	-0.466	0.865	0.191	-0.454	0.544	0.546	0.885	-0.192
	Reproduction	-0.539	0.848	0.719	0.549	*0.961	-0.129	0.838	0.686
90 days									
A. fischeri	Microtox test	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
L. sativum	Liquid	-0.185	-0.102	-0.506	-0.680	0.055	-0.013	-0.094	-0.580
	Solid	-0.563	0.284	-0.074	-0.255	0.519	0.231	0.387	-0.235
F. candida	Mortality	0.164	0.026	0.796	0.721	0.162	-0.233	0.381	0.899
	Reproduction	-0.011	0.047	0.912	0.627	0.433	-0.321	0.648	*0.985
180 days									
A. fischeri	Microtox test	*-0.986	-0.076	0.338	*0.986	-0.912	0.710	-0.384	-0.158
L. sativum	Liquid	0.214	0.579	-0.208	-0.221	0.445	0.021	0.227	-0.600
	Solid	0.308	-0.912	-0.729	-0.286	0.001	-0.819	-0.676	-0.221
F. candida	Mortality	**-0.994	-0.020	0.443	**0.992	-0.912	0.776	-0.297	-0.043
	Reproduction	0.664	0.630	-0.209	-0.673	0.845	-0.241	0.510	-0.301

Table S13. Correlations between ecotoxicological tests results of the experiment and total and bioavailable PAHs content in soil with SLW- derived biochar addition. Statistically important coefficients were marked with *red (p=0.95) or **green (p=0.99) or ***violet (p=0.999).

		Total PAHs						Bioavailable PAHs					
		Σ16	R2	R3	R4	R5	R6	Σ16	R2	R3	R4	R5	R6
0 days													
A. fischeri	Microtox test	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
L. sativum	Liquid	0.245	-0.351	0.274	0.377	0.561	0.457	*0.979	0.805	0.277	0.349	0.765	0.034
	Solid	0.781	0.725	0.948	0.196	0.333	0.319	0.476	0.716	-0.613	-0.614	0.354	0.103
F. candida	Mortality	0.826	*0.966	0.842	0.306	0.276	0.351	-0.239	0.012	-0.534	-0.616	-0.401	-0.311
	Reproduction	0.796	0.182	0.737	0.759	0.880	0.839	0.723	0.620	0.165	0.166	0.314	-0.410
30 days													
A. fischeri	Microtox test	-0.078	-0.028	-0.503	0.052	0.219	0.124	-0.710	-0.819	-0.585	-0.526	0.322	-0.222
L. sativum	Liquid	0.914	0.939	0.765	0.631	*0.966	**0.992	0.694	0.482	0.813	0.836	0.945	-0.489
	Solid	0.551	0.597	0.484	0.096	0.701	0.791	0.600	0.342	0.751	0.855	0.753	-0.831
F. candida	Mortality	-0.493	-0.459	-0.346	-0.841	-0.338	-0.215	-0.018	-0.119	0.047	0.186	-0.221	-0.647
	Reproduction	0.219	0.294	-0.020	-0.177	0.552	0.610	0.064	-0.230	0.273	0.432	0.688	*-0.982
90 days													
A. fischeri	Microtox test	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
L. sativum	Liquid	0.665	-0.012	0.643	0.253	0.662	0.586	*0.985	0.126	0.711	0.708	0.466	-0.526
	Solid	0.493	-0.483	0.480	-0.231	0.682	0.626	0.837	0.034	0.653	0.641	0.170	-0.083
F. candida	Mortality	-0.308	-0.280	-0.276	-0.527	-0.201	-0.105	-0.936	0.277	-0.904	-0.909	-0.214	0.785
	Reproduction	0.085	-0.583	0.119	-0.732	0.304	0.392	-0.611	0.537	-0.795	-0.807	0.016	0.879
180 days													
A. fischeri	Microtox test	*0.985	-0.216	0.429	0.736	-0.214	0.227	0.437	0.467	0.028	-0.814	0.100	-0.765
L. sativum	Liquid	-0.229	0.681	0.875	-0.721	-0.940	-0.948	-0.924	-0.903	-0.700	0.205	-0.143	0.237
	Solid	-0.259	-0.834	-0.638	0.102	0.742	0.631	0.358	0.403	-0.188	-0.239	0.802	-0.318
F. candida	Mortality	*0.989	-0.174	0.380	0.767	-0.185	0.246	0.477	0.497	0.140	-0.757	0.002	-0.704
	Reproduction	-0.684	0.770	0.503	*-0.962	-0.692	-0.931	*-0.984	**-0.994	-0.486	0.660	-0.309	0.670

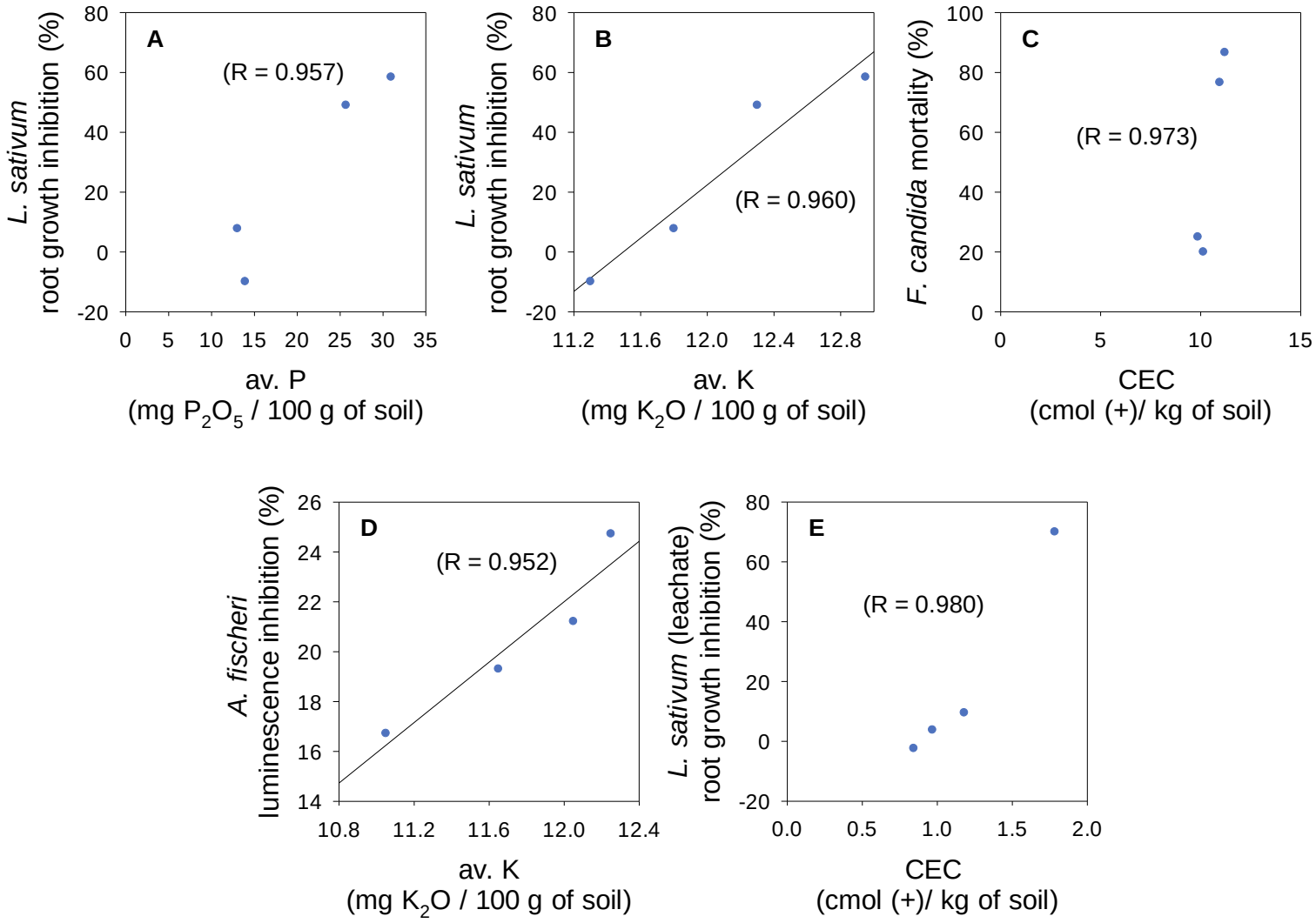

Fig. S1. Positive correlations between soil chemical properties and ecotoxicological tests results.

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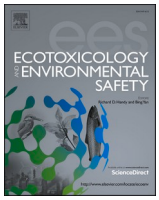

Effect of carrier gas change during sewage sludge or sewage sludge and willow pyrolysis on ecotoxicity of biochar-amended soil

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ABSTRACT

Different pyrolysis conditions determine the properties of the biochar. The properties of biochar may affect directly or indirectly their influence on living organisms. The aim of this study was to determine the toxicity of biochar obtained under different conditions (temperature: 500 or 700 °C, carrier gas: N_2 or CO_2, feedstock: sewage sludge or sewage sludge/biomass mixture) after adding to the soil in long-term pot experiment (180 days). Biochars were added to the podzolic loamy sand at a 2% (w/w) dose. Samples were collected at the beginning of the experiment and after 30, 90 and 180 days. The bacteria *Aliivibrio fischeri* (luminescence inhibition – Microtox), the plant *Lepidium sativum* (root growth and germination inhibition test – Phytotoxkit F), and the invertebrate *Folsomia candida* (mortality and reproduction inhibition test – Collembolan test) were used as the test organisms. In the long-term perspective for most tests, changing the carrier gas from N_2 to CO_2 resulted in reduced toxicity of the biochar. A particularly beneficial effect of changing the gas to CO_2 was observed for the solid-phase test with *L. sativum*. The CO_2 during pyrolysis had the least beneficial effect on toxicity towards *A. fischeri*.

1. Introduction

The growing acceptance of biochar (BC) as a soil additive requires the investigation of its safety towards soil organisms (Visioli et al., 2016). BC obtained from the pyrolysis of various types of biomass and organic/organic-mineral waste may contain toxic substances, potentially harmful to the environment (Godlewska et al., 2021; Hilber et al., 2017). Before using especially for agricultural purposes, BC should therefore be subjected to a thorough ecotoxicological analysis, preferably using organisms from different trophic levels (Ameloot et al., 2013; Godlewska et al., 2021; Hilber et al., 2017; Lehmann et al., 2011).

One of the organic-mineral wastes that are eagerly used to produce biochar is sewage sludge (SL). It is a material loaded with many toxic substances, such as polycyclic aromatic hydrocarbons (PAHs) and heavy metals (HMs), but also pharmaceuticals and other personal care products (Agrafioti et al., 2013). At the same time, SL contains a number of valuable components that, when returned to soils, fit perfectly into the rules of circular economy. Studies have shown that the conversion of SL to biochar is even a better direction, as it helps to stabilize the organic matter contained in it, reduce uncontrolled leaching of nutrients but also reduce the bioavailability of pollutants and eliminate biological hazards (parasite eggs and pathogenic microorganisms) (De la Rosa et al., 2019; Figueiredo et al., 2018; Kończak et al., 2019a; Kończak and Oleszczuk, 2020; Madej et al., 2016).

By manipulating the pyrolysis conditions, material with controlled physical and chemical properties can additionally be obtained, such as those that affect the content and bioavailability of toxic substances (Kończak et al., 2019b). In addition to temperature, which is the most common parameter used to manipulate the properties of biochar, the use of different gases during pyrolysis (He et al., 2020; Kim et al., 2019; Kwon et al., 2015; Lee et al., 2017) or mixing different feedstocks together (Ding and Jiang, 2013; Huang et al., 2017; Kończak and Oleszczuk, 2020) have become increasingly important in recent years. These actions are conducive to obtaining the desired biochar properties and reducing the contaminants bioavailability(He et al., 2020; Kończak et al., 2019a; Zhou et al., 2014). The increase in surface area due to these

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treatments can lead to an increased adsorption/sequestration of contaminants, which are very often present in biochars (Wang et al., 2017). Our previous studies confirm such trends (Kończak et al., 2019b). Thus, the change in biochar-pollutant interaction may determine the toxicity of biochars (Godlewska et al., 2019). So far, studies have focused on evaluating the persistence and bioavailability of PAHs in soils fertilized with biochar from biomass (De la Rosa et al., 2016; de Resende et al., 2018; Kuśmierz et al., 2016; Maienza et al., 2017; Rombolà et al., 2019), waste materials (De la Rosa et al., 2016) or mixed raw materials (Godlewska and Oleszczuk, 2022). However, there are no studies how the carrier gas, which affects the properties of biochars such as pore structure, size and shape (Kończak et al., 2019b) and pH, salinity and functional groups (Aktar et al., 2022), will determine the toxicity of biochar in the soil fertilized with it in the long term perspective. Moreover, biochar added to the soil changes its physical and chemical properties (water retention capacity, porosity, pH, organic carbon content and salinity), which in addition to pollution can directly or indirectly affect the organisms (Joseph et al., 2021).

Previous studies (2020) that were conducted directly on biochar (ignoring the effect of soil) showed that changing the carrier gas from N_2 to CO_2 resulted in reduced toxicity of biochars to *Aliivibrio fischeri*, *Lepidium sativum*, and *Folsomia candida*. However, close to real (using natural soil) and long-term studies are needed to precisely determine the potential effect on living organisms resulting from the application of biochar obtained under varying gas conditions.

The aim of this study was to determine the effect of changing the carrier gas from N_2 to CO_2 on the toxicity to bacteria (*Aliivibrio fischeri*), plants (*Lepidium sativum*) and arthropods (*Folsomia candida*) of biochar-amended soil. The biochars were prepared from SL and co-pyrolyzed SL and willow at 500 and 700 °C. Both the solid phase and the leachates obtained from the biochar-amended soils were evaluated. The proposed research hypothesis is that the biochars obtained in CO_2, due to better surface properties (determining the binding of contaminants) (Kończak et al., 2019b) than the biochars obtained in N_2, will exhibit lower toxicity to tested organisms.

2. Materials and methods

2.1. Pot experiment

All data regards to conditions of biochars production including feedstock and biochar properties are presented elsewhere (Kończak et al., 2019a, 2019b). The selected physico-chemical properties of biochars used in the experiment are also presented in SI (Table S1). The total and water-extractable content of heavy metals determined in feedstock are presented in SI (Table S2). The soil was classified as podzolic soil lying on marl substrate with the granulometric composition of loamy sand (according to World Reference Base for Soil Resources) situated in a warm temperate climate zone. Soil for the experiment was sampled from arable area under limited anthropogenic pressure (51°12′06′'N 23°16′06′'E). The characteristic of soil is presented in internet supporting information (Table S3). Dry soil was mixed with biochars at dose of 2% w/w (dry weight). Next, the soil-BC mixture was placed in plastic containers (20 L) and soil water holding capacity (WHC) was adjusted to 60%. The WHC was maintained by weighing the pots regularly and adding the water losses. The experiment was carried out in the dark in the room temperature (23 ± 2 °C in). At the beginning of the experiment (0) and after 30, 90 and 180 days samples of soil and biochar-amended soil were collected. Each variant had four pots related to the sampling time (each in three replications). In particular periods of time, the entire soil from every set (three replications) was mixed together, dried and stored in the fridge before chemical and ecotoxicological assessment. In Table S4 and S5 the chemical properties of biochar-amended soil were presented. The concentration of polycyclic aromatic hydrocarbons in investigated samples was presented and discussed in our previous paper (Godlewska and Oleszczuk, 2022).

2.2. Ecotoxicological tests

For evaluation of the toxicity of leachates from control and biochar-amended soil two tests were carried out with bacteria (*Aliivibrio fischeri*) and plants (*Lepidium sativum*). The leachates were obtained according to the procedure described in EN 12457–2 protocol (EN 12457-2, 2002). Detailed description of the procedure is presented in our previous paper (Godlewska et al., 2022). The Microtox® Toxicity Test with *Aliivibrio fischeri* was carried out according to the test protocol (Johnson, 2005). The evaluation of leachates toxicity to *Lepidium sativum* was performed according to OECD procedure (OECD, 1984). The Petri dish was filled out with paper filter and 2 mL of leachate was added. Then, 15 seeds of *L. sativum* were placed in each Petri dish. After 3 days (at 23 °C and darkness) the seedlings root growth was measured. The control comprised of seeds placed in Petri dish with 2 mL of deionized water added.

In solid phase test, plants (*L. sativum*) and springtails (*Folsomia candida*) were used as a test organisms. Phytotoxkit F test (*L. sativum*) was used according to ISO guideline 18763 (ISO 3, 1876, 2016). Sample (about 120 g) was placed in Phytotoxkit F plastic plate with paper filter. Ten *L. sativum* seeds were placed in a row in the top of the filter and closed with the flat cover. After 3 days (at 23 °C and darkness) the root growth was measured using Image Tool 3.0 for Windows (UTHSCSA, San Antonio, USA). The control comprised of soil without additions of biochar. To evaluate the toxicity of samples to *F. candida* the ISO guideline 11267 was applied (ISO 7, 1126, 1999). Soil sample was placed in Petri dish and weighted. Then 10 adult *F. candida* were placed in each dish. After 28 days in constant temperature and light conditions specified in test guideline the adults and juveniles were counted in each dish. During test period once in a week a deionized water was added based on weight to keep the moisture content in test containers.

The bioassays were performed in three replicates. RMANOVA analysis results were presented in Table S6. Detailed information about statistical evaluation of the results are presented in supporting internet information.

2.3. Statistical analysis

Mean values were taken from a triplicate data set. The differences between ecotoxicological endpoints in particular terms (and between terms) and variants were evaluated using a one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. Repeated measures analysis of variance (ANOVA) was performed to investigate whether sampling time had an effect on the tests' results, as the within subject factor (Table S6). Pearson's correlation coefficient test was performed to investigate the relations between physico-chemical properties and tests' results with Statistica 5.0. Significance was set at *$P \geq 0.05$.

3. Results

3.1. Solid-phase toxicity of BC-amended soil

The addition of SL-derived biochar to soil had differential effects on *L. sativum* root growth (Fig. 1A). BC500-N2 stimulated root growth by 11% while BC700-N2 inhibited root growth by 30%. An increase of the harmful effect of BC on root growth was observed at the next 30 and 90 days. After 180 days, the toxic effect on *L. sativum* decreased 32 (BC500-N2) and 24% (BC700-N2) compared to the earlier term. No statistically significant differences ($P > 0.05$) were noted between BC500-N2 and BC700-N2. However, the toxicity after 180 days was statistically significantly different ($P \leq 0.05$) for BC500-N2 and not significantly different for BC700-N2 compared to the beginning of the study ($P > 0.05$).

A significant difference ($P \leq 0.05$) between BC500-N2 and BC700-N2 was also observed for *F. candida*. The addition of BC500-N2 had no adverse effect on *F. candida* mortality, whereas BC700-N2 caused 50%

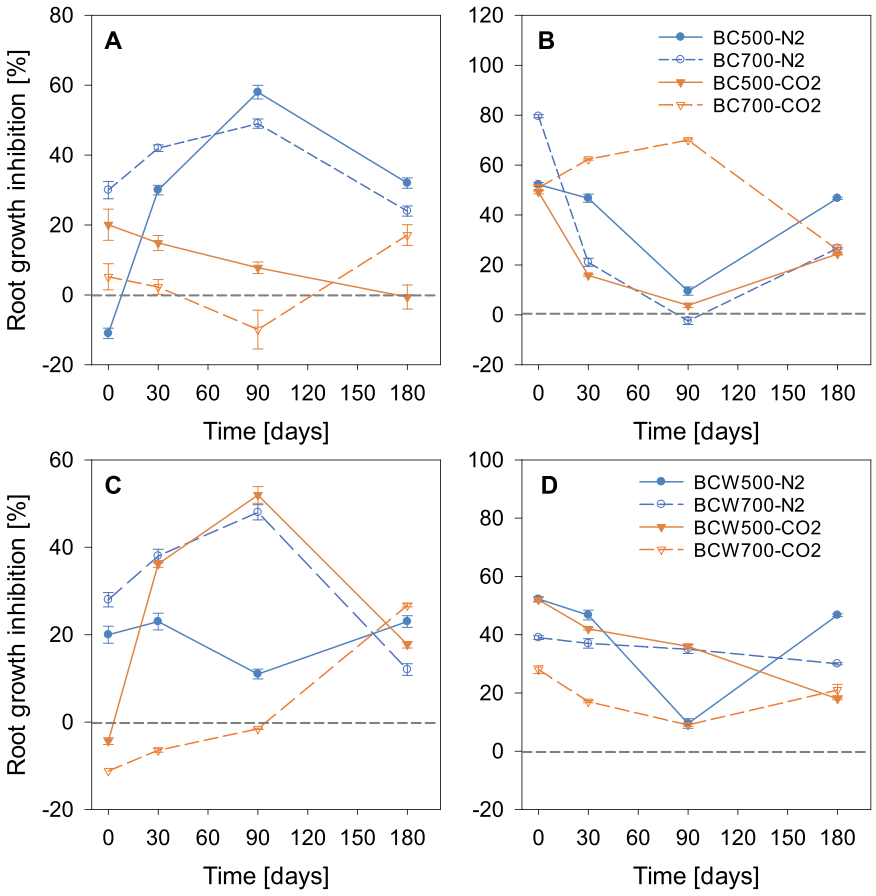

Fig. 1. *Lepidium sativum* root growth inhibition (positive change) or stimulation (negative change) (%) in solid- phase (A, C) and liquid-phase (B, D) test during the experiment with SL-biochar-amended soil.

mortality (Fig. 2A). After 30 days, both BCs had toxic effects, increasing *F. candida* mortality to 77 (BC500-N2) and 87% (BC700-N2) (Fig. 2A). The differences between the biochars were not statistically significant ($P > 0.05$). On the next two dates (Fig. 2A), *F. candida* mortality decreased to the level at the beginning of the study. The level of the mortality observed in BC500-N2-amended soil did not differ statistically ($P > 0.05$) with the control soil and was higher in BC700-N2-amended soil than in control soil (Fig. 2A).

In BC500-N2-amended soil, stimulation of *F. candida* reproduction was observed at the beginning of the study, whereas an 84% inhibition was noted in BC700-N2-amended soil (Fig. 2B). On subsequent dates (30–180 days), *F. candida* reproduction varied significantly in BC500-N2-amended soil, while it decreased by 36% (relative to the beginning) after 30 days in BC700-N2. It remained at this level until the end of the study. Finally, after 180 days, reproduction inhibition was 23% and 61% for BC500-N2 and BC700-N2, respectively, and was 244% higher (BC500-N2) and 27% lower (BC700-N2) than at the beginning of the study (Fig. 2B).

3.2. Toxicity of leachates from BC-amended soil

Addition of SL-derived biochars obtained at 500 and 700 °C to the soil completely reduced its toxicity to *A. fischeri* (Fig. 3A). After 30 days, the biochars showed luminescence inhibition in the range of 21–25% (not significant differences between BC500-N2 and BC700-N2), followed again after 90 days by a complete reduction of toxicity in the soil with the biochars. At the last term, only non-significant ($P > 0.05$) inhibition in BC500-amended soil (>10%) or no effect in BC700-amended soil was observed.

Leachates from soil with biochar had a toxic effect on *L. sativum*, inhibiting growth for BC500-N2 by 52% and for BC700-N2 by 80% (Fig. 1B). At subsequent times (30 and 90), the toxic effect was progressively reduced to 10% for BC500-N2. However, no significant differences ($P > 0.05$) with the control soil were noted in soil with BC700-N2. After 180 days, leachate toxicity increased to levels of 47% and 27% in soil with BC500-N2 and BC700-N2, respectively.

3.3. Influence of CO_2 on solid- phase toxicity of BC-amended soil

Changing gas from N_2 to CO_2 during pyrolysis of SL-derived biochar had various effects on *L. sativum* root growth in solid phase tests (Fig. 1A). The addition of BC500-CO2 inhibited root growth (by 20%), whereas BC700-CO2 had no significant ($P > 0.05$) effect on *L. sativum* relative to the control soil. Root growth inhibition in BC500-CO2-amended soil decreased gradually with time, eventually reaching the level observed for the control soil. The BC700-CO2-amended soil also showed a gradual decrease in root growth inhibition that reached a stimulatory value after 90 days, followed by a significant ($P \leq 0.05$) increase in toxicity to 17% on day 180 of the experiment (Fig. 1A). For most of the experiment, less root growth inhibition was observed in soil with CO_2-derived biochar than in soil with N_2-derived biochar. Depending on the term, root growth inhibition was 50–102% (BC500) and 29–120% (BC700) lower in soil with CO_2-derived biochar than N_2-derived biochar.

The addition of both BC-CO2 biochars at the beginning of the experiment had no adverse effect on *F. candida* mortality in the fertilized soil (Fig. 2A). However, in the following 90 days, a significant increase in *F. candida* mortality to 50–60% was observed, which was not significantly different ($P > 0.05$) between these biochars. Finally, after 180 days, BC700-CO2 showed a further increase (to a level of 75%), while

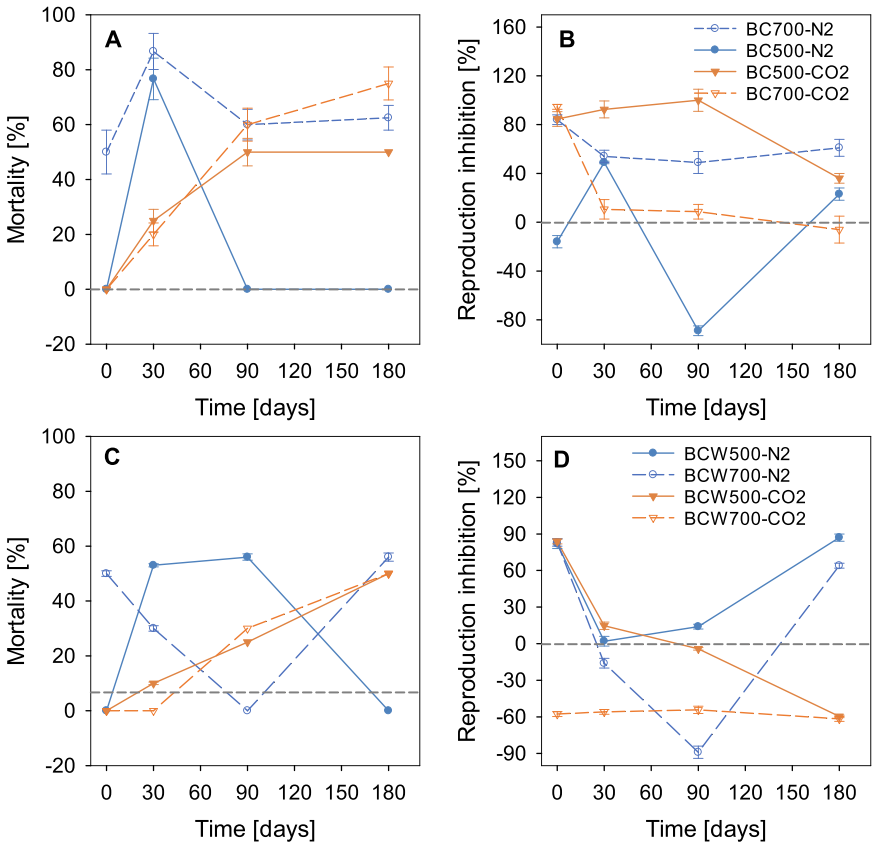

Fig. 2. *Folsomia candida* mortality (A, C) or reproduction inhibition (B, D) (%) during 180 days of the experiment with SL-biochar-amended soil.

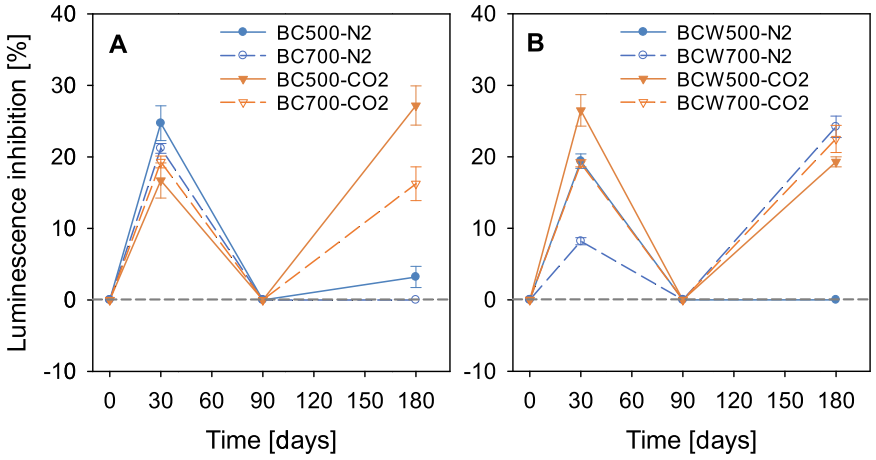

Fig. 3. *Aliivibrio fischeri* luminescence inhibition (%) of leachates from (A) SL-biochar- and (B) SL/biomass-biochar amended soil.

BC500-CO2 still expressed mortality at a level similar to that after 90 days (Fig. 2A). Significant differences ($P \leq 0.05$) were observed in the pattern of *F. candida* mortality between BC-N2 and BC-CO2 depending on the temperature at which the biochar was received and the experimental term (Fig. 2A).

The effect of carrier gas on *F. candida* reproduction was also evident (Fig. 2B). At the beginning of the study, both biochars obtained in CO_2 inhibited reproduction at high levels of 85 (BC500-CO2) and 95% (BC700-CO2), which was comparable only to BC700-N2. Further level of toxicity depended on the temperature of biochar production. For BC500-CO2, reproduction inhibition remained constant (no significant differences ($P > 0.05$) between terms), followed by a sharp decrease to 36% between 90 and 180 day of the experiment (Fig. 2B). For BC700-CO2, a significant decrease ($P \leq 0.05$) to 11% occurred after 30 days of experiment, after which reproduction inhibition remained constant until the end of the study, and was comparable to the control soil (Fig. 2B). The effect of CO_2 was temperature dependent. For most terms, BC500-CO2 showed a higher adverse effect on reproduction than BC500-N2. The opposite trend was observed for BC700-CO2/N2, where only at the beginning of the study no statistically significant ($P > 0.05$) differences were found between BC700 obtained in CO_2 and N_2. At other terms, BC700-CO2 was less toxic than BC700-N2.

3.4. *Influence of CO_2 on leachate toxicity of BC-amended soil*

The development of leachate toxicity to *A. fischeri* between the

biochars obtained in CO_2 and N_2 followed the same trend throughout the study period (Fig. 3A). The differences between the biochars were only observed at day 30 of the experiment (significant difference between BC500-N2 and BC500-CO2) and after 180 days of testing (for all biochars). At the last term, both biochars obtained in CO_2 inhibited the luminescence of *A. fischeri* significantly and more than biochars obtained in N_2 (Fig. 3A).

Leachate from biochars-amended soil obtained in CO_2 had a toxic effect on *L. sativum* (49–51%). There were no significant differences ($P > 0.05$) between the biochars obtained at 500 and 700 °C. However, clear differences were noted at subsequent test dates. Between the beginning of the study and day 90 of the experiment, there was a significant ($P \leq 0.05$) decrease in BC500-CO2 toxicity (up to 4%) and an increase in BC700-CO2 toxicity (up to 70%). This was followed by an increase and decrease in BC500-CO2 and BC700-CO2 to 24% and 26%, respectively, between days 90 and 180 of the experiment (Fig. 1B). These values were not significantly different ($P > 0.05$) between BC500-CO2 and BC700-CO2 as observed at the beginning of the study. Analyzing the effect of carrier gas, it was found that biochar obtained at 500 °C in CO_2 was, for most of the terms, less toxic than the same biochar but obtained in N_2. However, the opposite trend was observed for biochars obtained at 700 °C.

3.5. Influence of CO_2 on solid-phase toxicity of co-pyrolyzed biochar-amended soil

Changing the gas from N_2 to CO_2 during co-pyrolysis had different effect on root growth inhibition depending on temperature and experimental period. At the beginning of the study, co-pyrolyzed in CO_2 biochars stimulated root growth at levels of 4–11% in contrast to the biochars obtained in N_2, which showed root growth inhibition ranging from 20% to 28% (Fig. 1C). An increase in toxicity of soil with BCW500-CO2 was observed in subsequent test dates, above the level observed for BCW500-N2. In soil fertilized with BCW700-CO2, the stimulating effect of this biochar decreased, but by day 90 of the study, it was below the values observed for BCW700-N2. Finally, after 180 days, the toxicity of BCW500-CO2 and BCW500-N2 biochars to *L. sativum* was not significantly different between them ($P > 0.05$). However, significant differences ($P \leq 0.05$) were observed between the biochars obtained at 700 °C in CO_2 and N_2 (Fig. 1C). However, in general, in the last term of the study, toxicity was very similar between all biochars (obtained at 500/700 °C, in N_2/CO_2) and ranged from 12% to 27%.

No adverse effect of changing N_2 to CO_2 for biochar obtained at 500 °C was also observed regards to *F. candida* mortality (Fig. 2C). The significant differences ($P \leq 0.05$) were noted between biochars obtained at 700 °C in CO_2 and N_2. BCW700-CO2 had no effect on *F. candida* mortality, whereas BCW700-N2 significantly increased *F. candida* mortality. At successive terms, the change in *F. candida* mortality was very dynamic and differed significantly between the experimental variants. Finally, after 180 days, only BCW500-N2 showed no negative effect on *F. candida* mortality. Mortality of the test organisms in soil with the other biochars did not differ significantly ($P > 0.05$) between them and ranged from 50% to 56%.

A completely different trend in the effect of biochars obtained under different gas conditions was observed for the reproduction of *F. candida* (Fig. 2D). Biochars obtained at 500 °C in both CO_2 and N_2 atmospheres inhibited reproduction at 82–84%. After 30 days, there was a significant decrease ($P \leq 0.05$) in reproduction inhibition to the control soil level, which, for both biochars, persisted until 90 day of the experiment. Between 90 and 180 days of the study, BCW500-N2 showed a sharp increase in reproduction inhibition (to the level at the beginning of the study), while BCW500-CO2 showed a stimulatory (at 60% level) effect on *F. candida* reproduction. The biochars obtained at 700 °C in N_2 and CO_2 showed a different trend. Biochar BCW700 at the beginning of the study had toxicity similar to that observed for biochars obtained at 500 °C. Over the next 90 days, a significant ($P \leq 0.05$) decrease in its toxicity to a level that stimulated *F. candida* reproduction was noted, followed by a significant increase in toxicity to 64%. Biochar BCW700-CO2, on the other hand, stimulated *F. candida* reproduction at a constant level (from 54% to 62%) throughout the study period.

3.6. Influence of CO_2 on leachate toxicity of co-pyrolyzed biochar-amended soil

Changing the carrier gas from N_2 to CO_2 during co-pyrolysis of the biochars did not affect their toxicity to *A. fischeri* (Fig. 3B). After 30 days, increased luminescence inhibition was observed for all biochars (obtained in N_2 and CO_2). Biochars obtained in CO_2 showing lower toxicity than those in N_2. In the next 90 days, the toxicity decreased to the level at the beginning of the study and no significant differences ($P > 0.05$) between the biochars obtained in N_2 and CO_2 were observed. After 180 days, luminescence inhibition increased again (to levels ranging from 19% to 24%) for most biochars except BCW500-N2. No significant differences ($P > 0.05$) were found between biochars obtained at 700 °C in N_2 and CO_2.

Addition of both biochars to soil increased leachate phytotoxicity to > 28% (Fig. 1D). Changing the carrier gas from N_2 to CO_2 did not significantly ($P > 0.05$) affect the toxicity of biochar obtained at 500 °C (61% and 52%), and decreased it by 28% for biochar obtained at 700 °C (from 39% to 28%). In the subsequent terms, the effect of carrier gas also varied with pyrolysis temperature. In the case of biochars obtained in N_2 and CO_2 at 500 °C, there was a gradual decrease in the toxicity of the extracts until day 90 of the study. However, there was a significant increase ($P \leq 0.05$) in the phytotoxicity of the extracts to 25 (BCW500-N2) and 18% (BCW500-CO2) by the last date. Throughout the study period, biochar obtained in CO_2 had significantly lower toxicity than biochar obtained in N_2. The phytotoxicity of biochars obtained at 700 °C in N_2 and CO_2 differed significantly ($P \leq 0.05$) for almost the entire study period.

4. Discussion

Studies on the effect of biochar on the toxicity of soils fertilized with these materials mainly focus on biomass-derived biochars (Godlewska et al., 2021). Studies in relation to SL-derived biochar are still scarce (George, 2022). Tables S7-S9 collect the results of ecotoxicological tests against *A. fischeri, F. candida* and different plant species. It is difficult to relate the obtained results to the present study due to different doses, conditions for biochar production or sample preparation for ecotoxicological evaluation (different conditions for preparing leachates). In the case of plants, there are also other factors like the type of plant tested and its individual sensitivity. The toxicity observed in the present study against the test organisms was lower (Faria et al., 2018; Figueiredo et al., 2019; Gascó et al., 2016; Gondek et al., 2014; Kończak et al., 2020; Marks et al., 2014b, 2014a; Mierzwa-Hersztek et al., 2017; Mierzwa--Hersztek et al., 2018; Tomczyk et al., 2020; Xing et al., 2019; You et al., 2019; Zielińska and Oleszczuk, 2015), higher (Mierzwa-Hersztek et al., 2018; Tomczyk et al., 2021) or similar (Cernecka et al., 2017; Tomczyk et al., 2021) to studies of other authors, suggesting that the toxicity observed in the present study were usually lower than those reported so far in the literature. In addition, the information obtained indicates the specific behavior of biochar, which makes it difficult to develop a uniform strategy for the ecotoxicological evaluation of biochar obtained from sewage sludge and imposes the need to treat every biochar individually.

The Pearson correlation (Table S10) was performed to examine the effects of chemical properties (Table S4 and S5) of BC-N2 and BC-CO2-amended soil on soil toxicity for every experimental term. Occasionally, positive significant correlations (Table S10, Fig. S1) were found between av. K and *A. fischeri* and *L. sativum* (solid test), between av P and *L. sativum* (solid test), between CEC and *F. candida* mortality, and between TOC and *L. sativum* (liquid phase). Additionally, negative

relationships were observed between av P and *L. sativum* (solid phase) and *F. candida* mortality, and between av Mg and *L. sativum* (liquid phase). Av. Mg, K, and P can have positive effects especially to plants (Du et al., 2022), but also exert toxic effects when in excess (Niu et al., 2013), as evidenced by the varying directions of the observed correlations depending on the term. After 30 days, *F. candida* mortality was correlated (Table S10) with CEC (0.973, $p = 0.95$) and av. P (−0.965, $p = 0.95$). Increased CEC may be related to the presence of higher HMs content, which could negatively affect *F. candida* (Fountain and Hopkin, 2001), while the decrease in mortality with increased av. P is a result of increased food amount. After 30 days, a positive correlation was also noted (Table S10) between av. K and luminescence inhibition of *A. fischeri* (0.952, $p = 0.95$). The correlation between av. K and luminescence inhibition stands in contrast to a study by other authors (Berglind et al., 2010), where an increase in luminescence was observed with increasing potassium concentration in solution. Potassium is required by *A. fischeri* for NADH-oxidase activation and facilitates the stabilization of pH at optimal levels in the bacteria (Berglind et al., 2010). The observed effect may have been related to the synergistic effect of other substances present in the solution which could potentially be influenced by av. K.

Table S11 shows the relationships between the toxic effect and the content of C_{tot} and C_{free} PAHs. Previous studies show that PAHs contained in biochar can be potentially toxic to living organisms (Alkio et al., 2005; Intani et al., 2019; Stefaniuk et al., 2016). Similar to the physico-chemical properties, varied direction of the potential effects of PAHs on the organisms studied was observed. Positive relationships that may indicate negative effects on organisms were recorded only for 4- and 5-ring PAHs and $\Sigma 16C_{tot}$ and 4-, 5-, and 6-ring C_{free} PAHs. Although heavy PAHs (>4 rings) are hardly soluble in water and thus less available for organisms, they are characterized by higher toxicity, which should be explained by the observed positive relationships.

Co-pyrolysis of SL with biomass has been proposed previously (Ding and Jiang, 2013; Huang et al., 2017; Jin et al., 2017; Kończak et al., 2019b; Wang et al., 2018), but biochar from co-pyrolysis has not been extensively studied with respect to ecotoxicology (Gondek et al., 2014, 2017a, 2017b; Kończak et al., 2020). There are even fewer studies that would consider the toxicity of the soil to which such biochar has been added (Godlewska et al., 2022). For co-pyrolyzed biochars (BCW), significant relationships were observed even less frequently than for BC pyrolyzed biochar, which mainly took positive values (TOC, DOC, CEC and av. P) (Table S12). Negative relationships were recorded between pH and *A. fischeri* luminescence inhibition and *F. candida* mortality, and between av. Mg and *A. fischeri* luminescence inhibition. For PAHs, significant positive correlations with ecotoxicological test results were recorded occasionally, for *F. candida* mortality (R2, $\Sigma 16$ - C_{tot}) and *L. sativum* - liquid phase (R5, R6 and $\Sigma 16$ - C_{tot}; $\Sigma 16$ - C_{free}). Previous studies have also shown that PAHs in biochar can negatively affect *F. candida* and plants (Conti et al., 2018; Intani et al., 2019). For example, Conti et al. (2018) studied the toxicity of biochars obtained by gasification against *F. candida* and suggested PAHs as one of their toxicity factors. Similarly, Intani et al. (2019) found that the soluble fraction of PAHs may be responsible for the toxic effects of corncob biochar. Surprisingly, however, there are quite a few negative correlations between PAHs and *F. candida* reproduction (Table S13), suggesting a positive effect of biochar on this endpoint. Indeed, previous studies show a clear negative effect of PAHs on *F. candida* reproduction (Conti et al., 2018; Nota et al., 2009; Stefaniuk et al., 2016).

To determine whether changing the carrier gas from N_2 to CO_2 reduced the toxicity of the biochar-amended soil to the test organisms, a toxicity comparison was presented as a heatmap (Fig. 4). When toxicity BC/W-CO2 < BC/W-N2 the green color was selected, when toxicity BC/W-CO2 > BC/W-N2 the red color was selected. The absence of significant differences ($P > 0.05$) between BC/W-N2 and BC/W-CO2 was indicated by yellow. In the case of biochars obtained from SL (Fig. 4A), it can be observed that the effect of the change in carrier gas was mainly determined by the type of test, but also by the term and the biochars' temperature. For the solid-phase test toward *L. sativum*, the gas change from N_2 to CO_2 was very favorable. The change of gas from N_2 to CO_2 in the case of the other tests was very differentiated in most cases having no effect or increasing toxicity and only in a few cases having a positive effect (Fig. 4). The change to CO_2 was unfavorable for *F. candida* reproduction for biochar obtained at 500 °C, whereas for biochar obtained at 700 °C a favorable effect appeared at 30 days and at 180 days. The luminescence of *A. fischeri* was usually not affected by the gas change, only after 180 days a clear negative effect of gas change on CO_2 can be seen. A differentiated effect of gas change on CO_2 was observed in the test with *L. sativum* leachate, where an adverse effect appeared for biochar obtained at 700 °C at term after 30 and after 90 days, a positive effect was observed for biochar BC700 at the beginning of the test and BC500 after 30 and after 180 days, and in other cases no effect was found.

A completely different trend was observed for the co-pyrolyzed biochars (Fig. 4B). In most tests, changing the carrier gas from N_2 to CO_2 was also beneficial, except for the test with *A. fischeri*, where an adverse effect of the gas change was observed (BCW700 after 30 days and BCW500 after 180 days) or no significant differences were noted between the biochars obtained in N_2 and CO_2. For the other tests, the change of gas to CO_2 appeared to be beneficial mainly between 0 and 90 days, while after 180 days a beneficial effect was observed only in the *F. candida* reproduction test for both biochars.

5. Conclusions

In the long-term perspective and for most tests, changing the carrier gas from N_2 to CO_2 resulted in a reduction in soil toxicity. This indicates that the use of CO_2 may be a direction affecting the reduction of toxicity of biochars obtained from sewage sludge. The beneficial effect of the gas

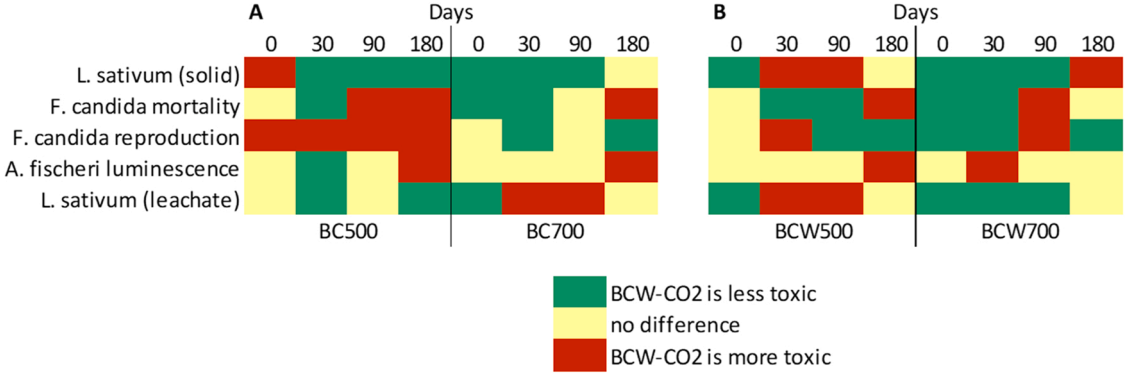

Fig. 4. The heatmap presenting comparison of toxicity of biochars from (A) SL-biochars and (B) SL/biomass-biochars produced in N_2 and CO_2.

change was particularly evident for the co-pyrolyzed biochars. However, the effect of carrier gas change was mainly determined by the organism tested. The factors mainly responsible for the toxicity of biochar-amended soil were the PAHs content, the content of available forms of Mg, K and P and the organic carbon content. PAHs had a particularly significant effect for biochar obtained from sewage sludge compared to co-pyrolyzed biochar.

CRediT authorship contribution statement

Paulina Godlewska: Methodology, Investigation, Writing – original draft. **Magdalena Kończak**: Methodology, Review. **Patryk Oleszczuk**: Conceptualization, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2022.114224.

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INTERNET SUPPORTING INFORMATION

Effect of carrier gas change during sewage sludge or sewage sludge and willow pyrolysis on ecotoxicity of biochar-amended soil

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MATERIALS AND METHODS

Sewage sludge

Sewage sludge (SL) was obtained from municipal wastewater treatment plant where SL is stabilized biologically by aerobic fermentation and chemically by threated with lime. SL were collected during autumn 2016 from municipal sewage treatment plant localized in Chełm (southeastern part of Poland, 510705600N, 232804000E, population of 64 000 people). Sewage treatment plant were located on agricultural areas and used mainly urban wastewater without the great impact of the industry.

Biochar preparation

Briefly, the sewage sludge (SL) before pyrolysis was grinded and sieved through a 2 mm sieve. SL was obtained from municipal wastewater treatment plant localized in Chełm (50°20′04″N 23°29′49″E) which uses mainly urban wastewater with limited influence of wastewater from industry. The willow was provided by a biomass-producing farm. Freshly cut willow was air-dried for two weeks and then cut into small pieces and sieved through 2 mm sieve. Mixtures of SL and willow (6:4 w/w) were obtained by mixing both materials in glass bottles (1000 mL) for 24 h in the dark at 10 rpm (Rotax 6.8. VELP, Italy). SL alone and SL with willow were pyrolysed in 500, 600 or 700°C, with the heating rate 10°C/ min. Temperature was held for 3 h (slow pyrolysis). During the pyrolysis the oxygen free atmosphere was maintained by constant flow of N_2. The physico-chemical properties of SL- and SL/biomass-derived biochar are presented in Table S1.

Table S1. The physico-chemical properties of biochars used in the experiment.

	pH	Ash content (%)	C (%)	OC (%)	H (%)	N (%)	O (%)	O/C	H/C	(O+N)/C	S_{BET} (m^2/g)	d (nm)	V_t (m^3/g)	V_{micro} (m^3/g)	V_{meso} (m^3/g)
BC500-N2	9.4	64.1	26.3	26.2	0.99	3.26	5.38	0.15	0.45	0.26	69.7	5.98	0.104	0.0189	0.0854
BC700-N2	12.4	71.4	24.5	24.4	0.29	2.10	1.71	0.05	0.19	0.13	89.2	6.18	0.137	0.0240	0.1139
BCW500-N2	10.8	46.4	44.6	44.2	1.66	3.33	3.93	0.07	0.44	0.13	74.6	4.66	0.087	0.0207	0.0663
BCW700-N2	12.5	50.9	46.2	46.0	0.62	2.09	0.21	0.02	0.16	0.04	104.1	4.93	0.115	0.0269	0.0881
BC500-CO2	9.2	59.2	25.1	24.5	0.68	3.17	11.8	0.35	0.32	0.46	71.1	5.59	0.099	0.0198	0.0798
BC700-CO2	9.8	69.7	22.7	22.1	0.16	1.86	2.64	0.1	0.09	0.17	83.5	5.86	0.122	0.0206	0.1018
BCW500-CO2	9.3	43.3	44.7	44.2	1.58	3.35	7.08	0.12	0.42	0.18	88.7	4.14	0.091	0.0262	0.0656
BCW700-CO2	9.8	48.6	47.7	47.2	0.59	2.49	0.69	0.01	0.15	0.06	152.5	3.61	0.137	0.0468	0.0908

pH- in H_2O- 1:10 (w/v)

Table S2. The total and water-extractable metal content of biochar and biochar's feedstock used in the experiment.

Feedstock	Zn	Cu	Cr	Ni	Cd	Pb
			Total content (mg/kg)			
SL	968 ± 57	158 ± 11.7	41.1 ± 3.91	27.4 ± 1.8	1.14 ± 0.11	22.9 ± 1.9
W	32.7 ± 4.1	4.58 ± 0.59	0.39 ± 0.08	1.05 ± 0.21	0.32 ± 0.04	0.81 ± 0.04
			Water-extractable metal (mg/L)			
SL	11.0 ±0.5	0.73 ±0.02	0.12 ±0.02	2.39 ±0.3	0.26 ±0.002	0.31 ±0.01
W	5.24 ±0.22	0.27 ±0.03	0.04 ±0.001	11.0 ±0.5	0.23 ±0.01	0.10 ±0.02

SL – sewage sludge, W – willow.

Table S4. Chemical properties of control soil

pH	Total N content (%)	Available nutrients (mg/ 100 g of soil)			Exchangeable cations content (mg/ 100 g of soil)				Hydrolytic acidity (mmol H^+ /100 g of soil)
6.84 in H_2O	0.064	P_2O_5	K_2O	Mg	Mg	Na	K	Ca	1.25
6.80 in KCl		12.2	11.7	2.2	3.50	2.87	10.6	101	

pH in H_2O- 1:10 (m/v), pH in 1M KCl- 1:2.5 (m/v), total N – Kjeldahl method, available P and K – Egner-Riehm method, available Mg – Schachtschabel method, exchangeable cations- Kappen method, hydrolytic acidity- Kappen method

Table S5. Chemical properties of soil with biochar pyrolyzed in N_2.

	pH	EC	TOC	DOC	CEC	Available		
						Mg	K	P
BC500-N2								
0 days	6.9	130.6	1.1	10.9	11.0	4.9	12.7	16.4
30 days	6.9	131.6	0.8	7.9	11.0	4.2	12.3	20.0
90 days	7.1	155.5	1.2	11.3	10.3	3.9	13.0	31.0
180 days	6.6	188.8	1.0	3.7	10.4	2.4	12.4	30.3
BC700-N2								
0 days	7.2	111.1	1.0	10.1	11.0	3.9	11.9	14.7
30 days	7.0	182.7	1.0	9.1	11.2	3.2	12.1	14.2
90 days	7.2	196.9	0.8	8.6	8.9	3.9	12.3	25.7
180 days	6.5	292.0	0.7	3.7	9.6	2.5	12.7	25.6
BCW500-N2								
0 days	6.9	91.6	1.2	9.1	9.4	2.1	15.4	15.0
30 days	7.1	161.5	1.4	8.4	11.4	2.9	17.2	17.9
90 days	7.0	174.4	1.7	13.0	11.7	2.6	16.4	27.0
180 days	6.9	177.5	1.4	4.9	10.4	2.0	14.3	20.0
BCW700-N2								
0 days	7.1	87.5	1.6	10.1	10.9	4.3	14.2	13.0
30 days	6.7	136.1	0.7	8.9	11.4	3.5	15.4	14.1
90 days	6.9	169.9	0.8	10.5	10.8	3.7	14.6	21.1
180 days	6.1	210.8	1.8	11.6	9.6	2.7	14.1	17.4

pH- in water 1:10 (w/v), EC (µS/cm), TOC (%), DOC (mL/g), CEC (mmol/kg), available P (mg P_2O_5/ 100 g of soil) and K (mg K_2O/ 100 g of soil) – Egner-Riehm method, available Mg (mg Mg/ 100 g of soil) – Schachtschabel method

Table S6. Chemical properties of soil with biochar pyrolyzed in CO_2.

	pH	EC	TOC	DOC	CEC	Available		
						Mg	K	P
BC500-CO2								
0 days	6.8	169.8	0.9	12.0	11.5	2.6	12.4	18.4
30 days	7.0	196.4	0.6	8.4	9.9	0.1	11.1	26.3
90 days	7.0	92.1	1.0	8.6	11.0	3.8	11.8	13.0
180 days	7.4	92.6	1.2	12.2	10.9	0.1	11.1	27.9
BC700-CO2								
0 days	7.0	189.8	1.0	12.6	11.9	2.7	11.8	20.2
30 days	6.3	316.0	1.0	15.7	10.1	2.5	11.7	28.2
90 days	7.2	146.7	1.8	14.1	11.6	3.3	11.3	13.9
180 days	6.3	111.8	0.8	11.7	9.1	2.5	13.4	28.7
BCW500-CO2								
0 days	7.0	124.5	1.2	8.7	11.7	3.0	11.4	14.8
30 days	7.0	130.3	1.5	18.4	11.6	2.1	15.3	24.5
90 days	7.3	104.5	1.3	10.3	10.8	0.1	15.4	25.0
180 days	6.1	180.4	2.2	11.6	9.3	2.7	14.5	26.4
BCW700-CO2								
0 days	7.1	66.3	1.1	9.1	10.6	3.4	12.3	12.4
30 days	7.4	68.5	0.9	9.4	10.3	2.5	12.3	15.7
90 days	7.8	72.8	0.8	9.9	8.0	0.1	12.4	22.7
180 days	6.1	116.7	1.2	11.8	9.1	2.2	11.8	16.3

Table S7. RMANOVA results

Source of variation		d.f.	F ratio	p value
A. fischeri	Microtox	3	10.33	0.000
L. sativum	Water	3	3.32	0.037
	Solid	3	2.12	0.013
F. candida	Mortality	3	2.88	0.060
	Reproduction	3	4.70	0.012

Table S8. *Aliivibrio fischeri* luminescence inhibition test (Microtox) results from multiple studies with biochars produced from sewage sludge or co-pyrolysed sewage sludge with plant biomass.

Biochar	Pyrolysis temperature (ºC)	Experiment	Soil type	Luminescence inhibition	Reference
Sewage sludge	500	Lab Biochar-amended soil leachate (1:10)	Podzolic loamy sand	15%	[1]
	600			18%	
	700			<10%	
Sewage sludge (1)	300	Lab Biochar-amended soil leachate (1:4)	Sandy acid soil	30% (dose 0.5%), 50% (dose 1.0%) and 42% (dose 2.0%); control soil inhibited luminescence at 49%	[2]
Sewage sludge (2)	300			49% (dose 0.5%), 49% (dose 1.0%) and 47% (dose 2.0%); control soil inhibited luminescence at 49%	
Sewage sludge (3)	300			39% (dose 0.5%), 38% (dose 1.0%) and 39% (dose 2.0%); control soil inhibited luminescence at 49%	
Sewage sludge (A)	200	Biochar's leachate (1:100)	Without soil	22%	[3]
Sewage sludge (A) + rape straw	200			34%	
Sewage sludge (A) + wheat straw	200			28%	
Sewage sludge (A) + sawdust	200			25%	
Sewage sludge (A) + bark	200			17%	
Sewage sludge (B)	200			<10%	
Sewage sludge (B) + rape straw	200			25%	
Sewage sludge (B) + wheat straw	200			23%	
Sewage sludge (B) + sawdust	200			23%	
Sewage sludge (B) + bark	200			13%	
Sewage sludge + wheat straw	300	Biochar's leachate (1:20)	Without soil	Stimulation of luminescence 26%	[4]
	600			Stimulation of luminescence 25%	
Sewage sludge + sawdust	300			Stimulation of luminescence 25%	
	600			Stimulation of luminescence 26%	
Sewage sludge + bark	300			Stimulation of luminescence 18%	
	600			Stimulation of luminescence 23%	
Sewage sludge	500, 600 or 700	Biochar's leachate (1:10)	Without soil	0-94%, toxicity increased with increasing temperature	[5]
Sewage sludge + willow (8:2)	500, 600 or 700			0-91%, toxicity increased with increasing temperature	

Sewage sludge + willow (6:4)	500, 600 or 700			0-92%, toxicity increased with increasing temperature	
Sewage sludge	300	Biochar's leachate	Without soil	48%	[6]
	500			<10%	
	700			<10%	
	900			<10%	

Table S9. *Foslomia candida* mortality and reproduction inhibition test results from multiple studies with biochars produced from sewage sludge or co-pyrolysed sewage sludge with plant biomass.

Biochar	Pyrolysis	Experiment	Soil type	Effect	Reference
Sewage sludge	500ºC	Lab Dose: 1% (30 t/ha) at the beginning	Loamy sand	Mortality 20-90% Reproduction inhibition 100%	[7]
		Lab Dose: 1% (30 t/ha) after 6 months		Mortality decreased 10% Reproduction stimulation 140-180%	
Sewage sludge	500, 600 or 700ºC	Lab Dose: 1% (30 t/ha)	Loamy sand	Reproduction inhibition 30-80%, toxicity increased with increasing temperature	[8]
Sewage sludge + willow (8:2)	500, 600 or 700ºC			Reproduction stimulation 5-20% (500, 600 ºC) or inhibition 60% (700ºC)	
Sewage sludge + willow (6:4)	500, 600 or 700ºC			Reproduction stimulation 18-30% (500, 600 ºC) or inhibition 50% (700ºC)	
Sewage sludge	550	Dose: 0.5, 1.3, 3.2, 8, 20, and 50%,	Hypercalcic Calcisol	Reproduction stimulation at dose 1.3% and 3.2% or inhibition at 50% dose	[9]
Sewage sludge	500	Dose: 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 20.0%	ND	Reproduction inhibition 18-60%, toxicity increased with increasing dose	[10]

Table S10. Germination and root growth inhibition test results on plants from multiple studies with biochars produced from sewage sludge or co-pyrolysed sewage sludge with plant biomass.

Biochar	Pyrolysis	Experiment	Soil type	Plant	Effect	Reference
Sewage sludge	200ºC Residence time: 30 min	Biochar's leachate (1:100)	Without soil	*L. sativum*	Germination inhibition 0-25% Root growth inhibition 24-38%	[11]
Sewage sludge + rape straw					Germination inhibition 15-20% Root growth inhibition 9-34%	
Sewage sludge + wheat straw					Germination inhibition 5% Root growth inhibition 32-34%	
Sewage sludge + sawdust					Germination inhibition 0-5% Root growth inhibition 34-37%	
Sewage sludge + bark					Germination inhibition 10-20% Root growth inhibition 15-34%	
Sewage sludge	500ºC	Lab Dose: 1% (30 t/ha)	Loamy sand	*L. sativum*	Germination inhibition 0-20% Root growth inhibition 0-10% or stimulation 60%	[7]
Sewage sludge KN	500ºC	Lab Dose: 1% (30 t/ha) aqueous extracts	Loamy sand	*L. sativum*	Germination inhibition 0-30% Root growth inhibition 0-5% or stimulation 10%	
Sewage sludge	500, 600 or 700ºC	Biochar's leachate (1:100)	Without soil	*L. sativum*	Root growth stimulation 55% or inhibition 39-57%, toxicity increased with increasing temperature	[8]
Sewage sludge + willow (8:2)	500, 600 or 700ºC				Root growth stimulation 10-98% or 5% inhibition, toxicity increased with increasing temperature	
Sewage sludge + willow (6:4)	500, 600 or 700ºC				Root growth stimulation 105% or inhibition 5-15%, toxicity increased with increasing temperature	
Sewage sludge	500, 600 or 700ºC	Dose:1%	ND	*L. sativum*	Root growth inhibition 6-8%	
Sewage sludge + willow (8:2)	500, 600 or 700ºC				Root growth stimulation <5%	
Sewage sludge + willow (6:4)	500, 600 or 700ºC				Root growth stimulation <5%	
Paper sludge+ wheat husks	Temp. 500ºC Residence time: 20 min	Lab Dose: 8% w/w Temp. 28ºC Duration: 1 month	Haplic Cambisols ST- sandy loam SA- sandy	Lettuce (*Lactuca sativa*) Lentil (*Lens culinaris*)	Lettuce: ST- 33% lower stem length. SA- no statistical difference Lentil: ST- 14% lower aerial biomass, 38% lower root dry weight. SA- higher aerial biomass, stem length and root dry weight 15-38%	[12]
Sewage sludge					Lettuce: ST- 32% lower lower stem length, 75% lower root dry weight. SA- 34% higher stem length Lentil: ST- no statistical difference. SA- 21% lower stem length	
Sewage sludge (from three different treatment plants)	Temp. 300ºC Residence time: 15 min	Lab Dose: 0.5 or 1 or 2% w/w Duration: 200 days	Loamy sand	*Poa pratensis* L.	1) From 60% to over 2.5- fold higher biomass depending on biochar dose. The higher dose, the higher biomass. 2) 80% higher biomass 3) 40-80% higher biomass	[13]
Sewage sludge	Temp. 300ºC	Field Dose: 15 t/ ha	Red Yellow Latosol (Typic Haplustox)	Corn (hybrid LG6030)	42% higher grain yield	[14]

	Residence time: 30 min	Duration: 2 years				
Sewage sludge	Temp. 300 or 500ºC Residence time: 30 min	Field Dose: 15 t/ ha Duration: 1 year	Clayey Oxisol (Typic Haplustox)	Corn	45% higher grain yield	[15]
Sewage sludge	Temp. 850ºC Residence time: 4 h	Field 20, 40 and 60 t/ha	Loamy sand	peanut (cv. No.16 Huayu)	the addition of biochar increased crop yield by 35-60%, the highest increase was noted for 40 t/ha addition	[16]
Sewage sludge	300, 500 or 700ºC	Lab aqueous extracts	Without soil	*Triticum aestivum* L.	No changes for seed germination Root growth inhibition- 6-18% Toxicity increased with increasing temperature	[17]
Sewage sludge	300, 500, 700 or 900ºC	Lab Dose: 5%	Sand	*Triticum* spp.	No changes for seed germination No changes for root length	[6]
Sewage sludge (different kinds: KN, KZ, CM, and SI)	500, 600 or 700ºC	Lab Dose: 1%	Standard soil (OECD)	*L. sativum*	KN, KZ, CM: 500ºC- stimulation of root growth (<10%), 600ºC and 700ºC- inhibition of root growth (<20%) SI: Stimulation of root growth (<20%)	[18]
Sewage sludge (different kinds: SS1, SS2, and SS3)	300ºC Residence time: 15 min	Lab aqueous extracts	Without soil	*L. sativum*	Inhibition 25% or stimulation 6% of root growth, depending of the sludge kind	[19]
Sewage sludge	550ºC Residence time: 15 min	Dose: 0.4, 0.9, 2.1, 4.9, 11.3 and 26 %, 10.6 t/ha to 676 t/ha	Fluventic Haploxerept, sandy loam agricultural soil	*Lactuca sativa* and *Lolium perenne*	No toxic effect, EC10 >26% (>676 t/ha) for aboveground or belowground biomass	[20]

Table S11. Correlations between ecotoxicological tests results of the experiment and chemical properties of soil with SL- derived biochar addition. Statistically important coefficients were marked with *red (p=0.95) or **green (p=0.99) or ***violet (p=0.999).

		pH	EC	TOC	DOC	CEC	Available		
							Mg	K	P
0 days									
A. fischeri	Microtox test	-	-	-	-	-	-	-	-
L. sativum	Liquid	0.923	-0.760	-0.088	-0.799	-0.600	0.306	-0.466	-0.784
	Solid	0.497	-0.189	-0.790	-0.245	-0.090	-0.451	-0.523	-0.291
F. candida	Mortality	0.906	-0.727	-0.171	-0.769	-0.570	0.236	-0.483	-0.761
	Reproduction	0.287	0.432	-0.663	0.375	0.559	-0.858	-0.782	0.354
30 days									
A. fischeri	Microtox test	0.086	-0.540	0.321	-0.274	0.810	0.944	*0.952	-0.589
L. sativum	Liquid	-0.879	0.515	0.513	0.727	-0.063	0.505	0.355	0.408
	Solid	0.737	-0.784	0.102	-0.723	0.882	0.478	0.617	*-0.980
F. candida	Mortality	0.546	-0.732	0.266	-0.589	*0.973	0.714	0.819	*-0.965
	Reproduction	0.870	-0.610	-0.844	-0.810	-0.163	-0.590	-0.472	-0.140
90 days									
A. fischeri	Microtox test	-	-	-	-	-	-	-	-
L. sativum	Liquid	0.401	-0.076	*0.980	0.925	0.728	*-0.958	-0.684	-0.487
	Solid	0.105	0.576	-0.625	-0.432	-0.810	0.876	*0.960	*0.957
F. candida	Mortality	0.317	0.026	0.085	-0.081	0.040	-0.499	-0.804	-0.688
	Reproduction	-0.422	-0.588	-0.197	-0.414	0.248	-0.230	-0.678	-0.833
180 days									
A. fischeri	Microtox test	0.671	-0.898	0.608	0.942	0.385	-0.811	-0.503	0.129
L. sativum	Liquid	-0.218	0.199	0.232	-0.631	0.270	0.360	0.059	0.708
	Solid	-0.760	0.664	-0.496	-0.842	-0.386	0.875	0.607	0.235
F. candida	Mortality	-0.160	-0.096	-0.532	0.536	-0.609	-0.003	0.321	-0.650
	Reproduction	0.322	0.699	-0.065	-0.531	0.290	-0.178	-0.443	-0.716

Table S12. Correlations between ecotoxicological tests results of the experiment and total and bioavailable PAHs content in soil with SL- derived biochar addition. Statistically important coefficients were marked with *red (p=0.95) or **green (p=0.99) or ***violet (p=0.999).

		Total PAHs						Bioavailable PAHs					
		Σ16	R2	R3	R4	R5	R6	Σ16	R2	R3	R4	R5	R6
0 days													
A. fischeri	Microtox test	-	-	-	-	-	-	-	-	-	-	-	-
L. sativum	Liquid	0.599	-0.138	0.754	*0.972	***0.999	0.889	*-0.982	-0.876	-0.552	-0.715	**0.997	0.552
	Solid	-0.218	-0.812	-0.007	0.563	0.662	0.346	-0.708	-0.856	0.229	-0.015	0.671	-0.202
F. candida	Mortality	0.529	-0.220	0.697	*0.959	**0.997	0.863	*-0.988	-0.907	-0.482	-0.660	**0.996	0.491
	Reproduction	-0.448	-0.652	-0.433	0.260	0.254	-0.236	-0.207	-0.345	0.333	0.119	0.282	-0.692
30 days													
A. fischeri	Microtox test	0.405	0.548	0.592	-0.205	-0.734	-0.579	0.353	0.366	-0.110	0.508	-0.702	0.908
L. sativum	Liquid	-0.402	-0.239	-0.357	-0.841	-0.754	-0.081	-0.660	-0.665	-0.046	0.877	-0.651	0.014
	Solid	0.474	0.465	0.648	0.361	-0.137	-0.669	0.728	0.755	-0.349	-0.414	-0.236	0.845
F. candida	Mortality	0.428	0.474	0.637	0.140	-0.404	-0.766	0.619	0.647	-0.381	-0.154	-0.476	*0.965
	Reproduction	0.666	0.516	0.544	*0.988	0.875	0.448	0.762	0.750	0.495	-0.587	0.847	-0.145
90 days													
A. fischeri	Microtox test	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
L. sativum	Liquid	-0.858	0.341	-0.429	-0.766	-0.763	-0.688	-0.645	-0.317	-0.897	-0.850	-0.313	0.591
	Solid	0.377	0.334	0.714	0.118	0.109	0.164	*0.968	0.888	0.691	0.887	0.847	*-0.969
F. candida	Mortality	-0.323	-0.069	*-0.987	0.025	0.052	-0.322	-0.797	-0.696	-0.620	-0.734	-0.867	0.488
	Reproduction	0.302	-0.737	-0.688	0.610	0.626	0.385	-0.721	-0.918	-0.108	-0.412	-0.921	0.690
180 days													
A. fischeri	Microtox test	0.444	-0.086	0.759	-0.411	-0.512	-0.689	0.844	0.882	-0.200	-0.631	0.774	*-0.963
L. sativum	Liquid	0.391	0.771	-0.163	*0.966	*0.973	0.669	-0.167	-0.268	0.921	**0.992	-0.397	0.465
	Solid	-0.335	0.190	-0.773	0.687	0.765	0.898	-0.798	-0.856	0.404	0.806	-0.873	0.945
F. candida	Mortality	-0.678	*-0.953	-0.195	-0.843	-0.828	-0.341	-0.158	-0.056	*-0.984	-0.889	0.034	-0.178
	Reproduction	0.010	0.156	0.131	-0.379	-0.320	-0.427	-0.056	-0.047	-0.110	-0.072	0.261	0.135

Table S13. Correlations between ecotoxicological tests results of the experiment and chemical properties of soil with SLW- derived biochar addition. Statistically important coefficients were marked with *red (p=0.95) or **green (p=0.99) or ***violet (p=0.999).

		pH	EC	TOC	DOC	CEC	Available		
							Mg	K	P
0 days									
A. fischeri	Microtox test	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
L. sativum	Liquid	-0.872	0.647	-0.232	-0.384	-0.349	-0.764	0.412	*0.968
	Solid	0.085	-0.004	0.739	0.708	-0.394	0.130	0.837	0.166
F. candida	Mortality	0.715	-0.138	**0.999	0.947	0.197	0.790	0.321	-0.408
	Reproduction	-0.393	0.731	0.378	0.126	0.041	-0.182	0.367	0.723
30 days									
A. fischeri	Microtox test	0.561	-0.084	0.860	0.721	0.081	*-0.963	-0.033	0.874
L. sativum	Liquid	-0.709	0.313	0.094	0.677	0.688	0.013	0.235	0.456
	Solid	-0.899	0.784	0.230	0.388	0.949	0.290	0.726	0.341
F. candida	Mortality	-0.466	0.865	0.191	-0.454	0.544	0.546	0.885	-0.192
	Reproduction	-0.539	0.848	0.719	0.549	*0.961	-0.129	0.838	0.686
90 days									
A. fischeri	Microtox test	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
L. sativum	Liquid	-0.185	-0.102	-0.506	-0.680	0.055	-0.013	-0.094	-0.580
	Solid	-0.563	0.284	-0.074	-0.255	0.519	0.231	0.387	-0.235
F. candida	Mortality	0.164	0.026	0.796	0.721	0.162	-0.233	0.381	0.899
	Reproduction	-0.011	0.047	0.912	0.627	0.433	-0.321	0.648	*0.985
180 days									
A. fischeri	Microtox test	*-0.986	-0.076	0.338	*0.986	-0.912	0.710	-0.384	-0.158
L. sativum	Liquid	0.214	0.579	-0.208	-0.221	0.445	0.021	0.227	-0.600
	Solid	0.308	-0.912	-0.729	-0.286	0.001	-0.819	-0.676	-0.221
F. candida	Mortality	**-0.994	-0.020	0.443	**0.992	-0.912	0.776	-0.297	-0.043
	Reproduction	0.664	0.630	-0.209	-0.673	0.845	-0.241	0.510	-0.301

Table S14. Correlations between ecotoxicological tests results of the experiment and total and bioavailable PAHs content in soil with SLW-derived biochar addition. Statistically important coefficients were marked with *red (p=0.95) or **green (p=0.99) or ***violet (p=0.999).

		Total PAHs						Bioavailable PAHs					
		Σ16	R2	R3	R4	R5	R6	Σ16	R2	R3	R4	R5	R6
0 days													
A. fischeri	Microtox test	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
L. sativum	Liquid	0.245	-0.351	0.274	0.377	0.561	0.457	*0.979	0.805	0.277	0.349	0.765	0.034
	Solid	0.781	0.725	0.948	0.196	0.333	0.319	0.476	0.716	-0.613	-0.614	0.354	0.103
F. candida	Mortality	0.826	*0.966	0.842	0.306	0.276	0.351	-0.239	0.012	-0.534	-0.616	-0.401	-0.311
	Reproduction	0.796	0.182	0.737	0.759	0.880	0.839	0.723	0.620	0.165	0.166	0.314	-0.410
30 days													
A. fischeri	Microtox test	-0.078	-0.028	-0.503	0.052	0.219	0.124	-0.710	-0.819	-0.585	-0.526	0.322	-0.222
L. sativum	Liquid	0.914	0.939	0.765	0.631	*0.966	**0.992	0.694	0.482	0.813	0.836	0.945	-0.489
	Solid	0.551	0.597	0.484	0.096	0.701	0.791	0.600	0.342	0.751	0.855	0.753	-0.831
F. candida	Mortality	-0.493	-0.459	-0.346	-0.841	-0.338	-0.215	-0.018	-0.119	0.047	0.186	-0.221	-0.647
	Reproduction	0.219	0.294	-0.020	-0.177	0.552	0.610	0.064	-0.230	0.273	0.432	0.688	*-0.982
90 days													
A. fischeri	Microtox test	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
L. sativum	Liquid	0.665	-0.012	0.643	0.253	0.662	0.586	*0.985	0.126	0.711	0.708	0.466	-0.526
	Solid	0.493	-0.483	0.480	-0.231	0.682	0.626	0.837	0.034	0.653	0.641	0.170	-0.083
F. candida	Mortality	-0.308	-0.280	-0.276	-0.527	-0.201	-0.105	-0.936	0.277	-0.904	-0.909	-0.214	0.785
	Reproduction	0.085	-0.583	0.119	-0.732	0.304	0.392	-0.611	0.537	-0.795	-0.807	0.016	0.879
180 days													
A. fischeri	Microtox test	*0.985	-0.216	0.429	0.736	-0.214	0.227	0.437	0.467	0.028	-0.814	0.100	-0.765
L. sativum	Liquid	-0.229	0.681	0.875	-0.721	-0.940	-0.948	-0.924	-0.903	-0.700	0.205	-0.143	0.237
	Solid	-0.259	-0.834	-0.638	0.102	0.742	0.631	0.358	0.403	-0.188	-0.239	0.802	-0.318
F. candida	Mortality	*0.989	-0.174	0.380	0.767	-0.185	0.246	0.477	0.497	0.140	-0.757	0.002	-0.704
	Reproduction	-0.684	0.770	0.503	*-0.962	-0.692	-0.931	*-0.984	**-0.994	-0.486	0.660	-0.309	0.670

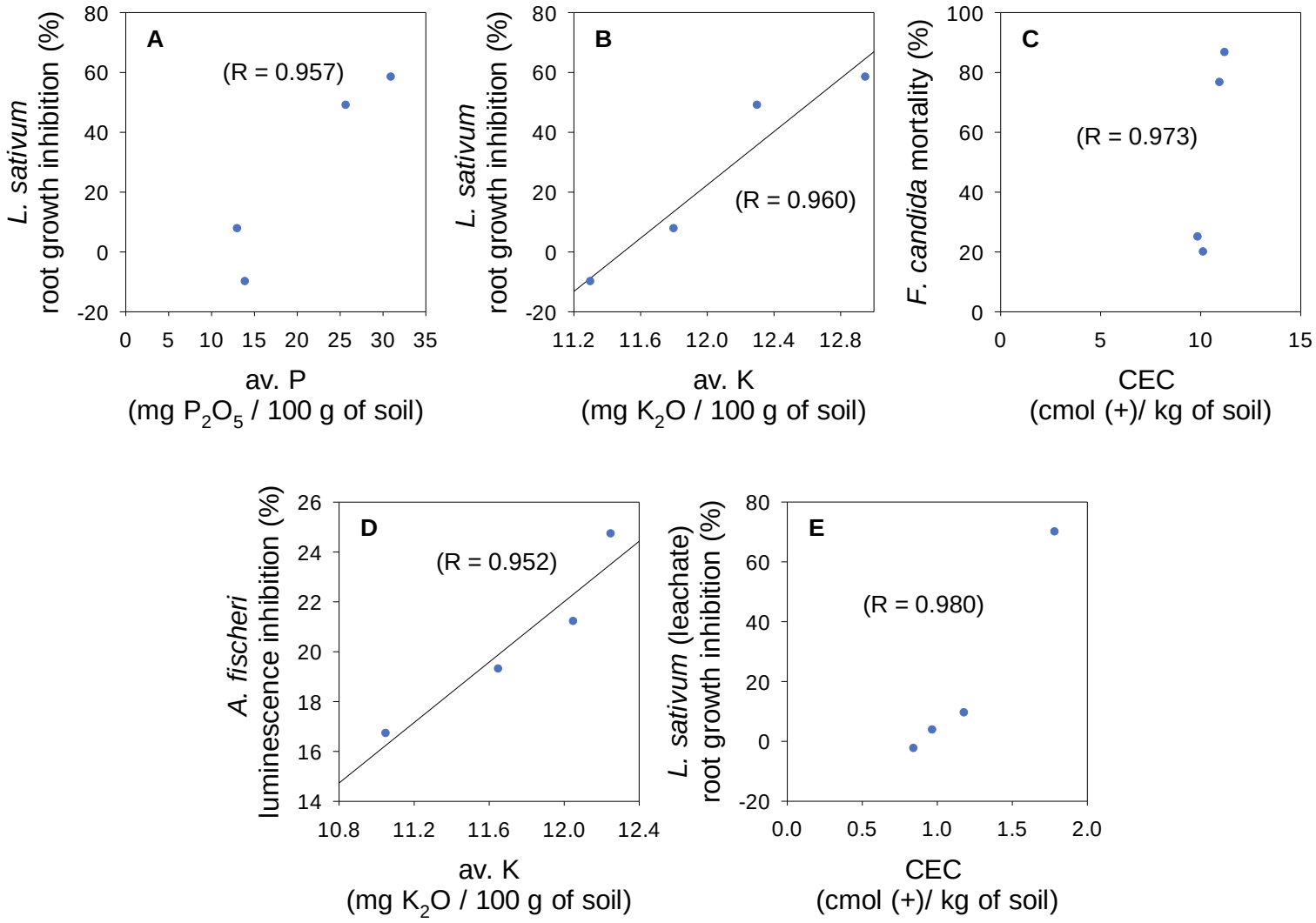

Fig. S1. Positive correlations between soil chemical properties and ecotoxicological tests results.