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**Wpływ warunków pirolizy na formowanie,
właściwości i oddziaływanie z zanieczyszczeniami
nano–biowęgla otrzymanych z różnych surowców
(The effect of pyrolysis conditions on the formation,
properties and interactions with contaminants of nano–
biochars derived from various feedstocks)**

Rozprawa doktorska przygotowywana pod kierunkiem naukowym
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Wykaz stosowanych skrótów w rozprawie doktorskiej

BC	biowęgiel (z ang. <i>biochar</i>)
b-BC	biowęgiel o wielkości cząstek $\sim \mu\text{m} - \text{mm}$ (z ang. <i>bulk biochar</i>)
n-BC	nano-biowęgiel (z ang. <i>nano-biochar</i>)
EC	przewodnictwo elektryczne (z ang. <i>electrical conductivity</i>)
CEC	pojemności wymiany kationów (z ang. <i>cation-exchange capacity</i>)
S_{BET}	powierzchnia właściwa
S_{micro}	powierzchnia mikroporów
V_p	całkowita objętość porów
V_{micro}	objętość mikroporów
WWA	wielopierścieniowe węglowodory aromatyczne (z ang. <i>polycyclic aromatic hydrocarbons</i>)
PTEs	pierwiastki potencjalnie toksyczne (z ang. <i>potentially toxic elements</i>)
WHC	zdolność zatrzymywania wody (z ang. <i>water holding capacity</i>)
TOC	całkowity węgiel organiczny (z ang. <i>total organic carbon</i>)
DOC	rozpuszczony węgiel organiczny (z ang. <i>dissolved organic carbon</i>)
C_{total} $\Sigma 16$ WWA	całkowite (ekstrahowane rozpuszczalnikami organicznymi) stężenie WWA
C_{free} $\Sigma 16$ WWA	stężenie swobodnie rozpuszczonych (biodostępnych) WWA
POM	polioksymetylen
WL	wiklina
RH	łuska ryżowa
OSR	słoma rzepakowa
PB	kiście palmowe
SSL	osad ściekowy
BAF	współczynnik bioakumulacji (z ang. <i>bioaccumulation factor</i>)
HRI	wskaźnik ryzyka zdrowotnego (z ang. <i>health risk index</i>)

1. Publikacje naukowe będące przedmiotem rozprawy doktorskiej

Prezentowana rozprawa doktorska oparta jest na spójnym tematycznie cyklu siedmiu artykułów naukowych opublikowanych w recenzowanych czasopismach znajdujących się na wykazie Ministerstwa Nauki i Szkolnictwa Wyższego (MEiN).

- D1 M. Raczekiewicz, I. Ostolska, O. Mašek, P. Oleszczuk**
"Effect of the pyrolysis conditions and type of feedstock on nano-biochars obtained as a result of ball milling"
Journal of Cleaner Production, 458, 142456,
<https://doi.org/10.1016/j.jclepro.2024.142456>
IF_{5-letni} = 10.7 MEiN: 140 pkt
- D2 M. Raczekiewicz, O. Mašek, Y. S. Ok, P. Oleszczuk**
"Size reduction of biochar to nanoscale decrease polycyclic aromatic hydrocarbons and trace metals content and bioavailability in nano-biochars"
Science of The Total Environment, 937, 173372,
<https://doi.org/10.1016/j.scitotenv.2024.173372>
IF_{5-letni} = 8.7 MEiN: 200 pkt
- D3 M. Raczekiewicz, A. Bogusz, B. Pan, B. Xing, P. Oleszczuk**
"Contrasting environmental impacts of nano-biochar and conventional biochar on various organisms"
Science of The Total Environment, 957, 177629,
<https://doi.org/10.1016/j.scitotenv.2024.177629>
IF_{5-letni} = 8.7 MEiN: 200 pkt
- D4 M. Raczekiewicz, P. Oleszczuk**
"Enhanced heavy metal removal using nano-engineered biochars synthesized under variable pyrolysis conditions and feedstocks"
Journal of Environmental Management, 389, 126072
<https://doi.org/10.1016/j.jenvman.2025.126072>
IF_{5-letni} = 8.6 MEiN: 200 pkt
- D5 M. Raczekiewicz, P. Oleszczuk**
"Boosting sewage sludge safety with nano-biochar for polycyclic aromatic hydrocarbons immobilization and ecotoxicity reduction"
Journal of Hazardous Materials, 492, 138224
<https://doi.org/10.1016/j.jhazmat.2025.138224>
IF_{5-letni} = 12.4 MEiN: 200 pkt
- D6 M. Raczekiewicz, D. Akachukwu, P. Oleszczuk**
"Sustainable soil remediation using nano-biochar for improved food safety and resource recovery"
Journal of Hazardous Materials, 494, 138537
<https://doi.org/10.1016/j.jhazmat.2025.138537>
IF_{5-letni} = 12.4 MEiN: 200 pkt
- D7 M. Raczekiewicz, D. Akachukwu, P. Oleszczuk**
"Nanoscale biochar outperforms conventional biochar in enhancing soil health and reducing risks from polycyclic aromatic hydrocarbon-contaminated soil"
Environmental Research (po pierwszym etapie recenzji, w trakcie decyzji)
IF_{5-letni} = 7.7 MEiN: 100 pkt

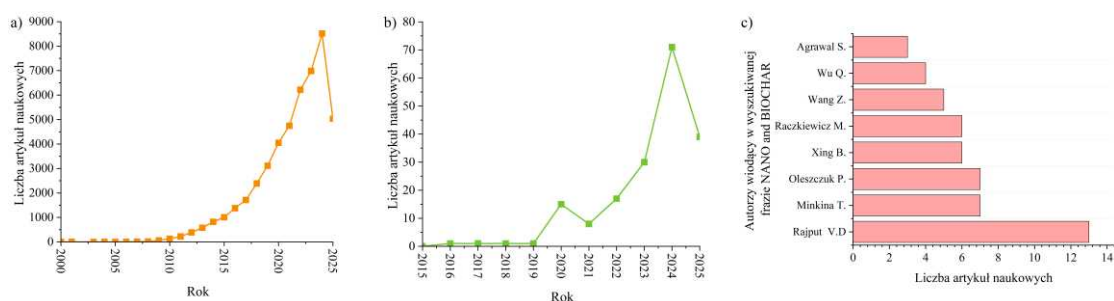
Sumaryczny IF_{5-letni}: 69.2

Sumaryczna liczba punktów MEiN: 1 240

2. Aktualny stan wiedzy

2.1. Wprowadzenie

Pierwsze wzmianki dotyczące biowęgla w literaturze naukowej pojawiły się na początku lat 2000 (**Rys. 1a**). Od tego czasu obserwowany jest wykładniczy wzrost zainteresowania tą tematyką, szczególnie zauważalny w ciągu ostatniej dekady. Według danych bibliometrycznych obejmujących lata 2015–2025, w bazie Scopus zarejestrowano 45 154 publikacji poświęconych biowęglowi, przy czym ponad 78 % z nich ukazało się po 2020 r. (**Rys. 1a**). Równoległy rozwój nanotechnologii przyczynił się do powstania nowego, szybko rozwijającego się nurtu badawczego łączącego klasyczne zastosowania biowęgla z osiągnięciami nanotechnologii – nano-biowęgla. Choć liczba publikacji dotyczących nano-biowęgla wynosi dotąd setki (184 publikacji w ostatniej dekadzie), obserwuje się znaczny wzrost zainteresowania, szczególnie od 2020 r. (**Rys. 1b**). Analizy dotychczas opublikowanych prac w tym zakresie, wskazują, że badania koncentrują się przede wszystkim na aspektach środowiskowych, takich jak oczyszczanie wód, remediacja gleby, „żywołność” nanocząstek i ich katalityczne właściwości.



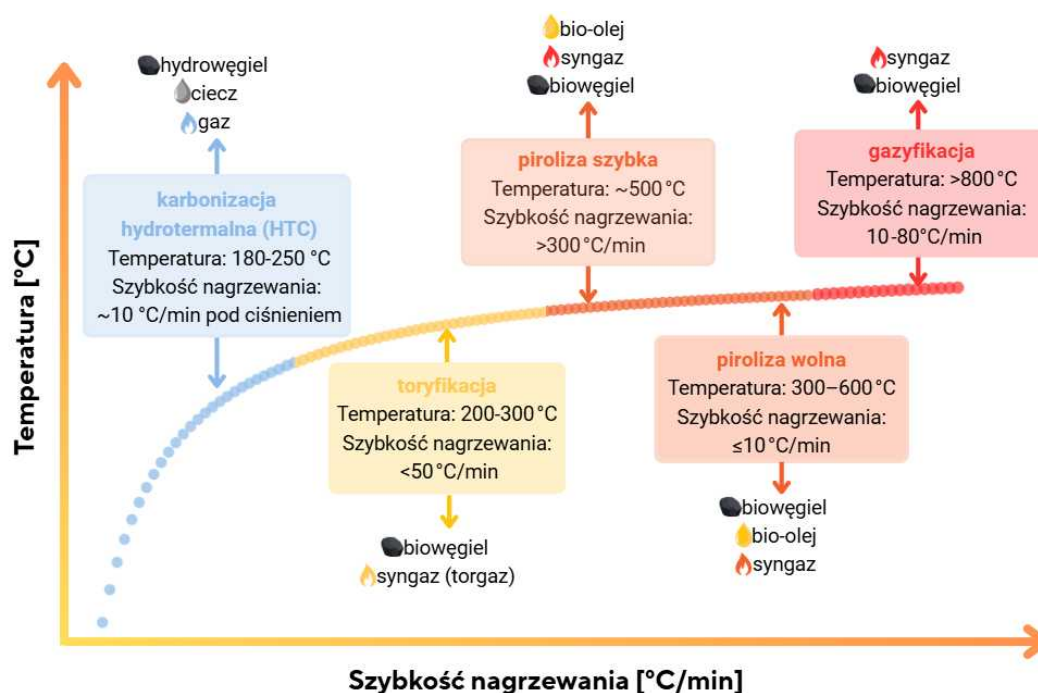
Rysunek 1. Analiza trendów publikacyjnych związanych z tematyką biowęgla i nano-biowęgla w bazie Scopus (stan z 22.06.2025). Panel (a) przedstawia roczną liczbę publikacji zawierających słowo kluczowe „BIOCHAR” w tytule, streszczeniu lub słowach kluczowych. Panel (b) ukazuje dynamikę wzrostu liczby prac odnoszących się do hasła „NANO” oraz „BIOCHAR” w tytule, streszczeniu lub słowach kluczowych w ostatniej dekadzie. Panel (c) pokazuje autorów o największej liczbie publikacji spełniających kryteria wyszukiwania hasła „NANO” and BIOCHAR”.

Tak dynamiczny rozwój tematyki zarówno biowęgla, jak i jego nano-odpowiednika wskazuje, że nano-biowęgiel stanowi obecnie jeden z aktualnie wiodących trendów badawczych. Ze względu na rosnące znaczenie tych materiałów zarówno w teorii, jak i w praktyce – szczególnie w obszarze ochrony środowiska, rolnictwa oraz inżynierii

materiałowej – uzasadnione jest podjęcie pogłębionych studiów nad procesem wytwarzania, właściwościami oraz aplikacjami nano–biowęgla. Tym samym tematyka niniejszej rozprawy odpowiada współczesnym wyzwaniom i trendom naukowym, uzasadniając podjęcie badań w tym zakresie.

2.2. Biowęgiel i nano–biowęgiel

Biowęgiel (BC, z *ang. biochar*) to termin odnoszący się do stałego, węglowego materiału pochodzenia organicznego, otrzymywanego w wyniku termochemicznej konwersji biomasy w warunkach ograniczonego dostępu tlenu [1]. Jest to materiał bogaty w węgiel elementarny, który – w odróżnieniu od tradycyjnego węgla drzewnego stosowanego jako paliwo – znajduje zastosowanie głównie w rolnictwie i ochronie środowiska, m.in. jako materiał do poprawy właściwości gleb oraz do sekwestracji węgla, adsorbent zanieczyszczeń organicznych i nieorganicznych, a także komponent do produkcji kompozytów węglowych, węgla aktywnego (z *ang. activated carbon*) czy nanomateriałów węglowych takich jak: kropki węglowe (z *ang. carbon dots*) czy też nano–biowęgiel (n–BC, z *ang. nano–biochar*) [2, 3]. Podstawowym procesem technologicznym wykorzystywanym do produkcji BC są różne rodzaje pirolizy (**Rys. 2**), czyli termochemiczny rozkład biomasy prowadzący do powstania trzech głównych frakcji: stałej (biowęgiel), ciekłej (bio–olej) oraz gazowej (syngaz). Proces pirolizy



Rysunek 2. Przegląd głównych metod termochemicznej konwersji biomasy prowadzących do otrzymania biowęgla oraz ich podstawowa charakterystyka procesowa.

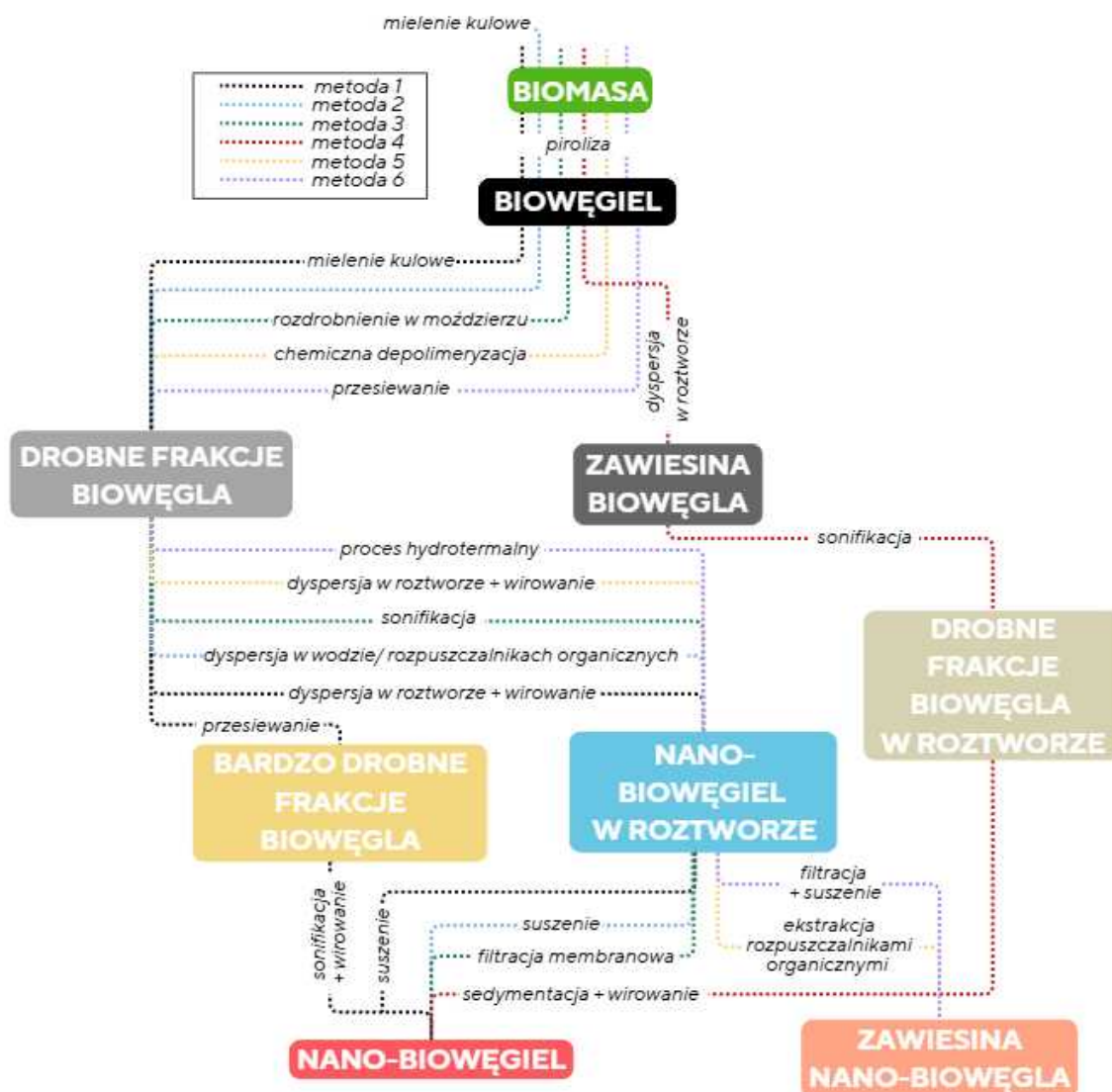
prorowadzony jest zwykle w zakresie temperatur od 300 do 700 °C w warunkach beztlenowych lub przy ograniczonym dostępie tlenu [4].

Do produkcji BC wykorzystuje się biomasę, rozumianą jako szeroką gamę surowców lignocelulozowych oraz organicznych materiałów odpadowych, zarówno pochodzenia roślinnego, jak i zwierzęcego [5]. Wśród najczęściej stosowanych surowców stosowanych do otrzymywania BC znajdują się pozostałości roślinne z upraw rolnych (np. słoma zbóż, łuski ryżu, kolby kukurydzy, liście trzciny cukrowej), odpady przemysłu drzewnego (np. zrębki, trociny), osady ściekowe z oczyszczalni komunalnych, odpady pochodzenia zwierzęcego (np. obornik, pomiot ptasi), pozostałości przemysłu spożywczego (np. makuchy poekstrakcyjne, łuski orzechów, wytloki owocowe i warzywne), a także biomasa glonów oraz różnego rodzaju odpady miejskie i przemysłowe o wysokiej zawartości materii organicznej [6, 7].

N-BC stanowi zmodyfikowaną formę BC, która została poddana dodatkowym procesom w celu uzyskania znacznie mniejszych cząstek o rozmiarach nanometrycznych [8]. Chociaż w literaturze często przyjmuje się, że n-BC to materiał o wielkości cząstek poniżej 100 nm, to jednak w praktyce zakres ten bywa znacznie szerszy. Jak wynika z przeglądu literatury, za n-BC uznaje się również BC o wielkości cząstek sięgającej nawet 600 nm. Średnie rozmiary cząstek opisywane jako n-BC mieszczą się zazwyczaj w przedziale od kilku do kilkuset nanometrów (około 4–500 nm) [9–11].

Istnieje sześć podstawowych, szeroko opisywanych w literaturze, metod otrzymywania n-BC, z których każda znajduje zastosowanie w odniesieniu do różnych typów biomasy (**Rys. 3**). Niezależnie od wybranej procedury, proces otrzymywania n-BC składa się z dwóch kluczowych etapów. W pierwszym etapie biomasa ulega pirolitycznej konwersji do BC w warunkach ograniczonego dostępu tlenu. Następnie otrzymany materiał poddawany jest procesowi frakcjonowania lub intensywnej redukcji rozmiaru cząstek, mających na celu uzyskanie struktur o rozmiarach w skali nano, zwykle w zakresie <100–600 nm, w zależności od zastosowanej metody [12]. Zastosowane techniki dezintegracji obejmują m.in. mechaniczne rozdrabnianie (np. mielenie kulowe, ultradźwięki), chemiczne trawienie, metody hydrotermalne oraz połączenia tych strategii [13–16]. Dobór odpowiedniej techniki zależy od specyfiki surowca użytego do otrzymania BC a następnie n-BC, pożądanych właściwości końcowego materiału oraz przewidywanego zastosowania środowiskowego. Każdy z wymienionych etapów

otrzymania n-BC – od warunków pirolizy, poprzez rodzaj surowca po fizykochemiczne frakcjonowanie BC – odgrywa kluczową rolę w kształtowaniu jego właściwości morfologicznych, powierzchniowych i funkcjonalnych.



Rysunek 3. Najczęściej wykorzystywane metody do otrzymywania nano-biowęgla z biomasy (zaadaptowane z [12]).

2.3. Wpływ rodzaju surowca i warunków pirolizy na właściwości biowęgla i nano-biowęgla

Właściwości fizykochemiczne BC, takie jak skład pierwiastkowy, porowatość, powierzchnia właściwa, pH, przewodnictwo elektryczne (EC), zawartość składników mineralnych oraz obecność powierzchniowych grup funkcyjnych, są determinowane przede wszystkim rodzajem użytego surowca oraz parametrami pirolizy [17]. Surowce o wysokiej zawartości ligniny oraz niskiej zawartości substancji lotnych, takie jak drewno

sosnowe czy łądygi konopi, prowadzą do uzyskania BC o większej stabilności, odporności chemicznej i rozwiniętej strukturze porowatej [18]. Z kolei surowiec bogaty w białka i lipidy, jak osady ściekowe czy obornik, sprzyja powstawaniu materiałów o podwyższonej zawartości azotu (N) oraz lepszych właściwościach sorpcyjnych, choć mogą one charakteryzować się mniejszą stabilnością chemiczną [19, 20]. Parametry procesu pirolizy, w tym temperatura, czas, szybkość ogrzewania, typ reaktora (rurowy, retortowy itd.) oraz obecność i rodzaj gazu nośnego (azot – N₂, argon – Ar, dwutlenek węgla – CO₂), w znacznym stopniu wpływają na charakterystykę otrzymanego BC [21]. Wzrost temperatury pirolizy powoduje zwiększenie zawartości węgla pierwiastkowego, aromatyczności, powierzchni właściwej i porowatości BC, jednocześnie obniżając zawartość wodoru (H), tlenu (O) oraz frakcji lotnych [22]. Procesy te są efektem skondensowania struktury węgla (C) oraz powstawania faz mineralnych (tlenków i węglanów), prowadzących do zwiększenia ilości popiołu i pH [23, 24]. Utrata azotu (N), wodoru (H) i tlenu (O) podczas pirolizy, dodatkowo prowadzi do koncentracji innych pierwiastków np. pierwiastków potencjalnie toksycznych (PTEs, z ang. *potentially toxic elements*), co jest szczególnie widoczne w materiałach, które zawierają podwyższone ich ilości (np. osady ściekowe) [25]. Rodzaj gazu nośnego istotnie wpływa na rozwój struktury porowatej oraz potencjał sorpcyjny BC [26, 27]. Typ pirolizy (powolna lub szybka) również istotnie determinuje właściwości uzyskanego materiału: powolna piroliza zwykle pozwala na otrzymanie BC o wyższej zawartości azotu (N), siarki (S), dostępnego fosforu (P), wapnia (Ca) czy magnezu (Mg), większej powierzchni właściwej oraz wyższej pojemności wymiany kationów (CEC, z ang. *cation-exchange capacity*) niż w przypadku BC otrzymywanego w procesie pirolizy szybkiej [28, 29].

Optymalizacja parametrów otrzymywania BC pozwala więc precyzyjnie dostosować jego właściwości do specyficznych zastosowań środowiskowych, takich jak oczyszczanie ścieków, remediacja gleb, obniżenie emisji gazów cieplarnianych, poprawa retencji wody czy magazynowanie składników odżywczych w glebie [30]. Pomimo licznych badań, nadal brakuje szczegółowych informacji na temat wpływu rodzaju surowca i parametrów pirolizy na właściwości n-BC, szczególnie w kontekście dalszej obróbki mechanicznej lub chemicznej, co wymaga dalszych badań. Aspekt ten został zbadany w ramach badań własnych będących częścią niniejszej rozprawy doktorskiej (*Publikacja DI*), której celem było określenie wpływu warunków pirolizy oraz rodzaju surowca na właściwości n-BC.

Szczegółowe wyniki i ich omówienie przedstawiono w kolejnych rozdziałach rozprawy doktorskiej.

M. Raczkiewicz, I. Ostolska, O. Mašek, P. Oleszczuk
"Effect of the pyrolysis conditions and type of feedstock on nano-biochars obtained as a result of ball milling"
Journal of Cleaner Production, 458, 142456

2.4. Oddziaływanie biowęgla i nano-biowęgla z zanieczyszczeniami

BC, a szczególnie n-BC stanowią obiecujące materiały do usuwania zanieczyszczeń organicznych i nieorganicznych z wód i gleb, dzięki swoim wyjątkowym właściwościom fizykochemicznym, takim jak porowatość, duża powierzchnia właściwa, obecności powierzchniowych grup funkcyjnych oraz trwałość w środowisku. Wspomniane właściwości determinują silniejsze oddziaływania między BC a różnorodnymi zanieczyszczeniami [31, 32]. Nanostrukturyzacja BC prowadzi do znacznego zwiększenia aktywności sorpcyjnej, dyspersji w środowisku wodnym oraz reaktywności powierzchniowej, co sprzyja bardziej efektywnej sorpcji PTEs (np. As, Cr, Pb, Cd, Zn) [33] oraz licznych związków organicznych, w tym antybiotyków, pestycydów i wielopierścieniowych węglowodorów aromatycznych (WWA) (PAHs, z *ang.* *polycyclic aromatic hydrocarbons*) [34]. Mechanizmy odpowiedzialne za wiązanie jonów metali przez BC i n-BC obejmują przede wszystkim kompleksowanie, wymianę jonową, adsorpcję powierzchniową oraz – w przypadku BC, które mają zasadowy charakter – również precypitację metali w postaci trudno rozpuszczalnych soli [35]. Z kolei sorpcja zanieczyszczeń organicznych zachodzi głównie poprzez oddziaływania hydrofobowe, interakcje π - π oraz mechanizm wypełniania porów [36]. W warunkach współobecności różnych klas zanieczyszczeń może dochodzić do konkurencji sorpcyjnej [37, 38], dlatego ważne jest dostosowanie składu chemicznego BC do konkretnego typu zanieczyszczeń poprzez ich modyfikacje. W ten sposób można uzyskać zwiększenie selektywności wobec związków organicznych i nieorganicznych [39]. Mimo wysokiej skuteczności, n-BC cechuje się większą mobilnością w środowisku, co może prowadzić do jego migracji oraz potencjalnej remobilizacji wcześniej zaadsorbowanych zanieczyszczeń [40]. Oddziaływania BC i n-BC z zanieczyszczeniami są złożone i wieloczynnikowe, i oprócz wcześniej wspomnianych właściwości BC, efektywność wiązania zanieczyszczeń przez BC zależy również od rodzaju zanieczyszczeń oraz parametrów środowiskowych (pH, reakcje redoks, obecność ligandów). Pomimo tych wyzwań,

właściwości sorpcyjne oraz możliwość modyfikacji czynią BC i n-BC wyjątkowo atrakcyjnymi materiałami w remediacji środowiska ukierunkowanej na kompleksowe usuwanie PTEs i zanieczyszczeń organicznych z różnych matryc. Należy jednak pamiętać, że zastosowanie BC i n-BC może pociągać za sobą także skutki uboczne, takie jak wpływ na skład i aktywność mikroorganizmów czy toksyczne oddziaływanie na organizmy żywe. Dlatego ich wykorzystanie w procesach remediacji powinno uwzględniać możliwe ryzyka, aby zapewnić bezpieczeństwo oraz efektywność działań środowiskowych [41].

2.5. Zastosowania biowęgla i nano-biowęgla

BC oraz n-BC są obecnie intensywnie badane w kontekście ich wykorzystania w wielu dziedzinach, w tym w inżynierii środowiska oraz rolnictwa. Szczególnie ważnym zastosowaniem BC i n-BC jest remediacja środowisk wodnych, ze względu na wysoką efektywność sorpcji szerokiej gamy zanieczyszczeń obecnych w wodach powierzchniowych i gruntowych. BC są skutecznym i ekonomicznym rozwiązaniem w procesach oczyszczania ścieków komunalnych oraz przemysłowych [42]. Istotnym aspektem ich zastosowania jest również immobilizacja zanieczyszczeń organicznych oraz nieorganicznych w glebie [43]. Zastosowanie BC i n-BC do gleb oprócz kierunkowej immobilizacji zanieczyszczeń bardzo często poprawia również strukturę i żyzność gleby, jej właściwości fizyczne, chemiczne i biologiczne [44], co przekłada się na poprawę warunków wzrostu roślin [45]. BC obniżają również zasolenie gleby poprzez zwiększenie CEC oraz adsorpcję sodu (Na), który zastępowany jest przez magnez (Mg) i wapń (Ca), dzięki czemu gleba staje się mniej zasadowa [46]. Dodatkowo, BC działa jako wielofunkcyjne źródło składników odżywczych [47], z jednej strony ograniczając wypłukiwanie nawozów azotowych oraz z drugiej będąc źródłem ważnych pierwiastków, takich jak żelazo (Fe), miedź (Cu) i cynk (Zn). Dodatek BC wpływa również na interakcje składników odżywczych w glebie poprzez ograniczenie ich mobilności oraz modyfikację cyklu i reakcji składników odżywczych w glebie [48].

Podsumowując, szerokie spektrum właściwości BC i n-BC sprawia, że materiały te znajdują zastosowanie nie tylko w ochronie i remediacji środowiska, lecz także w innych obszarach inżynierii środowiskowej i rolnictwa, przyczyniając się do rozwoju technologii wspierających zrównoważone gospodarowanie zasobami.

3. Cel i zakres badań

Głównym celem przeprowadzonych badań było opracowanie i otrzymanie nanomateriału węglowego w postaci n-BC oraz określenie jego właściwości fizykochemicznych i zdolności aplikacyjnych w kontekście potencjalnych zastosowań środowiskowych. Jednocześnie oceniano ryzyko środowiskowe związane z wykorzystaniem n-BC, w szczególności w odniesieniu do jego wpływu na rośliny, bakterie i bezkręgowce glebowe i wodne. Badania realizowane w ramach rozprawy doktorskiej zostały podzielone na cztery podstawowe etapy (Rys. 4).



Rysunek 4. Schemat przedstawiający główne etapy badań przeprowadzone w ramach rozprawy doktorskiej.

Pierwszy etap obejmował otrzymanie BC o wielkości cząstek rzędu mikro– do milimetrów ($\sim\mu\text{m}$ – mm) (b-BC, z ang. *bulk biochar*) oraz jego nano–odpowiedników (n-BC) i ocenę wpływu nanostrukturyzacji na właściwości fizykochemiczne tych materiałów. Badania przeprowadzono z wykorzystaniem szerokiej gamy surowców oraz w różnych warunkach pirolizy, w tym przy zmiennej temperaturze (450–750 °C) oraz z zastosowaniem różnych gazów nośnych (N_2 lub CO_2). W drugim etapie oceniono wpływ nanostrukturyzacji BC na zawartość w nich zanieczyszczeń, takich jak WWA oraz PTEs. Trzeci etap obejmował porównanie wpływu b-BC i n-BC na ekotoksyczność wobec różnych organizmów oraz roślin, co pozwoliło na ocenę potencjalnych skutków środowiskowych wynikających z ich zastosowania w środowisku. W czwartym etapie oceniano potencjał aplikacyjny n-BC w odniesieniu do b-BC w zakresie usuwania zanieczyszczeń z wody, immobilizacji zanieczyszczeń w glebie oraz zwiększenia potencjału wykorzystania odpadów na przykładzie osadów ściekowych z jednoczesnym obniżeniem ryzyka środowiskowego.

W ramach realizowanych badań sformułowano cztery główne hipotezy badawcze, które miały na celu weryfikację wpływu procesu nanostrukturyzacji BC na jego

właściwości fizykochemiczne, zawartość zanieczyszczeń natywnych, potencjalną ekotoksyczność oraz zdolności aplikacyjne w zakresie usuwania i immobilizacji zanieczyszczeń z różnych matryc środowiskowych. Celem postawionych hipotez było umożliwienie kompleksowej oceny potencjału n-BC jako nowego materiału przyjaznemu środowisku oraz zidentyfikowanie możliwego ryzyka związanego z jego wykorzystaniem. Poprzez otrzymanie b-BC i n-BC z surowców odpadowych zaproponowano rozwiązanie wpisujące się w zasady zrównoważonego rozwoju i gospodarki o obiegu zamkniętym. Poniżej przedstawiono szczegółowe hipotezy badawcze, które podlegały weryfikacji w ramach zaplanowanych etapów pracy:

1. Strukturyzacja do skali nanometrycznej wpłynie istotnie na właściwości fizykochemiczne n-BC, prowadząc do zwiększenia powierzchni właściwej, porowatości oraz zmian w strukturze porów. Mniejsze cząstki charakteryzują się zwykle większym stosunkiem powierzchni do objętości oraz większą reaktywnością [49]. W wyniku tych zmian, oczekuje się, że nanostrukturyzacja będzie prowadziła do poprawy parametrów sorpcyjnych n-BC, czyniąc go materiałem bardziej efektywnym niż materiału niemodyfikowanego w usuwaniu różnorodnych zanieczyszczeń ze środowiska. Kierunek i zakres tych zmian będą determinowane przez rodzaj zastosowanego surowca i warunki pirolizy, w tym temperaturę oraz atmosferę procesu, co obserwowane jest w przypadku b-BC [50] (*Publikacja D1*).
2. Strukturyzacja do skali nanometrycznej wpłynie na zawartość i biodostępność zanieczyszczeń natywnych BC, takich jak WWA oraz PTEs. Oczekuje się, że w wyniku zwiększenia powierzchni właściwej i zmniejszenia rozmiaru cząstek, biodostępność tych zanieczyszczeń może ulec zwiększeniu, co może potencjalnie wpływać na zwiększenie ich migracji po zastosowaniu do dodatku gleb. Jednocześnie przewiduje się, że proces nanostrukturyzacji może prowadzić do zmniejszenia całkowitej zawartości tych zanieczyszczeń w materiale, w wyniku ich usunięcia/degradacji w trakcie obróbki mechanicznej [51] (*Publikacja D2*).
3. Ekotoksyczność n-BC może być dwukierunkowa. Kierunek zmian toksyczności po obniżeniu rozmiaru będzie zależał od charakterystyki materiału oraz uzyskanych jego nowych właściwości. Z jednej strony, przewiduje się, że n-BC może wykazywać wyższą toksyczność w stosunku do organizmów wodnych oraz

roślin w porównaniu do b-BC, co wynika z mniejszych rozmiarów cząstek i lepszej dyspersji w środowisku co było wcześniej obserwowane w przypadku innych materiałów węglowych (np. nanorurki węglowe, fulereny, itd.) [52]. Z drugiej strony, nanostrukturyzacja może prowadzić do obniżenia zawartości zanieczyszczeń natywnych (np. WWA, PTEs), co może wpływać na jego interakcje z organizmami w środowisku wodno-glebowym (*Publikacja D3*).

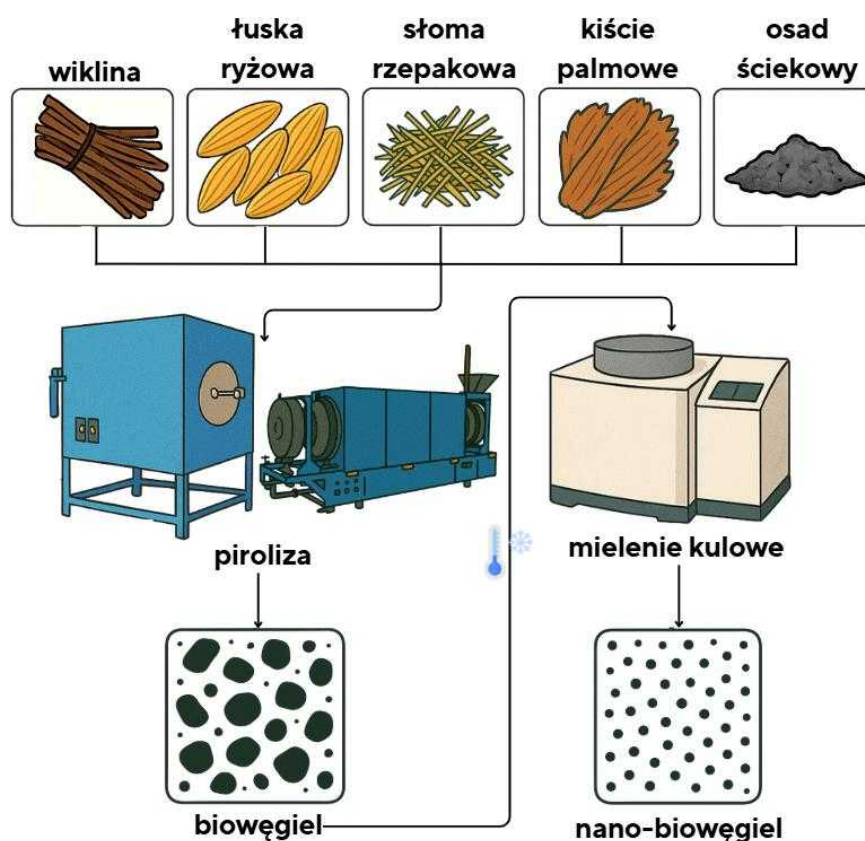
4. N-BC, dzięki zwiększonej powierzchni właściwej, będzie charakteryzował się wyższą efektywnością w usuwaniu wybranych zanieczyszczeń z roztworów wodnych, w tym PTEs (*Publikacja D4*). Przewiduje się również, że n-BC ze względu na wspomniane wcześniej właściwości będzie wykazywał lepsze zdolności do wiązania i immobilizacji zanieczyszczeń w różnych naturalnych (gleba) i antropogenicznych (osad ściekowych) matrycach środowiskowych, przyczyniając się do ograniczenia mobilności i biodostępności tych zanieczyszczeń. Dzięki takiemu działaniu możliwe będzie ponowne wykorzystanie obszarów zanieczyszczonych w celach rolniczych (np. uprawy roślin energetycznych), przy zmniejszonym ryzyku środowiskowym. Dodatkowo poprzez intensyfikację wykorzystania odpadów do środowiska zostaną zawrócone i ponownie wykorzystane składniki odżywcze i węgiel organiczny (*Publikacje D5–D7*).

Przedstawione powyżej cele badawcze ukierunkowane są na kompleksową ocenę potencjału n-BC jako innowacyjnego materiału środowiskowego. Realizacja badań pozwoli na lepsze zrozumienie mechanizmów odpowiedzialnych za właściwości n-BC i jego oddziaływanie z zanieczyszczeniami i innymi elementami środowiska, a także na określenie możliwości jego praktycznego zastosowania w ograniczaniu mobilności i biodostępności zanieczyszczeń. Otrzymane wyniki mogą przyczynić się do poprawy ochrony środowiska, wspierać działania w zakresie gospodarki o obiegu zamkniętym oraz realizować cele zrównoważonego rozwoju poprzez efektywne wykorzystanie odpadów. W dłuższej perspektywie korzyściami mogą być m.in. rekultywacja zanieczyszczonych terenów, zmniejszenie ryzyka środowiskowego oraz odzysk cennych składników odżywczych i węgla organicznego.

4. Opis koncepcji eksperymentalnej i zastosowanych metod badawczych

4.1. Preparatyka biowęgla i nano-biowęgla

W ramach rozprawy doktorskiej najpierw otrzymano dwanaście b-BC, z których następnie wytworzono n-BC. Szczegółowe informacje dotyczące rodzaju biomasy wykorzystanej do otrzymania BC oraz parametrów pirolizy przedstawiono w Tabeli 1. Na rysunku 5 przedstawiono schemat obrazujący etapy oraz kolejność otrzymywania b-BC, a następnie ich nanostrukturyzacji do postaci n-BC. Zastosowane surowce reprezentowały trzy główne grupy: biomasę drzewną (wiklina), odpady rolnicze (łuska ryżowa, słoma rzepakowa, kiście palmowe) oraz antropogeniczne bioodpady (osad ściekowy). Trzy spośród wszystkich wyprodukowanych b-BC uzyskano we współpracy z zespołem badawczym z UK Biochar Research w Szkocji (Tabela 1).



Rysunek 5. Poglądowy schemat kolejności i struktury procesu otrzymywania biowęgla (b-BC) oraz nano-biowęgla (n-BC).

Tabela 1. Szczegółowe parametry procesu otrzymywania biowęgla (b–BC) oraz miejsce pochodzenia surowca użytego do otrzymania b–BC.

Surowiec	Temperatura [°C]	Czas ogrzewania [min]	Narost temperaturowy [°C/min]	Przepływ gazu [L/min]	Gaz	Miejsce pochodzenia
wiklina	450	180	10	1	N ₂	Polska, Bezek
wiklina	550	180	10	1	N ₂	Polska, Bezek
wiklina	650	180	10	1	N ₂	Polska, Bezek
wiklina	750	180	10	1	N ₂	Polska, Bezek
łuska ryżowa	550	180	10	1	N ₂	Nigeria, Umudike
łuska ryżowa	550	15	98	10	N ₂	Szkocja, Edynburg
słoma rzepakowa	550	12	78	10	N ₂	Szkocja, Edynburg
kiście palmowe	550	180	10	1	N ₂	Nigeria, Umudike
osad ściekowy	550	13	86	10	N ₂	Szkocja, Edynburg
osad ściekowy	500	180	7	1	N ₂	Polska, Zamość
osad ściekowy	500	180	10	1	N ₂	Polska, Lublin
osad ściekowy	500	180	10	1	CO ₂	Polska, Lublin

B–BC otrzymano w poziomym piecu retortowym (Czyłok, Polska) lub obrotowym (Ansac Pty Ltd., Australia). W kolejnym etapie przygotowano n–BC poprzez mechaniczne rozdrobnienie uprzednio otrzymanych b–BC przy użyciu planetarnego młyna kulowego (PM100, Retsch GmbH, Niemcy). Przed mieleniem, b–BC mrożono przez 24 godziny w zamrażarce laboratoryjnej (–80 °C) w szczelnych, sterylnych pojemnikach przystosowanych do przechowywania w niskiej temperaturze. Próbkę b–BC (6 g) umieszczano następnie w naczyniu mielącym (125 ml) wykonanym z korundu wraz z siedmioma korundowymi kulami mielącymi o średnicy 20 mm. Stosunek masowy b–BC do kul wynosił 1:100. Mielenie prowadzono w temperaturze pokojowej ($T=22 \pm 2$ °C) przez 90 minut, przy prędkości 440 obrotów/min. W celu ograniczenia zwiększenia temperatury podczas procesu, kierunek obrotów zmieniano co 5 minut, stosując zaprogramowane przerwy.

4.2. Charakterystyka fizykochemiczna biowęgla i nano-biowęgla

W celu kompleksowej charakterystyki otrzymanych materiałów i oceny ich potencjału środowiskowego określono właściwości fizykochemiczne b-BC oraz n-BC, obejmujące parametry powierzchniowe i porowatość, wielkość cząstek, morfologię powierzchni, skład elementarny, grupy funkcyjne, pH i przewodnictwo elektryczne (EC), zawartość całkowitego i rozpuszczonego węgla organicznego (TOC i DOC) oraz potencjał zeta. Poniżej szczegółowo opisano metody zastosowane do oznaczenia powyższych właściwości. Szczegółowy opis poszczególnych metod przedstawiono w publikacjach stanowiących rozprawę doktorską.

— Charakterystyka powierzchniowa i porowatość

Odgazowane próbki b-BC i n-BC analizowano przy użyciu analizatora ASAP 2420 (Micromeritics, USA) metodą izoterm adsorpcji-desorpcji N₂ w niskiej temperaturze (77,3 K). Kluczowe parametry, takie jak powierzchnia właściwa (S_{BET}), powierzchnia mikroporów (S_{micro}), całkowita objętość porów (V_{p}), objętość mikroporów (V_{micro}) oraz średnia średnica porów, wyznaczano na podstawie danych z desorpcji azotu zgodnie z opisem podanym przez Michot i Villiéras [53].

— Pomiar wielkości cząstek

Średnicę hydrodynamiczną n-BC oznaczano za pomocą techniki dynamicznego rozpraszania światła (DLS) przy użyciu analizatora Zetasizer Nano ZS90 (Malvern Instrument Inc., UK) wyposażonego w uniwersalną sondę zanurzeniową. Średnicę hydrodynamiczną ważoną intensywnością obliczano, przyjmując równanie Stokesa-Einsteina dla cząstek sferycznych [54].

— Morfologia powierzchni

Morfologia b-BC/n-BC została określona za pomocą skaningowego mikroskopu elektronowego (Quanta 3D FEG, FEI, USA). Analizę wybranych próbek b-BC/n-BC z zaadsorbowanymi jonami Cd (II), Zn (II) lub Pb (II) przeprowadzono przy użyciu transmisyjnego mikroskopu elektronowego (TEM) Titan G2 60–300 kV (FEI Company) wyposażonego w emiter polowy (FEG), monochromator, układ trzech soczewek kondensora, detektor HAADF oraz spektrometr rentgenowski EDS. W celu zobrazowania przestrzennego rozkładu wybranych pierwiastków (C, Si, O, Cd, Zn, Pb) widma EDS zbierano punkt po punkcie w trybie STEM, uzyskując obrazy, na których różne kolory i intensywności odpowiadały stężeniom poszczególnych pierwiastków.

Identyfikację faz przeprowadzono metodą FFT z wykorzystaniem pakietu oprogramowania Gatan Digital Micrograph.

— Skład elementarny

Zawartość węgla (C), wodoru (H) i azotu (N), oznaczano przy użyciu analizatora CHN (Perkin–Elmer 2400, Akron, OH, USA). Zawartość popiołu określano zgodnie ze standardem ASTM D3176 [55]. Zawartość popiołu obliczano według równania (1):

$$\% \text{ Popiołu} = \frac{\text{masa po spopieleniu} - \text{masa tygła}}{\text{sucha masa próbki b-BC lub n-BC}} \cdot 100\% \quad (1)$$

Całkowitą zawartość tlenu (O) określano z wykorzystaniem równania (2):

$$\% O = 100\% - \% \text{ Popiół} - \%C - \%H - \%N \quad (2)$$

— Grupy funkcyjne

Grupy funkcyjne na powierzchni b-BC/n-BC oznaczano za pomocą widm spektroskopowych w podczerwieni z transformacją Fouriera (FTIR-ATR) w zakresie $400\text{--}4000\text{ cm}^{-1}$ z rozdzielczością 1 cm^{-1} (Nicolet 8700A, Thermo Scientific, USA) oraz spektroskopii fotoelektronów wzbudzanych promieniowaniem rentgenowskim (XPS) (system analityczny UHV Prevac, Polska).

— Wartości pH i przewodnictwa elektrycznego (EC)

Wartości pH oraz przewodnictwo elektryczne (EC) b-BC/n-BC oznaczano zgodnie z procedurą opisaną przez Kończak i in. [56]. Zawiesiny b-BC/n-BC (1 g b-BC/n-BC w 10 mL wody) wytrząsano przez 24 godziny na wytrząsarce orbitalnej (190 obrotów/min, $T = 22 \pm 2\text{ }^{\circ}\text{C}$) (Model 358 A, ELPINSC+, Polska). Pomiary pH oraz EC przeprowadzano za pomocą miernika cyfrowego (HQ430d Benchtop Single Input, HACH, USA lub CP-401, ELMETRON, Polska).

— Zawartość całkowitego węgla organicznego (TOC) oraz rozpuszczonego węgla organicznego (DOC)

Całkowitą zawartość węgla organicznego (TOC) w próbkach stałych oraz rozpuszczonego węgla organicznego (DOC) w ekstraktach wodnych oznaczano za pomocą analizatora TOC (TOC-VCSH, SHIMADZU, Japonia) wyposażonego w moduł do próbek stałych (SSM-5000). Ekstrakty wodne przygotowywano zgodnie z procedurą opisaną wcześniej [56].

— Potencjał zeta

Potencjał zeta zawiesin b-BC/n-BC wyznaczano na podstawie pomiarów mobilności elektroforetycznej oraz równania Smoluchowskiego (Zetasizer Nano ZS, Malvern Instruments, Wielka Brytania) [57]. Zawiesiny b-BC/n-BC o stężeniu $100 \text{ mg}\cdot\text{L}^{-1}$ w roztworze elektrolitu tła ($0,001 \text{ M NaCl}$) i dyspergowano przy pomocy ultradźwięków (VCX 130, Sonics, USA) przez 1 minutę ($T=22 \pm 2 \text{ }^{\circ}\text{C}$). Następnie potencjał zeta mierzono w zakresie pH od 2 do 10, regulując pH dodając $0,1 \text{ M HCl}$ lub NaOH .

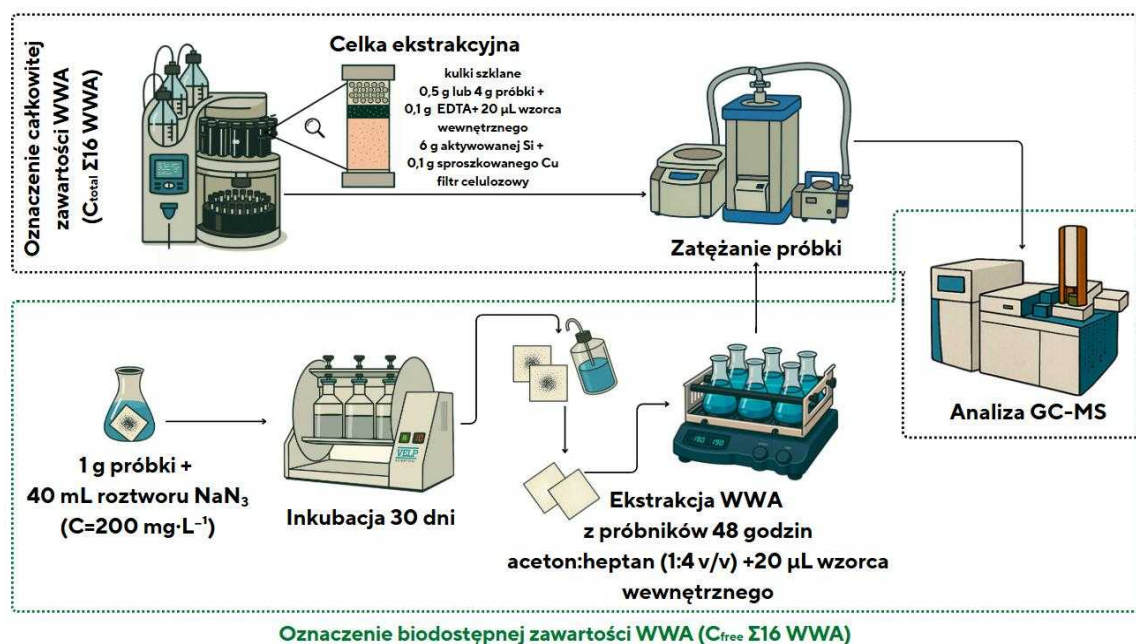
4.3. Oznaczenie pseudo-całkowitej i biodostępnej frakcji pierwiastków potencjalnie toksycznych

W celu określenia pseudo-całkowitej zawartości PTEs w badanych próbkach, 200 mg b-BC/n-BC /gleby lub 500 mg liści *Lactuca sativa L.*, mineralizowano w systemie mikrofalowym przy użyciu mieszaniny HNO_3 i HCl w stosunku 1:3 (v/v) przez 1,5 godziny w temperaturze 200°C (Start D, Microwave Digestion System, Milestone, Włochy). Otrzymane roztwory pozostawiono do ostygnięcia do temperatury pokojowej, a następnie filtrowano a otrzymany filtrat przenoszono ilościowo do kolby miarowej o pojemności 50 mL i uzupełniono wodą Milli-Q do kreski.

Frakcję biodostępną PTEs w badanych matrycach oznaczano w roztworze po ekstrakcji próbek przy pomocy wody Milli-Q lub roztworu chlorku wapnia (CaCl_2) o stężeniu $0,01 \text{ mol}\cdot\text{L}^{-1}$. Zawiesinę wytrząsano na wytrząsarce orbitalnej (Model 358 A, ELPINSC+, Polska) przez 24 godziny, po czym roztwory przepuszczano przez filtry strzykawkowe PTFE ($0,45 \text{ }\mu\text{m}$) (AlfaChem, Polska). Przed analizą ilościową i jakościową roztwory zakwaszono $1\% \text{ HNO}_3$ (v/v). Do analizy ilościowej stosowano spektrometr emisyjny ze wzbudzeniem w plazmie indukcyjnie sprzężonej (ICP-OES iCAP7400, Thermo Scientific, USA). Do wzbudzenia plazmy oraz nebulizacji próbek stosowano argon o wysokiej czystości ($99,9999\%$, AirLiquide, Polska). Oznaczenia zawartości PTEs przeprowadzano przy użyciu krzywej kalibracyjnej w zakresie od $0,001$ do $5 \text{ mg}\cdot\text{L}^{-1}$. Roztwory do krzywej kalibracyjnej przygotowano poprzez rozcieńczenie wielopierwiastkowego roztworu wzorcowego o stężeniu $1\,000 \text{ mg}\cdot\text{L}^{-1}$ (Centipur, Merck, Niemcy) wodą MilliQ, a następnie zakwaszono $1\% \text{ HNO}_3$ (v/v).

4.4. Oznaczenie całkowitej i biodostępnej frakcji wielopierścieniowych węglowodorów aromatycznych (WWA)

Całkowite (ekstrahowane rozpuszczalnikami organicznymi) stężenie WWA ($C_{\text{total}} \Sigma 16$ WWA) w badanych próbkach oznaczano w ekstrakcie uzyskanym przy użyciu przyspieszonego systemu ekstrakcji rozpuszczalnikowej (Dionex ASE 350, ThermoFisher Scientific, USA) [58]. Ekstrakcję prowadzono, przy użyciu n-heksanu w temperaturze 150 °C i pod ciśnieniem 14 MPa, w dwóch cyklach po 5 minut każdy. Po każdym cyklu stosowano płukanie przez 1 minutę przy przepływie 60% objętości komory pod ciśnieniem 1 MPa N_2 . Zawartość swobodnie rozpuszczonych (biodostępnych) WWA ($C_{\text{free}} \Sigma 16$ WWA) oznaczano przy wykorzystaniu pasywnych próbników wykonanych z polioksymetyleny (POM) [59]. Szczegóły dotyczące poszczególnych procedur przedstawiono na rysunku 6 oraz w publikacjach stanowiących element niniejszej rozprawy (Publikacje D2, D5, D7).



Rysunek 6. Poglądowy schemat oznaczania frakcji całkowitej i biodostępnej WWA.

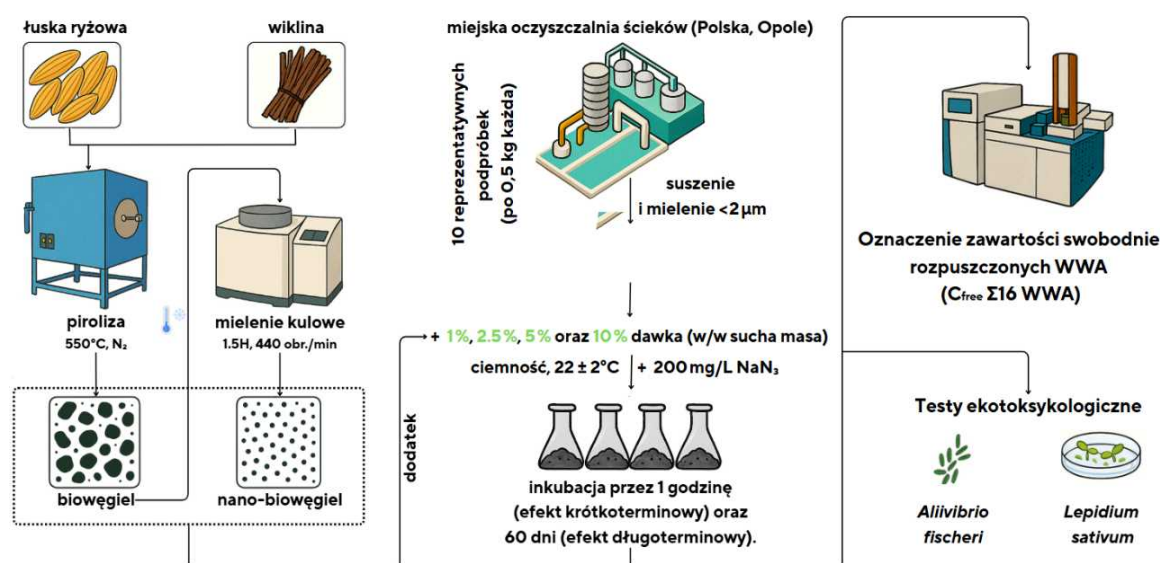
4.5. Określenie kinetyki adsorpcji oraz pojemności sorpcyjnej biowęgli i nano-biowęgli

Roztwory robocze o stężeniu $2\,000 \text{ mg} \cdot \text{L}^{-1}$ jonów Cd (II), Zn (II) i Pb (II) przygotowano poprzez rozpuszczenie soli $(\text{Cd}(\text{NO}_3)_2 \cdot 4 \text{ H}_2\text{O})$, $\text{Zn}(\text{NO}_3)_2 \cdot 6 \text{ H}_2\text{O}$ i $\text{Pb}(\text{NO}_3)_2$ (POCH, Gliwice, Polska) w wodzie Milli-Q. Roztwory te wykorzystano następnie do badań nad kinetyką adsorpcji i do przygotowania izoterm adsorpcji.

Szczegóły dotyczące całej procedury zostały przedstawione i opisane szczegółowo w *Publikacji D4*.

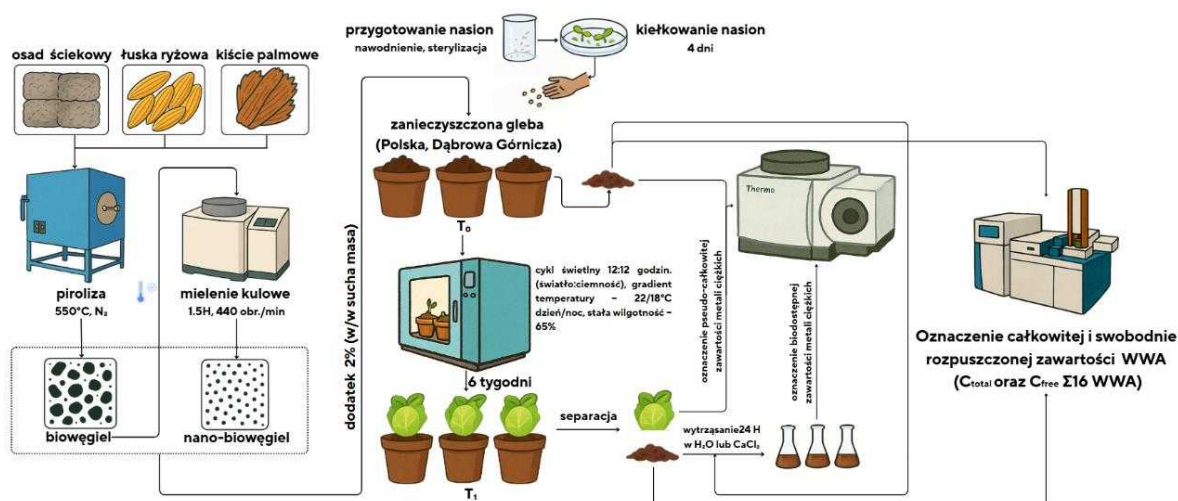
4.6. Immobilizacja zanieczyszczeń w glebie i osadzie ściekowym

Ze względu na odmienny charakter gleby i osadu ściekowego, eksperymenty wazonowe zostały przeprowadzone z zastosowaniem dostosowanych protokołów przygotowania próbek, mieszania oraz inkubacji, co umożliwiło uzyskanie wiarygodnych i porównywalnych wyników dla obu matryc.



Rysunek 7. Schemat przebiegu doświadczenia z wykorzystaniem osadu ściekowego oraz dodatku wybranych b-BC/n-BC.

Przebieg eksperymentu z osadem ściekowym przedstawiono na rysunku 7. Osad ściekowy mielono w młynku laboratoryjnym (<2µm) (TESTCHEM, Polska), a następnie dodano do niego 1%; 2,5%; 5% lub 10% b-BC lub n-BC (w/w, w przeliczeniu na suchą masę) [60]. Osad ściekowy bez dodatku stanowił kontrolę. Mieszaniny wytrząsano wstępnie przez 2 godziny na wytrząsarce orbitalnej (Model 358 A, ELPINSC+, Polska), a następnie do każdej próbki dodano 2 mL roztworu azydki sodu (200 mg·L⁻¹) w celu zahamowania aktywności mikrobiologicznej i wyeliminowania wpływu procesów biodegradacji na zawartość C_{total} oraz C_{free} Σ16 WWA. Uzyskaną zawiesinę dokładnie homogenizowano przy użyciu szklanej bagietki. Przygotowane mieszaniny umieszczano na wytrząsarce obrotowej (Rotax 6.8, VELP, Włochy) i wytrząsano (10 obrotów/min) przez 1 godzinę (w celu oceny efektu krótkoterminowego) oraz przez 60 dni (w celu oceny efektu długoterminowego). Wytrząsanie prowadzono w ciemności w temperaturze pokojowej (T= 24 ± 2 °C).



Rysunek 8. Schemat przebiegu doświadczenia wazonowego z wykorzystaniem gleby oraz dodatku wybranych b-BC/n-BC.

Eksperyment z wykorzystaniem gleby przeprowadzono w sposób przedstawiony na rysunku 8. B-BC lub n-BC dodawano do gleby w dawce 2% (w/w, w przeliczeniu na suchą masę), następnie homogenizowano dokładnie mieszając. Otrzymaną mieszaninę przenoszono do wazonów o pojemności 500 mL i na tak przygotowanym podłożu posadzono wcześniej otrzymane siewki sałaty (*Lactuca sativa L.*). Szczegółowy opis przygotowania nasion oraz przebiegu doświadczenia został przedstawiony w *Publikacji D6*. Po sześciu tygodniach liście i korzenie sałaty zebrano w celu analizy translokacji zanieczyszczeń. Próbkę gleby również zostały pobrane do dalszych analiz.

4.7. Testy ekotoksykologiczne

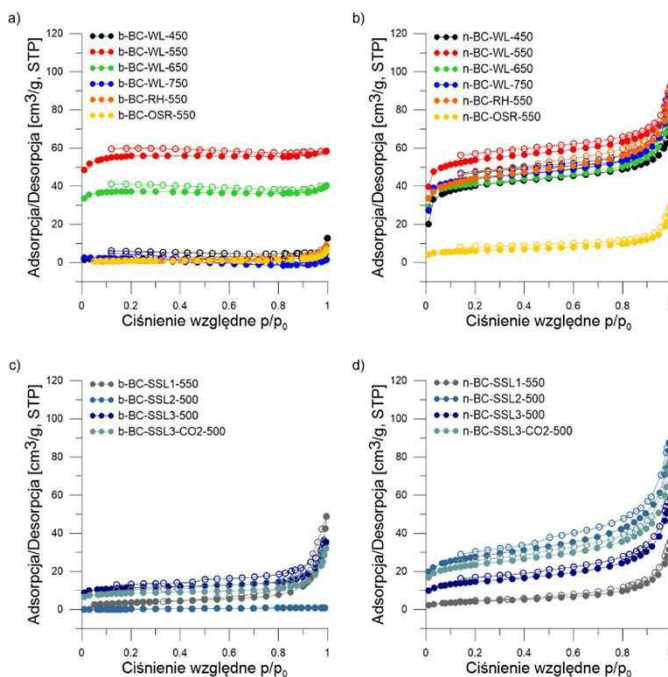
Wpływ b-BC/n-BC lub ich dodatków do matrycy środowiskowych na organizmy testowe oceniano przy użyciu testów ekotoksykologicznych fazy ciekłej lub stałej. Testy w fazie ciekłej badają wpływ odcieków (ekstraktów wodnych) uzyskanych z próbek na organizmy (głównie wodne) i mają charakter pośredni, ponieważ ocenie podlega jedynie część substancji chemicznych obecna w matrycy. Natomiast testy w fazie stałej oceniają bezpośredni wpływ całego materiału na organizmy testowe, przy czym odzwierciedlają one zmieniające się w czasie warunki środowiskowe. W celu oceny i porównania ekotoksyczności zastosowano pięć testów ekotoksykologicznych, które szerzej opisane zostały w *Publikacji D3* i *Publikacji D5*, i obejmowały następujące grupy organizmów *Aliivibrio fischeri* (Microtox®) [61], *Daphnia magna* [62, 63], *Folsomia candida* [64], *Lemna minor* [65] oraz *Lepidium sativum* [66]. Odcieki do testów w fazie ciekłej przygotowano zgodnie z protokołem EN 12457-2 (2002) [56].

5. Badania własne

5.1. Wpływ warunków pirolizy i rodzaju surowca na właściwości fizykochemiczne nano-biowęgla (*Publikacja D1*)

Celem pracy było określenie wpływu nanostrukturyzacji metodą mielenia kulowego na właściwości otrzymanych n-BC, wytworzonych z różnych surowców w zmiennych warunkach pirolizy (temperatura, gaz nośny). Na podstawie posiadanych danych starano się wskazać zależności, na podstawie których możliwe by było prognozowanie właściwości uzyskiwanych n-BC na podstawie kompleksowej charakterystyki b-BC. Opracowanie zależności między właściwościami wyjściowego b-BC a właściwościami uzyskiwanych z niego n-BC może stanowić podstawę do projektowania materiałów o określonych parametrach, dedykowanych do specyficznych zastosowań środowiskowych.

Wykazano, że rodzaj surowca miał największy wpływ na właściwości n-BC, następnie temperatura pirolizy i zastosowany gaz nośny. Proces mielenia prowadził do istotnego zwiększenia powierzchni właściwej n-BC (nawet o 4 160% względem b-BC), co zwiększa potencjał sorpcyjny materiału, szczególnie w przypadku n-BC otrzymanego z WL i RH (**Rys. 9**). Należy zaznaczyć, że efekt zwiększenia pola powierzchni był szczególnie widoczny dla n-BC, których odpowiedniki b-BC charakteryzowały się niskim polem powierzchni ($< 40 \text{ m}^2 \cdot \text{g}^{-1}$). Wzrost ten nie był jednak znaczący dla n-BC o wysokim polu powierzchni ($> 120 \text{ m}^2 \cdot \text{g}^{-1}$). Zaobserwowano także wyższą porowatość dla n-BC, która może wynikać z agregacji cząstek n-BC, a nie z rozwoju nowych porów. Analiza elementarna wykazała, że zawartość węgla (C), obniżyła się o 27–55% po redukcji rozmiaru cząstek n-BC otrzymanych



Rysunek 9. Izotermy adsorpcji/desorpcji azotu otrzymanych biowęgla (a i c) i nano-biowęgla (b i d).

z biomasy roślinnej (WL, RH OSR) oraz zwiększyła się o 9–15% w przypadku n-BC uzyskanych z odpadów antropogenicznych (SSL 1–3). W przypadku azotu (N) tendencja zależała głównie od surowca. Po zmniejszeniu rozmiaru, n-BC otrzymane z pozostałości rolniczych (RH i OSR), a także antropogenicznych odpadów (SSL 1–3) charakteryzowały się o 10–27% wyższą zawartością tego pierwiastka niż odpowiadające im b-BC. Wyjątek zaobserwowano dla n-BC otrzymanego z biomasy drzewnej (WL), w przypadku, którego odnotowano 59% obniżenie zawartości azotu (N). Redukcja rozmiaru do skali nano skutkowała obniżeniem zawartości tlenu (O) i wodoru (H) dla wszystkich badanych n-BC, podczas gdy zawartość popiołu uległa zwiększeniu w stosunku do b-BC. Efekt ten wyraźniej zaznaczał się w przypadku n-BC otrzymanych z biomasy roślinnej (WL, RH i OSR) niż w przypadku n-BC pochodzących z odpadów antropogenicznych (SSL 1–3).

Zmiana gazu nośnego z N_2 na CO_2 , nie zmieniła trendu zmian właściwości fizykochemicznych n-BC. Natomiast w przypadku zmiany temperatury, zaobserwowano pewne zróżnicowanie. Najmniejszy wpływ na skład pierwiastkowy po nanostrukturyzacji obserwowano dla n-BC otrzymanego w najniższej temperaturze (450 °C). Można to przypisać utrudnionemu rozrywaniu wiązań w strukturze BC, które słabną wraz ze wzrostem temperatury pirolizy [67]. Stosunki H/C i O/C świadczące odpowiednio o aromatycznym charakterze BC oraz ich polarności nie zmieniły charakteru bio-geochemicznego n-BC, który pozostał zbliżony do b-BC (**Publikacja D1, Rys. 2**). Wraz z obniżeniem wielkości cząstek stosunek H/C malał, a O/C zwiększał się, co wskazuje na większą aromatyczność oraz polarność n-BC w porównaniu do ich odpowiedników b-BC, co może wpływać na inne zachowanie się tych materiałów w środowisku, jak i oddziaływanie z zanieczyszczeniami w porównaniu do b-BC. Proces nanostrukturyzacji wpływał także na (1) morfologię materiału, prowadząc do zmiany struktury z cząstek włóknistych na zaokrąglone aglomeraty (**Publikacja D1, Rys. S1**), oraz (2) na rozmiar cząstek, który wynosił od 30 do 88 nm w zależności od zastosowanego surowca i warunków pirolizy (**Publikacja D1, Tabela 3**). Wyniki analiz FT-IR i Raman potwierdziły zmiany w strukturze chemicznej oraz obecności grup funkcyjnych w n-BC w stosunku do b-BC, co również może wpływać na ich właściwości sorpcyjne i środowiskowe (**Publikacja D1, Rys. 3–4**). Obserwowano zmiany w potencjale zeta, wskazujące na różnice w ładunku powierzchniowym w zależności od pH i składu powierzchni BC. Uzyskane wyniki wskazują, że nanostrukturyzacja prowadzi

do obniżenia wartości potencjału zeta w stosunku do b-BC, co podkreśla większe znaczenie powierzchniowych grup funkcyjnych i związków mineralnych w kształtowaniu właściwości elektrokinetycznych n-BC. Niezależnie od rodzaju surowca, n-BC charakteryzowały się większą liczbą miejsc aktywnych, co wynikało ze zwiększonej powierzchni właściwej (S_{BET}) i liczby mikroporów (V_{micro}). Wszystkie testowane b-BC były silnie zasadowe ($\text{pH} > 8$), a ich nanostrukturyzacja do n-BC znacząco obniżyła wartości pH, co może być związane ze stopniową utratą zasadowych grup funkcyjnych na powierzchni BC i/lub ekspozycją w wyniku mielenia kulowego. Obniżenie rozmiaru cząstek BC spowodowała zwiększenie EC, co z jednej strony może ograniczać zastosowanie większych dawek n-BC jako dodatku do gleby ze względu na prawdopodobieństwo zwiększenia poziomu zasolenia gleby oraz wpływać negatywnie na niektóre organizmy glebowe i wodne. Z drugiej natomiast strony może zwiększać dostępność składników pokarmowych i wpływać na retencję wody oraz jonów w glebie.

Podsumowując, zastosowanie nanostrukturyzacji przedstawioną metodą umożliwia kontrolowane kształtowanie właściwości n-BC, co pozwala na dostosowanie materiału do zastosowań środowiskowych, w tym usuwania zanieczyszczeń z wód i gleb, przy jednoczesnym uwzględnieniu ograniczeń związanych z zasoleniem i potencjalną toksycznością materiału. Badania wykazały, że właściwości n-BC zależą w znacznym stopniu od właściwości b-BC. O ile na podstawie uzyskanych wyników możliwe jest przewidywanie kierunku zmian poszczególnych właściwości (lub ich braku), to na obecnym etapie nie jest jeszcze możliwe oszacowanie ich skali ilościowej. Wymaga to dalszych analiz oraz dopracowania metod prognozowania w oparciu o sztuczną inteligencję. Wyniki dotychczasowych analiz są jednak obiecujące i wskazują na potencjał dalszych badań, tym bardziej że są one jednymi z pierwszych, które w sposób systematyczny podjęły tę problematykę.

5.2. Wpływ redukcji rozmiaru do skali nano na zawartość oraz biodostępność wybranych zanieczyszczeń w nano-biowęglu ***(Publikacja D2)***

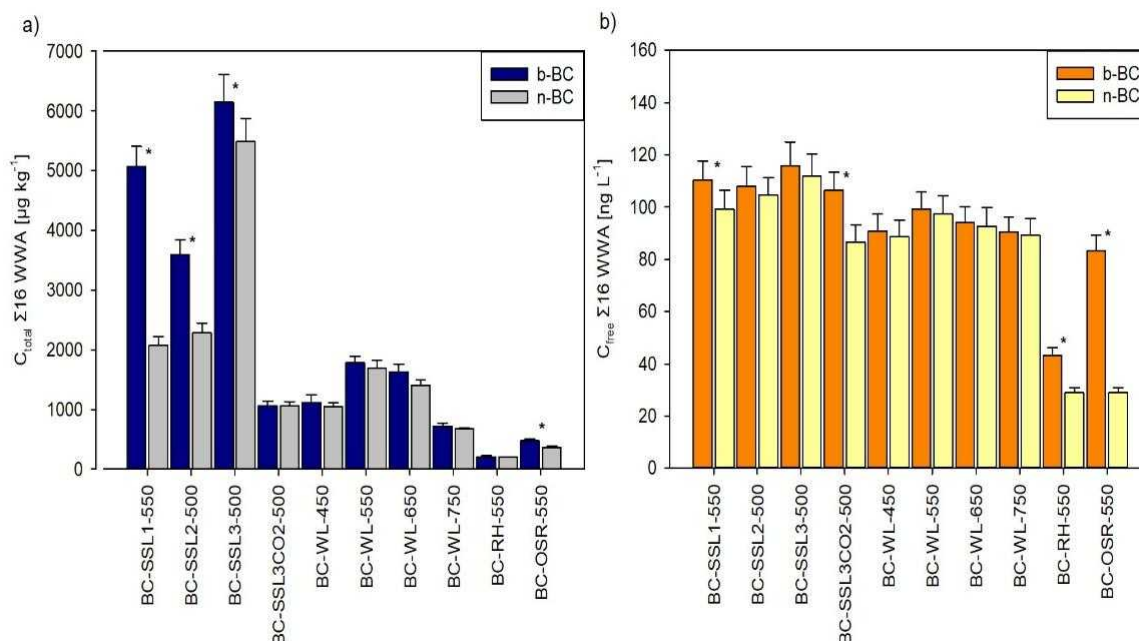
Celem pracy było określenie wpływu nanostrukturyzacji na zawartość i biodostępność WWA oraz PTEs w n-BC otrzymanych z różnych surowców w zmiennych warunkach pirolizy (temperatura, gaz nośny). Podobnie jak we wcześniejszych badaniach, starano się ustalić, czy możliwe jest prognozowanie zmian wspomnianych parametrów na

podstawie kompleksowej charakterystyki wyjściowych b-BC oraz opracowanie zależności pozwalających na projektowanie n-BC dedykowanych do bezpiecznych zastosowań środowiskowych o obniżonej zawartości zanieczyszczeń.

W ramach badań wykazano, że proces mielenia prowadzi, ale w przypadku tylko niektórych n-BC, do istotnego ($p \leq 0,05$) obniżenia $C_{\text{total}} \Sigma 16$ WWA. W odniesieniu do rodzaju materiału wyjściowego, największe istotne statystycznie ($p \leq 0,05$) obniżenie $C_{\text{total}} \Sigma 16$ WWA po nanostrukturyzacji odnotowano dla n-BC otrzymanych z SSL. W tym przypadku zawartość $C_{\text{total}} \Sigma 16$ WWA obniżyła się od 11–59%, w zależności od rodzaju osadu (1–3) (**Rys. 10**). Dla n-BC wytworzonych z materiałów roślinnych (WL, RH i OSR), istotny spadek zawartości $C_{\text{total}} \Sigma 16$ WWA zaobserwowano jedynie w n-BC otrzymanym z OSR (24%). Temperatura pirolizy (450–750 °C), jak i zastosowany gaz nośny (N_2 lub CO_2) nie miały istotnego wpływu ($p > 0,05$) na finalną zawartość $C_{\text{total}} \Sigma 16$ WWA w n-BC. Zastosowanie CO_2 jako gazu nośnego podczas pirolizy okazało się wystarczająco skuteczne w ograniczaniu zawartości $C_{\text{total}} \Sigma 16$ WWA w b-BC, przez co nanostrukturyzacja nie prowadziła już do istotnych zmian ich zawartości w n-BC. Należy podkreślić, że po procesie mielenia kulowego utrzymała się zależność zawartości $C_{\text{total}} \Sigma 16$ WWA od temperatury pirolizy, wcześniej zaobserwowana w b-BC. Może to stanowić podstawę do prognozowania zawartości $C_{\text{total}} \Sigma 16$ WWA w n-BC wytwarzanym w różnych temperaturach na podstawie wartości początkowych określonych dla b-BC.

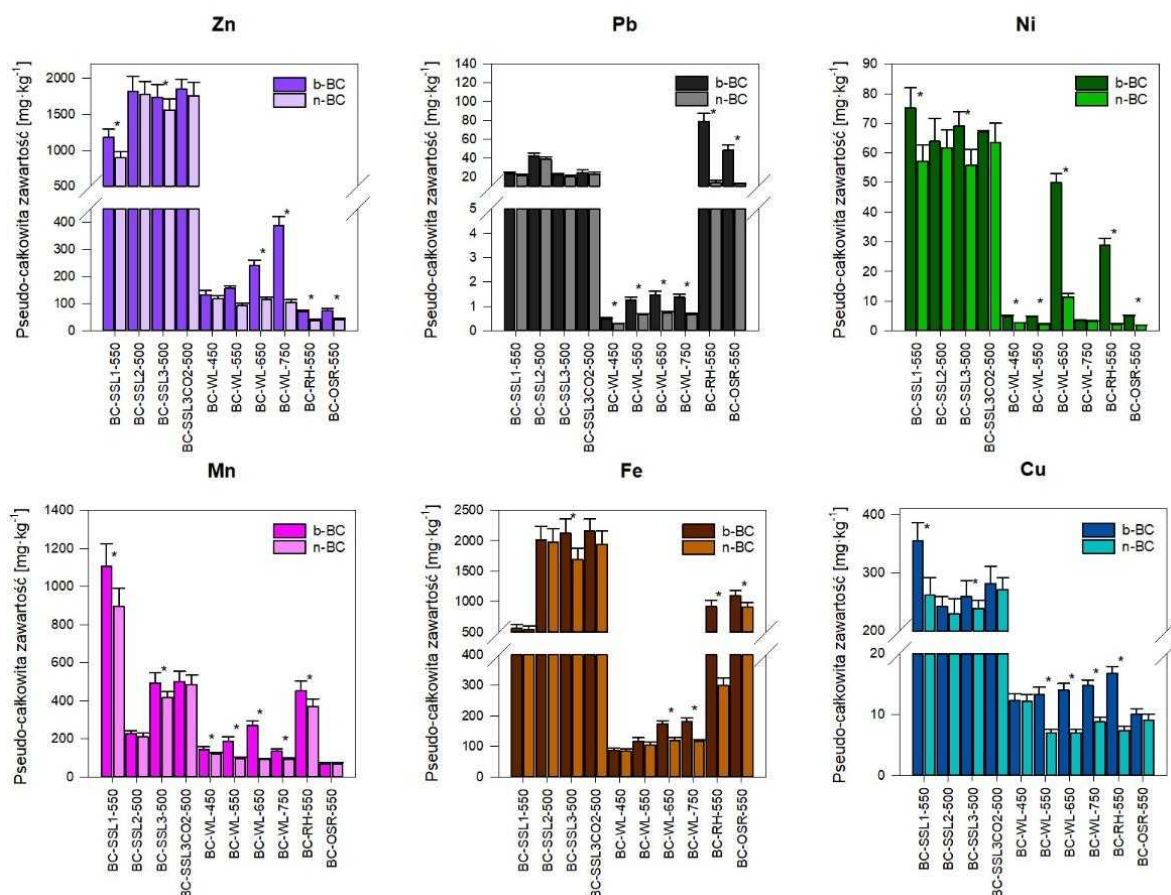
Zawartość $C_{\text{free}} \Sigma 16$ WWA uległa również istotnemu obniżeniu dla wybranych n-BC. Co ciekawe jednak zmiany te dotyczyły w większości innych n-BC niż obserwowano to w odniesieniu do $C_{\text{total}} \Sigma 16$ WWA. Porównując zawartość $C_{\text{free}} \Sigma 16$ WWA, w n-BC otrzymanym z różnych materiałów wyjściowych, proces nanostrukturyzacji spowodował istotne statystycznie ($p \leq 0,05$) obniżenie (10–65%) zawartości $C_{\text{free}} \Sigma 16$ WWA dla n-BC otrzymanych z SSL, RH i OSR. Nie stwierdzono również istotnego wpływu temperatury na kierunek i zakres zmian zawartości $C_{\text{free}} \Sigma 16$ WWA. Zmiana gazu nośnego z N_2 na CO_2 wpłynęła na zawartości $C_{\text{free}} \Sigma 16$ WWA w n-BC. W przypadku n-BC otrzymanych w atmosferze CO_2 mielenie istotnie ($p \leq 0,05$) obniżyło zawartość $C_{\text{free}} \Sigma 16$ WWA o 19%, podczas gdy dla n-BC wytwarzanych w N_2 taki efekt nie wystąpił ($p > 0,05$). Wskazuje to, że podczas pirolizy w atmosferze CO_2 nie tylko zmieniają się właściwości otrzymywanego b-BC w stosunku do prowadzenia pirolizy w atmosferze N_2 , ale także

obecność CO₂ ma istotny wpływ na „zachowanie” i trwałość WWA w tym materiale. Obserwowane zamiany wynikały ze zmniejszenia aromatyczności powierzchni n-BC (wyrażonej wyższymi wartościami stosunku O/C), co ograniczało zdolność sorpcyjną materiału wobec WWA (**Publikacja D2, Tabela S3**). Ponadto, zaobserwowano zmiany w profilu C_{free} Σ16 WWA, gdzie następowało zwiększenie udziału związków 2- i 3-pierścieniowych przy jednoczesnym obniżeniu udziału 5- i 6-pierścieniowych, co może wskazywać na degradację mechano-chemiczną zachodzącą podczas mielenia oraz reakcje wolnorodnikowe sprzyjające rozkładowi cząsteczek WWA [68, 69]. Nie odnotowano przy tym wyraźnych zależności względem rodzaju surowca użytego do otrzymania BC ani warunków pirolizy. Zmniejszenie rozmiaru b-BC do formy nano wpływało w sposób zróżnicowany na wartości *log* K_d badanych WWA. W przypadku niektórych związków (np. naftalen) proces mielenia sprzyjał zwiększeniu współczynnika *log* K_d, a zatem jego powinowactwa do n-BC. Z kolei dla związków cięższych, takich jak benzo[a]piren, obserwowano obniżenie się tego parametru, co sugeruje obniżenie powinowactwa do tych WWA (**Publikacja D2, Rys. 5**). Obserwowane różnice między poszczególnymi WWA w kierunku zmian wartości *log* K_d, może wynikać ze zwiększenia hydrofilowości powierzchni n-BC po mieleniu kulowym, co szczególnie ma wpływ na bardziej hydrofobową cząsteczkę benzo[a]pirenu niż mniej hydrofobową cząsteczkę naftalenu. Dodatkowo, zaobserwowany efekt może być powiązany z obecnością grup funkcyjnych na powierzchni BC, które po procesie nanostrukturyzacji stają się bardziej dostępne dla mniejszych cząsteczek, takich jak naftalen. Ich rozmiary sprzyjają łatwiejszemu dostępowi do centrów sorpcyjnych i umożliwiają efektywniejsze tworzenie oddziaływań z powierzchniowymi grupami funkcyjnymi. W przeciwieństwie do tego, większe i bardziej rozbudowane strukturalnie cząsteczki, takie jak benzo[a]piren, mogą doświadczać ograniczeń sterycznych, które redukują ich zdolność do bezpośredniego oddziaływania z tymi miejscami wiązania.



Rysunek 10. Całkowita (C_{total} $\Sigma 16$ WWA) (a) oraz biodostępna (C_{free} $\Sigma 16$ WWA) zawartość $\Sigma 16$ WWA (b) w b-BC i n-BC, gdzie * oznacza statystycznie istotną zmianę ($p \leq 0,05$). Słupki błędów przedstawiają odchylenie standardowe średniej ($n=3$).

Na rysunku 11 przedstawiono wpływ mielenia kulowego na zawartości PTEs w n-BC. Zaobserwowano, że mielenie obniżało istotnie ich zawartość, szczególnie w n-BC uzyskanych z biomasy roślinnej (WL, RH i OSR), gdzie obniżenie ich zawartości wahało się od 11–92% w zależności od rodzaju PTEs i surowca użytego do pirolizy. Analizując wpływ temperatury pirolizy, zaobserwowano, że największe obniżenie zawartości PTEs (31–77%) wystąpiło w przypadku n-BC wytwarzanego z WL w temperaturze 650 °C. Obniżenie zawartości PTEs można wiązać z usuwaniem słabiej związanych form mineralnych oraz modyfikacją struktury i właściwości powierzchniowych n-BC, które sprzyjają ich desorpcji z matrycy węglowej, a także z adsorpcją PTEs na powierzchni kul mielących, co dodatkowo ograniczało ich zawartość w uzyskanym materiale. Powyższe przypuszczenia wymagają jednak dodatkowych badań. Mimo wszystko jednak wskazują ewidentnie, że proces mielenia kulowego znacząco obniża zawartość PTEs w otrzymanym n-BC. Na uwagę zasługuje również brak obecności jonów metali w ekstrakcie wodnym otrzymanym z n-BC i b-BC. Brak wymywania się jonów PTEs sugeruje, że proces mielenia kulowego nie zwiększa ich mobilności, eliminując ryzyko ich uwolnienia do środowiska w trakcie i po zastosowaniu n-BC (**Publikacja D2, Tabela S8**).



Rysunek 11. Pseudo-calkowita zawartość wybranych pierwiastków potencjalnie toksycznych [$\text{mg} \cdot \text{kg}^{-1}$] w b-BC i n-BC, gdzie * oznacza istotną statystycznie zmianę ($p \leq 0,05$). Słupki błędów przedstawiają odchylenie standardowe średniej ($n=5$).

Podsumowując przedstawione powyżej informacje można stwierdzić, że nanostrukturyzacja metodą mielenia kulowego sprzyja obniżaniu zawartości i biodostępności WWA oraz PTEs w otrzymanych n-BC, co znacząco zwiększa potencjał n-BC do zastosowań środowiskowych, przy jednoczesnym ograniczeniu ryzyka wtórnej emisji zanieczyszczeń. Proces mielenia kulowego nie tylko zmniejsza rozmiar cząstek do skali nano, ale dodatkowo sprzyja preferencyjnemu usuwaniu lub degradacji WWA o wyższej liczbie pierścieni oraz znacząco obniża zawartość PTEs, szczególnie w n-BC z biomasy roślinnej. Ważnym podkreślenia jest również fakt, że w wyniku mielenia nie dochodzi do ułatwienia uwalniania jonów PTEs do fazy wodnej.

5.3. Porównanie profilu ekotoksykologicznego biowęgla i nano-biowęgla w różnych układach środowiskowych (*Publikacja D3*)

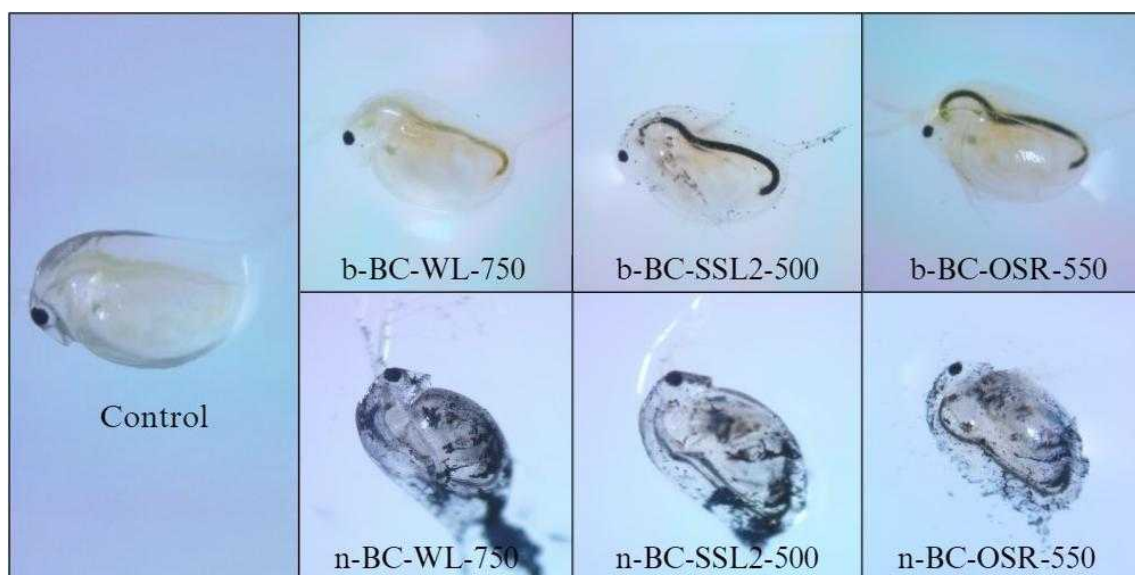
Celem pracy była ocena toksyczności n-BC w stosunku do zróżnicowanej grupy organizmów wodnych i glebowych reprezentujących zróżnicowany profil troficzny uwzględniający bakterie (*Aliivibrio fischeri*), rośliny (*Lemna minor*, *Lepidium sativum*) oraz bezkręgowce (*Daphnia magna*, *Folsomia candida*). Zastosowanie organizmów należące do różnych grup troficznych, pozwala uzyskać kompleksową informację na temat potencjalnych zagrożeń związanych z wprowadzaniem n-BC do środowiska. Zrozumienie interakcji między organizmami pochodzących z różnych poziomów troficznych jest również istotne dla przewidywania wpływu n-BC na środowisko w ujęciu całościowym, a analiza tych interakcji może pokazać zmiany w dynamice ekosystemów oraz potencjalne efekty kaskadowe wynikające z obecności n-BC.

Zmniejszenie rozmiaru do skali nano znacząco zwiększyło toksyczność n-BC wobec *Aliivibrio fischeri*, szczególnie dla n-BC otrzymanych z RH i SSL, co wynikało z większej aktywności powierzchniowej oraz niższego pH tych n-BC. Redukcja rozmiaru w przypadku n-BC otrzymanych w atmosferze CO₂ spowodowała 30-krotny wzrost toksyczności w porównaniu do jego odpowiednika b-BC. Należy również podkreślić, że n-BC wykazywały podobne trendy zmian toksyczności w zależności od warunków pirolizy jak b-BC, co podkreśla, że rodzaj surowca, temperatura pirolizy i gaz nośny mają istotny wpływ na ekotoksyczność n-BC.

Testy z wykorzystaniem *Lemna minor* wykazały, że dla wszystkich testowanych stężeń b-BC/n-BC średnie tempo wzrostu (μ_i) było zbliżone do kontroli, co wskazuje na brak wpływu redukcji rozmiaru na wzrost biomasy. Wyjątkiem były jedynie niektóre n-BC z SSL oraz odpadów rolniczych (RH i OSR), gdzie przy najwyższym stężeniu (100 mg·L⁻¹) odnotowano stymulację tempa wzrostu frondów (o 20–44%) w porównaniu do kontroli, co sugeruje na pozytywny wpływ n-BC na wzrost tych roślin wynikający z większej dostępności składników odżywczych w n-BC. W przypadku n-BC dodanym do roztworu w najwyższym stężeniu nastąpił statystycznie istotny ($p \leq 0.05$) wzrost powierzchni frondów po 7 dniach, który był szczególnie widoczny dla n-BC otrzymanych z SSL i odpadów rolniczych (RH i OSR). Podobnego efektu nie obserwowano natomiast dla b-BC, które nie wpływały istotnie na powierzchnię frondów w stosunku do kontroli. Różnice między b-BC były niewielkie w zależności od surowca

i zastosowanych stężeń, podczas gdy wzrost powierzchni frondów dla n-BC był determinowany tymi parametrami. Może to sugerować, że mniejszy rozmiar cząstek sprzyja uwalnianiu substancji stymulujących wzrost roślin, co potwierdza istotnie statystycznie korelacja między zawartością popiołu i azotu (N) w n-BC a tempem wzrostu *L. minor* przy najwyższych stężeniach.

Szczególnie interesujące wyniki uzyskano dla *Daphnia magna*, w przypadku których wstępna ocena nie wykazała negatywnego wpływu zarówno b-BC, jak i n-BC na te organizmy. Na podstawie obserwacji mikroskopowych, zaobserwowano wyraźne „oblepianie” *D. magna* przez cząstki n-BC, czego nie obserwowano w przypadku b-BC (**Rys. 12**). Takie zachowanie n-BC może prowadzić do wystąpienia toksyczności chronicznej. Przeprowadzone testy toksyczności chronicznej polegającej na ocenie zdolności do reprodukcji *D. magna* potwierdziły pierwotne przypuszczenia. Wykazano, że zarówno n-BC, jak i b-BC ograniczały reprodukcję *D. magna*, przy czym n-BC powodował istotnie większe ograniczenia niż b-BC już przy wyższych zastosowanych stężeniach ($>10 \text{ mg}\cdot\text{L}^{-1}$). Ponadto w przypadku n-BC efekt toksyczny pojawiał się już przy najniższym stężeniu ($2 \text{ mg}\cdot\text{L}^{-1}$), czego nie obserwowano dla b-BC (**Publikacja D3, Tabela 1**).



Rysunek 12. Zdjęcia *Daphnia magna* w kontroli oraz w zawiesinach wybranych b-BC i n-BC.

Zarówno ekstrakty wodne z b-BC jak i n-BC nie wpływały istotnie na kiełkowanie *Lepidium sativum* (**Publikacja D3, Tabela S6**). W porównaniu do b-BC, n-BC charakteryzowały się wyraźnie silniejszym działaniem toksycznym wobec *L. sativum*,

przejawiającym się większym stopniem hamowania wzrostu korzeni (**Publikacja D3, Rys. 5**). W przypadku n-BC otrzymanych z SSL ich toksyczność była wyższa o 18,3–42% niż w odpowiadających im b-BC, natomiast dla n-BC wytwarzanych z WL, RH i OSR efekt stymulujący obserwowany dla b-BC przekształcił się w działanie toksyczne (hamowanie wzrostu korzeni o 10,4–24,6%). Pomimo podobnych trendów zależnych od rodzaju surowca i warunków pirolizy, n-BC wykazywały silniejsze efekty toksyczne niż odpowiadające im b-BC. Powodem zwiększonej toksyczności po mieleniu w stosunku do *L. sativum* może być zwiększona wartość EC. Wyraźnie zwiększony poziom EC, mógł przekraczać tolerancję roślin na zasolenie, szczególnie przy wyższych stężeniach n-BC, zwiększając obserwowany efekt toksyczny.

W badaniach dotyczących wpływu b-BC oraz n-BC na *Folsomia candida* wykazano, że zarówno b-BC, jak i n-BC nie wpływały istotnie na śmiertelność *F. candida* (**Publikacja D3, Tabela S7**). Wpływ BC na *F. candida* zaznaczył się jednak w przypadku reprodukcji. Zmniejszenie rozmiaru do n-BC istotnie wpływało na reprodukcję *F. candida* (**Publikacja D3, Rys. 6**). Przy dawce 1% efekt stymulujący był wyraźnie słabszy dla n-BC (o 30–56% mniejszy) niż dla odpowiadających im b-BC, choć w obu przypadkach obserwowano podobne zależności względem rodzaju surowca i warunków pirolizy (m.in. silniejsze działanie b-BC i n-BC z SSL oraz wzrost efektu wraz z temperaturą pirolizy). Przy wyższej dawce (5%) zmniejszenie rozmiaru cząstek nasilało efekt toksyczny – n-BC powodowały silniejsze hamowanie reprodukcji (o 15–81%) niż b-BC. Mechanizm ten można wiązać ze zwiększoną powierzchnią właściwą (S_{BET}) i wyższą zawartością DOC w n-BC, które sprzyjają większej mobilności i biodostępności zanieczyszczeń (np. WWA, PTEs), a także adsorpcji składników odżywczych z gleby, ograniczającej ich dostępność dla organizmów. W efekcie, mimo niższej całkowitej zawartości WWA i PTEs w n-BC, ich działanie toksyczne było silniejsze niż w przypadku b-BC, co wskazuje, że redukcja rozmiaru cząstek może zwiększać ryzyko toksyczności przy wyższych dawkach.

Podsumowując, przeprowadzone badania pozwoliły w sposób systematyczny ocenić ekotoksyczność n-BC, wskazując, że surowiec, warunki pirolizy i wielkość cząstek mają kluczowy wpływ na interakcje n-BC z organizmami. Stwierdzono, że wpływ ten jest bardzo zróżnicowany i determinowany szeregiem parametrów (właściwości BC, warunki otrzymania), ale również rodzajem organizmu testowego, jak i zastosowanego parametru

do oceny toksyczności. Kolejnym istotnym parametrem jest dawka BC. Powyższe sugeruje, że problem oceny toksyczności jest bardzo złożony i wymaga jeszcze szeregu badań. Wyniki podkreślają również potrzebę uwzględniania nie tylko parametrów wytwarzania n-BC, ale również różnych organizmów testowych oraz wskazują na konieczność badań długoterminowych. Badania z *D. magna* wykazały bowiem, że wstępne i niepełne badania mogą prowadzić do mylnych wniosków mających konsekwencje w przyszłości.

5.4. Wpływ nanostrukturyzacji biowęgla na jego zdolności sorpcyjne wobec wybranych jonów pierwiastków potencjalnie toksycznych w środowisku wodnym (*Publikacja D4*)

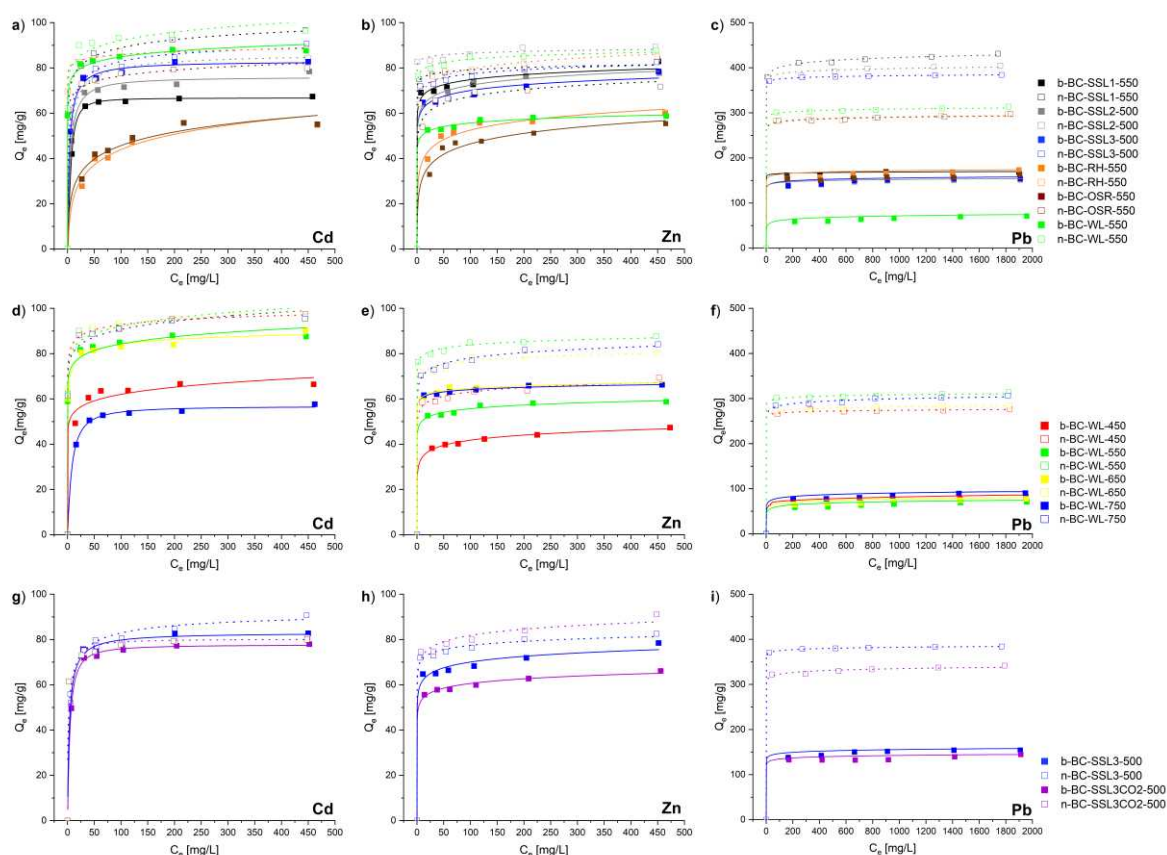
Celem niniejszych badań było określenie zdolności sorpcyjnych n-BC w usuwaniu jonów kadmu (Cd), cynku (Zn) i ołowiu (Pb) z roztworów wodnych w zależności od zastosowanego surowca oraz warunków pirolizy. Uzyskane wyniki porównano z parametrami sorpcji uzyskanymi dla odpowiadających im b-BC. Na podstawie parametrów pirolizy oraz właściwości fizykochemicznych n-BC określano efektywność sorpcji oraz zaproponowano możliwe mechanizmy odpowiedzialne za ten proces. Lepsze zrozumienie zależności między warunkami otrzymania b-BC, a zdolnościami sorpcyjnymi n-BC w stosunku do wybranych metali jest kluczowe dla optymalizacji wytwarzania n-BC oraz zastosowania go w procesach remediacji środowiska i usuwania zanieczyszczeń z wód.

Oceniając kinetykę adsorpcji, stwierdzono, że jest to proces trójfazowy przebiegający następująco: (1) początkowa szybka sorpcja, (2) wolniejsza faza osiągnięcia równowagi oraz (3) plateau. Badania kinetyczne wykazały, że adsorpcja jonów Pb (II) osiągała równowagę szybciej na b-BC (6 h) niż na n-BC (24 h), co wynikało z różnic w strukturze porów oraz energii hydratacji jonów Pb (II). Kinetykę adsorpcji jonów metali najlepiej opisywał model pseudo-drugiego rzędu (PSO, z ang. *pseudo-second order*), wskazując na chemisorpcję jako główny mechanizm adsorpcji badanych jonów metali na powierzchni b-BC/n-BC (***Publikacja D4, Rys. 1***). Zastosowanie modelu dyfuzji wewnątrzcząsteczkowej (IPD, z ang. *intraparticle diffusion model*) wykazało, że ten typ oddziaływania nie był jedynym czynnikiem ograniczającym szybkość adsorpcji, a proces przebiegał w kilku etapach związanych z zajmowaniem miejsc adsorpcyjnych, o różnej dostępności w strukturze porowatej (***Publikacja D4, Rys. 2***). Zmniejszenie rozmiaru BC

do formy nano zwiększyło szybkość adsorpcji jonów Cd (II) i Zn (II), ale obniżyło szybkość względem jonów Pb (II), co było związane z większym promieniem jonowym Pb (II) niż Cd (II) i Zn (II), co utrudniało dyfuzję Pb (II) do mikroporów n-BC. Wyliczone wartości kinetyczne w oparciu o model PSO dla badanych układów adsorpcji z wykorzystaniem b-BC wykazały, że szybkość adsorpcji przebiegał w następującej kolejności (od najszybszej do najwolniejszej): Pb (II) > Zn (II) > Cd (II). W układach z n-BC kolejność uległa jednak zmianie i kształtowała się w następujący sposób: Zn (II) > Cd (II) > Pb (II). Uwzględniając surowiec użyty do otrzymania BC stwierdzono, że najszybciej adsorpcja jonów Cd (II) zachodziła na BC otrzymanych z WL zarówno w przypadku b-BC, jak i n-BC. Dla pozostałych dwóch metali szybkość adsorpcji w zależności od surowca zależała jednak od jego formy (b-BC/n-BC). Jony Zn (II) najszybciej adsorbowały się na b-BC otrzymanych z SSL, natomiast w przypadku n-BC proces najszybciej przebiegał na tych otrzymanych z WL. Z kolei jony Pb (II) najszybciej adsorbowały się na b-BC pochodzącym z SSL oraz na n-BC wytwarzanym z pozostałości rolniczych (RH i OSR). Zarówno dla b-BC, jak i n-BC, zwiększenie temperatury pirolizy z 450 do 650 °C zwiększało szybkość adsorpcji, po czym przy dalszym zwiększaniu pirolizy do 750 °C powodowało jej obniżenie. Zmiana gazu nośnego z N₂ na CO₂ przyspieszyła adsorpcję jedynie jonów Cd (II), co przypisano większej liczbie grup tlenowych na powierzchni BC (**Publikacja D4, Rys. 1**).

Izotermy adsorpcji jonów Cd (II), Zn (II) i Pb (II) na b-BC i n-BC (**Rys. 13**), wykazały szybkie nasycenie miejsc adsorpcyjnych przy niskich stężeniach, co potwierdziło najlepsze dopasowanie do modelu Langmuira. Wskazuje to, że adsorpcja w badanych układach doświadczalnych opiera się na dominacji adsorpcji monowarstwowej. Zmniejszenie rozmiaru do nanoskali zwiększyło zdolność adsorpcji jonów Cd (II), Zn (II) i Pb (II) odpowiednio o 2,1– 53,9%, 14,3–48,3% i 71,9–332% w porównaniu do b-BC. Ponadto, na podstawie uzyskanych danych stwierdzono, że zdolności adsorpcyjne b-BC i n-BC zależały głównie od surowca użytego do ich otrzymania. Największy zwiększenie zdolności adsorpcji jonów Cd (II), w zależności od użytego surowca po nanostrukturyzacji, zaobserwowano dla n-BC otrzymanych z pozostałości rolniczych (RH i OSR). Niemniej jednak, najwyższą ogólną zdolność adsorpcji spośród wszystkich n-BC osiągnęły, podobnie jak w przypadku b-BC, BC otrzymane z WL (93,1 mg·g⁻¹) i SSL (91,8 mg·g⁻¹). W przypadku jonów Zn (II), największy wzrost pojemności adsorpcyjnej po nanostrukturyzacji zaobserwowano dla n-BC otrzymanych z WL i RH

(Rys. 13). Jednakże, to n-BC wytworzone z SSL wykazywały najwyższe pojemności adsorpcyjne względem Zn (II) osiągając wartości o 22,3–45,2% wyższe niż n-BC wytworzone z biomasy roślinnej (WL, RH i OSR). Spośród analizowanych jonów PTEs, największy wzrost pojemności adsorpcyjnej po nanostrukturyzacji, a zarazem najwyższe wartości adsorpcji dla wszystkich badanych n-BC, odnotowano dla Pb (II), którego pojemność adsorpcyjna była istotnie wyższa niż w przypadku pozostałych jonów. Podobnie jak w przypadku jonów Cd (II) i Zn (II), n-BC pochodzące z SSL wykazały najwyższe pojemności, które były średnio o 33% wyższe niż pojemności sorpcyjne n-BC otrzymanych z innych surowców (WL, RH i OSR). Pojemności adsorpcyjne n-BC pochodzących z SSL wzrosły o 148–167% w porównaniu do odpowiadających im b-BC. Jednak najbardziej znaczące zwiększenie pojemności adsorpcyjnej (332%) Pb (II) do b-BC po mieleniu kulowym zaobserwowano dla n-BC z WL.



Rysunek 13. Izoterm adsorpcji jonów Cd (II), Zn (II) i Pb (II) na b-BC i n-BC. Pozycje (a–c) przedstawiają porównanie różnych surowców, (d–f) ilustrują porównanie różnych temperatur pirolizy, natomiast (g–i) ilustrują porównanie różnych gazów nośnych.

Warunki pirolizy nie determinowały w sposób istotny pojemności adsorpcyjnej b-BC, a tym n-BC wobec jonów PTEs. W przypadku jonów Cd (II) temperatura pirolizy (450–

750 °C) nie miała istotnego wpływu na pojemności adsorpcyjne n-BC, które utrzymywały się na podobnym poziomie (89,5–93,1 mg·g⁻¹), jednocześnie będąc o 232–332% wyższymi niż dla odpowiedników b-BC. W przypadku Zn (II) i Pb (II) najwyższe wartości adsorpcji uzyskano dla n-BC otrzymanego w temperaturze 550 °C (odpowiednio 88,0 mg·g⁻¹ i 314,3 mg·g⁻¹), co stanowiło wzrost o 48,3% i 332% względem b-BC. Nie stwierdzono jednak wyraźnej zależności od temperatury pirolizy. Dla n-BC zmiana atmosfery pirolizy z N₂ na CO₂, podobnie jak w przypadku b-BC, skutkowała obniżeniem zdolności adsorpcyjnej wobec wszystkich analizowanych jonów PTEs o 10–16%, w zależności od ich rodzaju.

Nanostrukturyzacja nie wpłynęła na ogólny mechanizm adsorpcji. W obydwu przypadkach (b-BC i n-BC) adsorpcja opierała się na kompleksowaniu chemicznym (chemisorpcja) z udziałem powierzchniowych grup funkcyjnych zawierających tlen, takich jak grupy karboksylowe (–COOH) i hydroksylowe (–OH), co potwierdziły analizy XPS i FTIR (**Publikacja D4, Rys. S2 oraz S6**). W układach z zaadsorbowanymi metalami, obserwowano wyraźne obniżenie intensywności ich sygnałów (**Publikacja D4, Rys. S2 oraz S6**), co wskazuje na tworzenie się silnych kompleksów między metalem a wspomnianymi grupami funkcyjnymi. Dodatkowo, procesy wytrącania (precypitacja) metali w postaci nierozpuszczalnych wodorotlenków i węglanów (np. Pb(OH)₂, PbCO₃, Cd(OH)₂, Zn(OH)₂) (**Publikacja D4, Rys. S6**) również uczestniczyły w adsorpcji jonów metali, co było możliwe dzięki lokalnym wzrostom pH na granicy faz BC–roztwór. W efekcie adsorpcja jonów Cd (II), Zn (II) i Pb (II) na b-BC i n-BC przebiegała głównie przez chemisorpcję, wspomaganą wytrącaniem, co skutecznie zwiększało wydajność usuwania jonów Cd (II), Zn (II) i Pb (II) z roztworów wodnych. Ponadto analiza głównych składowych (PCA, z ang. *Principal Component Analysis*) potwierdziła, że powierzchnia właściwa (S_{BET}), objętość porów (V_p) i zawartość popiołu, wraz z powierzchniowymi grupami funkcyjnymi, są czynnikami determinującymi efektywność adsorpcji na badanych BC, a mechanizmy sorpcji dla Cd (II), Zn (II) i Pb (II) są podobne. Wyniki potwierdzają, że n-BC, dzięki korzystnym właściwościom powierzchniowym i strukturalnym, wykazują znacznie większy potencjał adsorpcyjny niż b-BC w usuwaniu PTEs z wód, co wskazuje na jego przydatność w zastosowaniach środowiskowych związanych z oczyszczaniem wód zanieczyszczonych metalami ciężkimi.

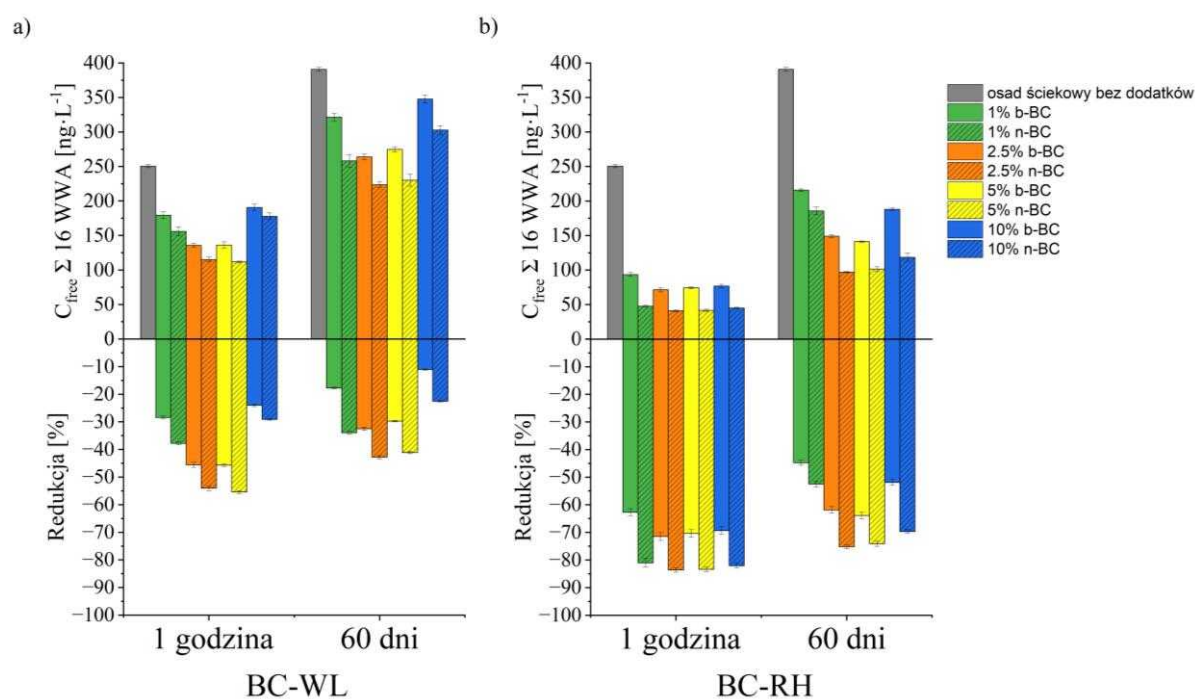
Podsumowując, na podstawie przeprowadzonych badań stwierdzono, że nanostrukturyzacja znacząco zwiększa zdolności adsorpcyjne n-BC. Proces ten jest szczególnie widoczny w przypadku n-BC, które w formie b-BC charakteryzują się stosunkowo niską wydajnością sorpcji. W przypadku b-BC charakteryzujących się wstępną wysoką zdolnością adsorpcyjną, obniżenie do rozmiaru nano skutkuje brakiem zmiany zdolności adsorpcyjnych lub pewnym wzrostem jednak nie tak znaczącym jak dla form nisko efektywnie sorpcyjnych. Szczególnie duży wzrost efektywności zaobserwowano w usuwaniu Pb (II), co potwierdza, że zmniejszenie rozmiaru cząstek zwiększa skuteczność selektywnej adsorpcji. Dzięki łatwemu przygotowaniu, wykorzystaniu tanich i dostępnych surowców oraz wysokiej efektywności usuwania jonów PTEs, n-BC mogą stanowić obiecujący i ekonomicznie efektywny materiał do oczyszczania ścieków, oferując realne zastosowanie w ochronie środowiska na skalę przemysłową. Istotne w tym zakresie są dalsze badania uwzględniające wpływ różnych parametrów środowiskowych na wydajność adsorpcji.

5.5. Zwiększenie bezpieczeństwa osadów ściekowych za pomocą nano-biowęgla poprzez immobilizację wielopierścieniowych węglowodorów aromatycznych i redukcję ekotoksyczności (Publikacja D5)

Celem badań opisanych w niniejszej pracy była ocena skuteczności zastosowania n-BC otrzymanych z RH lub WL jako dodatku do osadów ściekowych w obniżaniu biodostępności WWA ($C_{\text{free}} \Sigma 16$ WWA). W ramach badań określono również wpływ dodanego n-BC na właściwości ekotoksykologiczne osadów. Analizę tych zanieczyszczeń uzasadnia ich zróżnicowany charakter chemiczny, co przekłada się na ich różne powinowactwo do materii organicznej i potencjał migracyjny w środowisku.

Początkowa zawartość $C_{\text{free}} \Sigma 16$ WWA w SSL wynosiła $251 \pm 2,4 \text{ ng} \cdot \text{L}^{-1}$ i uległa zwiększeniu po inkubacji do $391 \pm 2,94 \text{ ng} \cdot \text{L}^{-1}$ z powodu dłuższego kontaktu wody z osadem, co ułatwiało uwalnianie WWA do fazy wodnej (**Rys. 14**). Sugeruje to, że dłuższy kontakt osadu z wodą w środowisku może zwiększać biodostępność WWA, a w rezultacie ryzyko środowiskowe. Jest to odmienne od wyników dotychczasowych badań glebowych, w których wydłużenie czasu kontaktu zanieczyszczeń z glebą zwykle prowadzi do ograniczenia ich dostępności [70]. Wykazano, że dodatek b-BC/n-BC skutecznie zmniejszał zawartość $C_{\text{free}} \Sigma 16$ WWA w osadzie. Największą efektywność redukcji zawartości $C_{\text{free}} \Sigma 16$ WWA po zastosowaniu b-BC/n-BC obserwowano po

1 godzinnej inkubacji, a po 60 dniach skuteczność wiązania C_{free} $\Sigma 16$ WWA uległa obniżeniu. Spadek ten można przypisać osiągnięciu stanu równowagi bądź wtórnej desorpcji WWA z powierzchni b-BC/n-BC do roztworu wodnego. Wykazano, że bez względu na formę (b-BC lub n-BC) wyższą efektywność redukcji C_{free} $\Sigma 16$ WWA wykazywały BC otrzymane z RH, obniżając C_{free} $\Sigma 16$ WWA odpowiednio o 52–72% w zależności od formy BC. Nanostrukturyzacja zwiększała skuteczność immobilizacji WWA, szczególnie dla n-BC otrzymanych z WL, gdzie w zależności od czasu inkubacji odnotowano wzrost efektywności o 19–33% po 1 godzinie i o 91–104% po 60 dniach. Podobnie, obniżenie wielkości cząstek n-BC pochodzących z RH doprowadziła do zwiększonej wydajności immobilizacji C_{free} $\Sigma 16$ WWA (ze wzrostem o 16,9–29,2% po 1 godzinie i 15,9–33,4% po 60 dniach). Optymalne wyniki uzyskiwano przy dawkach 2,5–5%. Wyższą skuteczność w immobilizacji WWA przez BC otrzymane z RH można przypisać wyższemu pH, szerszym porom, większej zawartości popiołu oraz wyższemu stosunkowi O/C tych materiałów niż BC otrzymanych z WL, co zwiększało interakcje chemiczne, wiązania wodorowe i oddziaływania polarne pomiędzy powierzchnią BC a cząsteczkami WWA.



Rysunek 14. Zawartość C_{free} $\Sigma 16$ WWA (górny panel) i procentowa redukcja w osadzie ściekowym (dolny panel) z dodatkiem b-BC-WL/n-BC-WL (a) i b-BC-RH/n-BC-RH (b). Słupki błędów przedstawiają odchylenie standardowe średniej ($n = 3$).

Dodatek b-BC i n-BC do SSL znacząco obniżył toksyczność odcieków wobec *A. fischeri* (15,9–63,1% w zależności od dawki, rodzaju i czasu inkubacji), przy czym najwyższe dawki (5–10%) były najbardziej skuteczne w redukcji toksyczności. Należy zaznaczyć, że b-BC i n-BC pochodzące z RH podobnie jak w przypadku C_{free} Σ16 WWA były bardziej efektywne w obniżaniu toksyczności niż BC otrzymane z WL, a formy n-BC działały skuteczniej niż b-BC, co wynikało z ich większej powierzchni właściwej i lepszego wiązania zanieczyszczeń. Po 60 dniach inkubacji obserwowano dalsze obniżenie toksyczności w układach z b-BC i n-BC otrzymanych z WL, natomiast dla b-BC i n-BC z RH efektywność była stabilna lub nieznacznie spadała, co może wynikać z uwalniania słabo związanych WWA z powierzchni BC. Podobnie jak w przypadku *A. fischeri*, dodatek b-BC i n-BC eliminował fitotoksyczność odcieków z SSL, które przed dodaniem b-BC/n-BC hamowały kiełkowanie *L. sativum* na poziomie 47–53%. Podobnie, dodatek b-BC/n-BC ograniczał inhibicję wzrostu korzeni *L. sativum*, przy czym formy n-BC, szczególnie te pochodzące z WL, były najbardziej skuteczne, obniżając inhibicję wzrostu korzeni o 43–70% po 1 godzinie i 17–46% po 60 dniach. Redukcja rozmiaru cząstek do skali nano znacząco poprawiała skuteczność obniżenia fitotoksyczności, a wyższe dawki sprzyjały lepszym efektom wzrostowym. W przeciwieństwie jednak do wyników otrzymanych dla *A. fischeri*, b-BC i n-BC z WL lepiej ograniczały toksyczność wobec roślin niż b-BC i n-BC otrzymane z RH. Obserwacje te mogą sugerować, że fitotoksyczność SSL zależy od szeregu czynników, takich jak dostępność składników odżywczych, zmiany pH i EC oraz interakcji mikrobiologicznych w obecności BC, które wymagają dalszych badań i wyjaśnienia.

Uzyskane w niniejszych badaniach wyniki potwierdzają, że zastosowanie n-BC jako dodatków do SSL może być skuteczną, tanią i przyjazną środowisku metodą redukcji toksyczności i biodostępności zanieczyszczeń organicznych, a także wspierania wzrostu roślin, co zwiększa potencjał bezpiecznego wykorzystania osadów w rolnictwie i innych zastosowaniach środowiskowych. Nanostrukturyzacja zwiększa istotnie ten efekt. Wyniki powyższych badań wskazują, że poprzez zastosowanie n-BC jako dodatku do osadu ściekowego minimalizuje się negatywny wpływ obecnych w nim zanieczyszczeń, jednocześnie zawracając do środowiska cenne składniki odżywcze oraz materię organiczną, co promuje bezpieczne wykorzystanie osadów w rolnictwie lub innych zastosowaniach środowiskowych (np. rekultywacji gleb lub gruntów zdegradowanych).

5.6. Zastosowanie nano-biowęgla w zrównoważonej remediacji gleb zanieczyszczonych pierwiastkami potencjalnie toksycznymi dla poprawy bezpieczeństwa żywności (*Publikacja D6*)

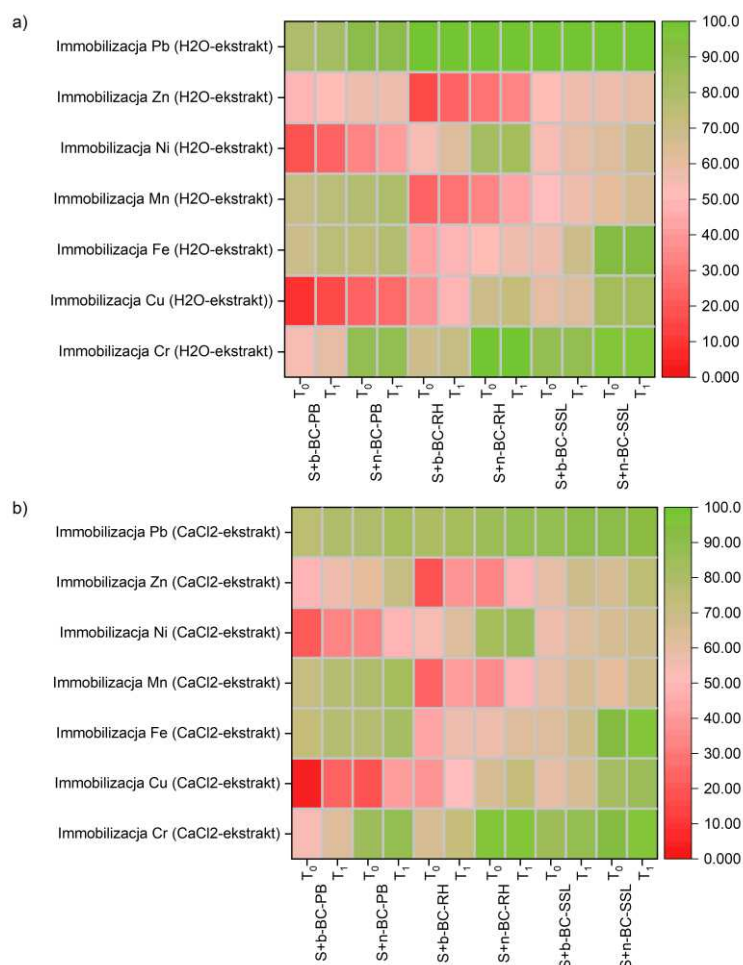
Celem pracy było określenie zdolności n-BC, otrzymanych z trzech różnych surowców (RH, PB i SSL) do immobilizacji wybranych PTEs w zanieczyszczonych glebach. Określano dodatkowo wpływ n-BC na wzrost roślin (*Lactuca sativa* L.) oraz akumulację przez nie PTEs. Przeprowadzono również kompleksową analizę korzyści i ryzyka stosowania n-BC, uwzględniając potencjalny wpływ na bezpieczeństwo żywności oraz zdrowie ludzi.

Zaobserwowano, że dodatek b-BC/n-BC do gleby zwiększał pH i EC roztworu glebowego. Zastosowanie obydwu form – b-BC i n-BC – zwiększyło także zawartość TOC i DOC w glebie. Co istotne, n-BC poprawiały powyższe parametry efektywniej niż b-BC, wspierając jakość gleby oraz jednoczesną immobilizację zanieczyszczeń (*Publikacja D6, Tabela 3*).

Dodatek b-BC/n-BC do gleby zanieczyszczonej PTEs skutecznie obniżał ich biodostępność zarówno określoną w roztworze wodnym (H_2O) jak i $CaCl_2$ Po aplikacji b-BC/n-BC odnotowano istotne obniżenie ($p \leq 0,05$) zawartości chromu (Cr), miedzi (Cu), żelaza (Fe), manganu (Mn), niklu (Ni), cynku (Zn) oraz ołowiu (Pb) we frakcji biodostępnej względem gleby kontrolnej. Kobalt (Co) i kadm (Cd) nie były wykrywane w tej frakcji, zarówno w glebie kontrolnej, jak i z dodatkiem b-BC/n-BC, co wynika z ich silnego wiązania z trwałymi frakcjami gleby [71]. W czasie T_0 (początek doświadczenia) obniżenie frakcji PTEs było 8–163% (H_2O) i 6–30% ($CaCl_2$) większe dla n-BC niż b-BC. Po 6 tygodniach (T_1) biodostępna zawartość badanych PTEs uległa dalszemu obniżeniu osiągając wartości niższe niż w glebie kontrolnej o 15–100% i 23–91% ($CaCl_2$) w przypadku b-BC, oraz o 26–100% (H_2O) i 40–97% ($CaCl_2$) dla n-BC (*Publikacja D6, Rys. 2*). Zaobserwowano, że w niektórych przypadkach (w zależności od PTEs lub rodzaju surowca, z którego otrzymano BC) wzrost efektywności był z upływem czasu bardziej zauważalny dla b-BC aniżeli n-BC. Należy jednak podkreślić, że n-BC należy uznać ogólnie za bardziej skuteczny dzięki wyższej efektywności początkowej wskazującej na szybsze działanie immobilizujące. Wynika to z większej liczby miejsc aktywnych w n-BC niż b-BC zdolnych do interakcji z zanieczyszczeniami

oraz mniejszego rozmiaru cząstek, co sprzyja ich lepszemu rozproszeniu w glebie. Tym samym zwiększeniu ulega dostępność powierzchni aktywnej dla potencjalnych interakcji z zanieczyszczeniami. Największą efektywność w immobilizacji badanych PTEs wykazywały b-BC i n-BC otrzymane z SSL (**Rys. 15**), co wynikało z wyższej zawartości popiołu i grup funkcyjnych w tych b-BC/n-BC, co z kolei sprzyjało adsorpcji i precypitacji metali. Analiza korelacji wykazała, że kluczowe właściwości b-BC i n-BC (**Publikacja D6, Rys. 5**) zwiększające immobilizację to zawartość popiołu, powierzchnia właściwa (S_{BET}) oraz szerokość porów. Zwiększenie tych parametrów zasadniczo zwiększało efektywność. Obecność popiołu może sprzyjać immobilizacji PTEs poprzez precypitację i wymianę jonową. Ponadto zwiększenie zawartości popiołu sprzyja zwiększeniu pH, co jest istotnym czynnikiem ograniczającym ich mobilność [72]. Większa powierzchnia właściwa i objętość porów zwiększały liczbę miejsc aktywnych, wzmacniając procesy sorpcji i kompleksacji metali. Analiza skupień potwierdziła, że rodzaj surowca do otrzymania b-BC/n-BC, w większym stopniu niż rozmiar cząstek decydował o wzorcu immobilizacji PTEs w badanej glebie (**Publikacja D6, Rys. 4**). Mimo, że największą immobilizację ogółem spośród analizowanych PTEs odnotowano dla Pb, największy wzrost efektywności po zmniejszeniu rozmiaru zaobserwowano dla Cu i Fe, co wynikało z małych promieni jonowych tych pierwiastków, umożliwiających penetrację do mikroporów, a przez to efektywną ich sorpcję.

Na podstawie modelowania NICA–Donnan dla gleb z podwyższoną zawartością DOC wykazano, że mechanizmy odpowiedzialne za immobilizację to głównie wymiana jonowa –dominująca dla Cr, Fe, Mn, N i– oraz precypitacja – kluczowa dla Cu, Zn, Pb – (**Publikacja D6, Tabela S5**). Wyższe pH gleby po zastosowaniu b-BC/n-BC sprzyjało powstawaniu trudno rozpuszczalnych wodorotlenków metali, a podwyższone EC oraz obecność DOC wspierały procesy kompleksowania i wymiany jonowej, ograniczając mobilność metali. Analiza FTIR i XPS potwierdziła obecność grup funkcyjnych (–OH, –COOH, C=O) na powierzchni b-BC i n-BC, które ułatwiały sorpcję PTEs (**Publikacja D6, Rys. S1 i S3**). Badania wykazały również, że n-BC dzięki większej powierzchni właściwej (S_{BET}), większej reaktywności powierzchni i większej liczbie grup funkcyjnych skuteczniej immobilizuje PTEs niż b-BC.



Rysunek 15. Heatmap wydajności immobilizacji różnych b-BC i n-BC dla pierwiastków potencjalnie toksycznych ekstrahowanych (a) H₂O i (b) CaCl₂. Skala kolorów przedstawia stopień immobilizacji, gdzie kolor czerwony oznacza najniższy, a zielony najwyższy stopień immobilizacji. Dane przedstawiono dla dwóch punktów czasowych: T₀ (etap początkowy) i T₁ (po 6 tygodniach).

Zastosowanie b-BC/ n-BC jako dodatku do gleby znacząco obniżyło translokację PTEs z gleby do liści sałaty, przy czym n-BC były bardziej skuteczne, obniżając zawartość metali o 13–135% efektywniej niż obserwowano to dla b-BC. Wynikało to z lepszej zdolności do immobilizacji PTEs w glebie po zastosowaniu n-BC niż b-BC, a przez to ograniczeniu ich dostępności dla roślin. Największą redukcję bioakumulacji odnotowano dla Pb i Mn, a najlepsze efekty uzyskano po zastosowaniu b-BC i n-BC otrzymanych z SSL, co było zgodne z ich wysoką skutecznością wiązania PTEs przez te BC (**Publikacja D6, Rys. 2**). Wskaźniki bioakumulacji (BAF, z ang. *bioaccumulation factor*) wyznaczone dla poszczególnych PTEs uległy istotnemu obniżeniu po zastosowaniu zarówno b-BC, jak i n-BC (**Publikacja D6, Rys. 6**). Zastosowanie b-BC/n-BC obniżyło również istotnie wartości wskaźnika ryzyka zdrowotnego

(HRI, z ang. *health risk index*) związanego ze spożyciem sałaty, redukując HRI poniżej wartości granicznych dla większości badanych PTEs (**Publikacja D6, Tabela 5**). Wskazuje to na ograniczenie ryzyka zdrowotnego, zwłaszcza dla dzieci, które zwykle są bardziej narażone na wpływ zanieczyszczeń ze względu na wyższe spożycie warzyw liściastych w przeliczeniu na masę ciała oraz większą podatność organizmu na toksyczne działanie PTEs. W przypadku b-BC redukcja HRI wynosiła od 12,6% do 100%, natomiast n-BC wykazał większą skuteczność od 1,1 do 2,5 razy większe niż w przypadku b-BC– i wynosiły od 29,7% do 100%. Po zastosowaniu b-BC i n-BC do gleby wartości HRI były < 1 dla wszystkich PTEs (oprócz Fe), zarówno dla dorosłych, jak i dla dzieci.

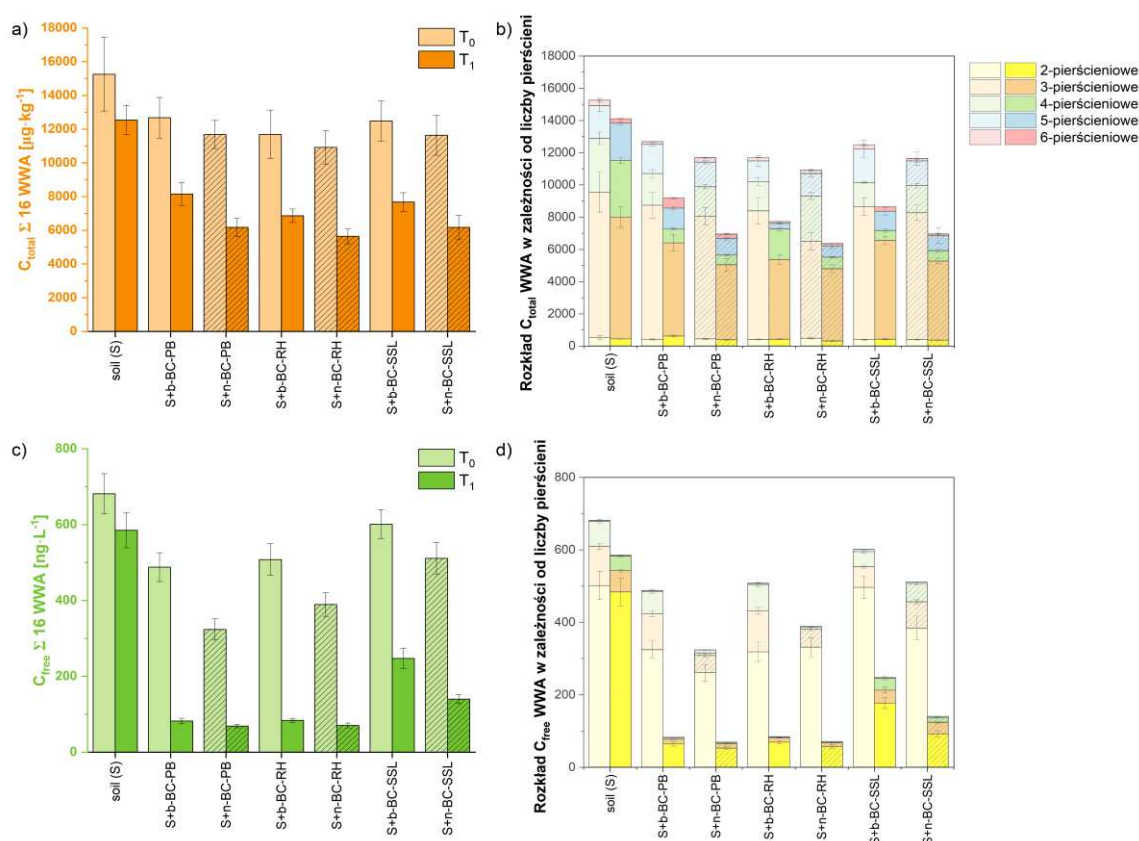
Podsumowując, n-BC, jak wskazują powyższe badania, może stanowić skuteczne narzędzie do ograniczania ryzyka związanego z obecnością PTEs w glebie oraz ich akumulacją w roślinach. Prowadzi to do zwiększenia bezpieczeństwa żywności, szczególnie warzyw spożywanych na surowo. Łącząc zaawansowaną wiedzę z zakresu nanomateriałów z badaniami rolniczymi i środowiskowymi, praca ta przedstawia całościowe podejście do zarządzania glebami zanieczyszczonymi PTEs. Wykorzystując lokalnie dostępne odpady do produkcji efektywnych materiałów remediacyjnych, podejście to nie tylko zwiększa efektywność oczyszczania gleb, lecz także wspiera gospodarkę o obiegu zamkniętym i zrównoważony rozwój, przekształcając odpady w cenne zasoby dla poprawy jakości środowiska i rolnictwa.

5.7. Nano-biowęgiel jako efektywniejszy środek poprawy jakości gleby skażonej WWA w porównaniu z tradycyjnym biowęgłem (Publikacja D7)

Celem niniejszej pracy było określenie skuteczności n-BC otrzymanych z RH, PB i SSL w immobilizacji WWA w glebach skażonych tymi związkami. Skuteczność n-BC porównywano ze skutecznością formy b-BC otrzymanych z tych samych surowców. Szczególną uwagę zwracano na interakcje n-BC z cząstkami gleby oraz długoterminową stabilność środowiskową n-BC. W pracy badano również wpływ parametrów n-BC, takich jak rozkład wielkości cząstek oraz rodzaj zastosowanego surowca do ich otrzymania, w celu opracowania protokołu wdrożeniowego dla kosztowo efektywnej remediacji gleb zanieczyszczonych WWA. Badania te poszerzają zakres zastosowania nanomateriałów węglowych w rekultywacji gleb, wprowadzając ulepszone podejścia

do remediacji, które zwiększa ich efektywność w porównaniu do metod konwencjonalnych z jednoczesnym zachowaniem zasad zrównoważonego rozwoju oraz gospodarki o obiegu zamkniętym.

Dodanie b-BC do gleby nie wpłynęło istotnie na zawartość C_{total} $\Sigma 16$ WWA na początku doświadczenia (T_0), podczas gdy n-BC spowodował obniżenie wartości C_{total} $\Sigma 16$ WWA w zakresie 23,4–28,4% (w zależności od surowca użytego do otrzymania BC) w porównaniu do gleby kontrolnej. Wynikało to z niższej zawartości WWA w n-BC niż w glebie i pojawienia się efektu „rozcieńczania” (**Rys. 16**). Po sześciu tygodniach (T_1) inkubacji w glebie z b-BC lub n-BC zawartość C_{total} $\Sigma 16$ WWA obniżyła się odpowiednio o 35,7–41,3% oraz 46,9–48,3% względem T_0 , przy czym n-BC wykazał większą o 17–32% skuteczność w obniżaniu zawartości C_{total} $\Sigma 16$ WWA niż b-BC, niezależnie od użytego surowca do produkcji n-BC. Zaobserwowano, że b-BC otrzymane z RH i SSL wykazały najwyższą skuteczność redukcji C_{total} $\Sigma 16$ WWA spośród badanych b-BC,



Rysunek 16. Zawartość C_{total} $\Sigma 16$ WWA (a) oraz ich rozkład ze względu na liczbę pierścieni (b), a także zawartość C_{free} $\Sigma 16$ WWA (c) oraz ich rozkład ze względu na liczbę pierścieni (d) w glebie oraz glebie z dodatkiem 2% b-BC/n-BC na początku eksperymentu (T_0) (jaśniejsze kolory) oraz po 6 tygodniach (T_1) (ciemniejsze kolory). Przerywane słupki oznaczają warianty z dodatkiem n-BC. Słupki błędów przedstawiają odchylenie standardowe średniej ($n = 3$).

natomiast wszystkie n-BC działały podobnie niezależnie od surowca (PB, RH i SSL), z którego zostały otrzymane.

Wykazano, że zastosowanie b-BC/n-BC spowodowało także istotne ($p \leq 0.05$) obniżenie zawartości C_{free} $\Sigma 16$ WWA w glebie. Istotne ($p \leq 0.05$) obniżenie C_{free} $\Sigma 16$ WWA obserwowano już na początku doświadczenia (T_0). Zawartość C_{free} $\Sigma 16$ WWA obniżyła się od 11,8–28,4% po zastosowaniu b-BC oraz od 25,0–52,5% po zastosowaniu n-BC, przy czym n-BC wykazał o 68–113% wyższą efektywność niż odpowiadające im b-BC. W przypadku C_{free} $\Sigma 16$ WWA najwyższą skuteczność immobilizacji na początku doświadczenia (T_0) wykazywały b-BC jak i n-BC otrzymane z PB. Wysoka efektywność BC otrzymanych z tego surowca była związana z wysoką zawartością węgla organicznego (TOC i DOC), co sprzyjało immobilizacji C_{free} $\Sigma 16$ WWA. Wykazano, że mimo niższej powierzchni właściwej (S_{BET}) b-BC i n-BC otrzymanych z PB, w porównaniu do BC z innych surowców, te były najbardziej skuteczne w redukcji C_{free} $\Sigma 16$ WWA, co również potwierdziła analiza PCA (**Publikacja D7, Rys. 3**). Po sześciu tygodniach (T_1) badań skuteczność immobilizacji C_{free} $\Sigma 16$ WWA uległa zwiększeniu w porównaniu do T_0 , osiągając poziomy 58,9–83,5% w zależności od rodzaju b-BC/n-BC, przy czym różnice między n-BC a b-BC malały, co wskazuje na osiągnięcie równowagi sorpcyjnej w dłuższym czasie przez b-BC. W czasie T_1 jedynie n-BC otrzymany z SSL wykazał większą skuteczność niż odpowiadający mu b-BC, co wynikało z jego najmniejszego rozmiaru cząstek spośród badanych n-BC. W pozostałych przypadkach różnice między b-BC a n-BC były wyraźnie mniejsze niż obserwowano to dla BC pochodzących z SSL.

Analiza zależności między poziomem immobilizacji a właściwościami fizykochemicznymi BC wykazała, że immobilizacja WWA w glebie była silnie powiązana z powierzchnią właściwą (S_{BET}), objętością porów (V_p) oraz wartościami EC, które były wyższe dla n-BC niż b-BC. Dane uzyskane techniką FTIR i XPS potwierdziły wyższą zawartość grup funkcyjnych w n-BC, co sprzyjało sorpcji WWA poprzez interakcje hydrofobowe i wiązania wodorowe. Zmiany właściwości gleby (TOC, DOC, pH, EC) po zastosowaniu b-BC/n-BC również korelowały z obniżeniem zawartości WWA.

Analiza wskaźnika zanieczyszczenia Nemerowa (NIPI), określająca ogólny stopień zanieczyszczenia badanej matrycy środowiskowej, wykazała wyraźne jego obniżenie po dodaniu b-BC i n-BC, przy czym n-BC, zwłaszcza otrzymane z PB i SSL, okazały się najbardziej skuteczne (**Publikacja D7, Tabela 1**). Wszystkie próbki mieściły

się w zakresie uznawanym za bezpieczny, jednak ze względu na możliwe dalsze uwalnianie zanieczyszczeń z opuszczonego zakładu przemysłowego konieczne są działania kontrolne, a zastosowanie n-BC może znacząco ograniczyć to ryzyko.

Podsumowując, stwierdzono, że zarówno b-BC, jak i n-BC istotnie wpływają na zawartość i biodostępność WWA w zanieczyszczonej glebie oraz związane z nimi ryzyko ekologiczne. Skuteczność tych materiałów zależała również od rodzaju surowca oraz rozmiaru cząstek. Wyniki potwierdziły, że n-BC był bardziej efektywny niż b-BC, obniżając zarówno $C_{total} \Sigma 16$ WWA, jak i $C_{free} \Sigma 16$ WWA, co przekładało się na obniżenie poziomu zanieczyszczenia i ryzyka środowiskowego w czasie. Ocena ryzyka ekologicznego wykazała także, że zastosowanie b-BC i n-BC obniżało poziom zanieczyszczenia WWA w glebie, przy czym skuteczniejsze w tym procesie ponownie okazały się n-BC, niezależnie od surowca użytego do ich otrzymania. Wydłużenie czasu kontaktu między BC a zanieczyszczoną glebą sprzyjało dalszemu obniżaniu ryzyka ekologicznego wyznaczonego w oparciu o wskaźnik zanieczyszczenia Nemerowa (NIPI).

6. Wnioski

1. Zastosowanie mielenia kulowego w celu otrzymania n-BC pozwala na istotne zwiększenie powierzchni właściwej (S_{BET}), objętości porów oraz liczby aktywnych miejsc sorpcyjnych na ich powierzchni, zmieniając jednocześnie morfologię cząstek na bardziej kuliste aglomeraty. Obniżenie rozmiaru skutkuje również zmniejszeniem pH oraz zmianą jego właściwości powierzchniowych w kierunku większej polarności i aromatyczności.
2. W trakcie mielenia dochodzi do istotnego obniżenia całkowitej zawartości oraz biodostępności WWA oraz PTEs. W wyniku nanostrukturyzacji zmienia się również profil WWA obecnych w materiale, przesuwając się w kierunku związków o niższej masie cząsteczkowej i mniejszej toksyczności, przy jednoczesnym zachowaniu wysokiego potencjału sorpcyjnego n-BC.
3. Badania nad toksycznością n-BC wykazały zróżnicowany wpływ na organizmy żywe w zależności od rodzaju surowca użytego do ich otrzymania, temperatury pirolizy, a także zastosowanego testu czy określanego parametru toksyczności. W świetle słów Paracelsusa, że „*wszystko jest trucizną i nic nie jest trucizną – to dawka czyni trucizną*”, kluczowe znaczenie ma odpowiedni dobór dawki n-BC. Właściwie dobrana ilość tego materiału może wykazywać efekt stymulujący na wzrost i rozwój organizmów testowych, podczas gdy zbyt wysokie dawki mogą działać hamująco.
4. Zastosowanie nanostrukturyzacji prowadzi do istotnego zwiększenia zdolności sorpcyjnych n-BC wobec kadmu Cd (II), cynku Zn (II) i ołowiu Pb (II). Efekt ten wynikał z uzyskania przez n-BC większej powierzchni właściwej i objętości porów, a tym samym zwiększonej ilości miejsc aktywnych zdolnych do interakcji z metalami, oraz bardziej ujemnego potencjału zeta niż odpowiedników b-BC, co sprzyja adsorpcji jonów metali na powierzchni n-BC oraz umożliwia ich częściową precypitację. Proces adsorpcji zachodził zgodnie z modelem Langmuira, wskazując na charakter monowarstwowy oraz modelem kinetycznym PSO, co sugeruje dominację mechanizmów chemisorpcji.
5. Wprowadzenie n-BC do SSL skutkuje znacznym obniżeniem stężenia i mobilności C_{free} Σ16 WWA oraz ogranicza biodostępność tych zanieczyszczeń, szczególnie w przypadku WWA o wyższej masie cząsteczkowej. Dodatek n-BC do osadów wpływa ponadto na obniżenie toksyczności odcieków z osadów ściekowych oraz eliminuje ich fitotoksyczność. Zastosowanie n-BC w dawkach

2,5–5% jest optymalne dla uzyskania najlepszego efektu detoksykacji osadów ściekowych. Współstosowanie osadu ściekowego i n-BC, może być więc skuteczną metodą ograniczenia ryzyka środowiskowego związanego z wykorzystaniem osadów ściekowych, z jednoczesnym wykorzystaniem ich potencjału nawozowego.

6. Nanostrukturyzacja zwiększa skuteczność n-BC w immobilizacji PTEs w glebie, redukując ich mobilność oraz biodostępność. Zastosowanie n-BC do gleby ogranicza również bioakumulację metali w roślinach, redukując wskaźnik HRI dla dorosłych oraz dzieci poniżej wartości granicznych. Zwiększa to bezpieczeństwo zdrowotne żywności uprawianej na glebach zanieczyszczonych PTEs.
7. N-BC skuteczniej obniżały zawartość C_{total} $\Sigma 16$ WWA i C_{free} $\Sigma 16$ WWA w glebach skażonych tymi związkami, ograniczając ich biodostępność oraz ryzyko migracji w profilu glebowym. Istotne efekty obserwowano już w początkowym okresie kontaktu gleby z n-BC (czego nie zaobserwowano dla b-BC), wskazując na wysoką aktywność sorpcyjną n-BC oraz szybkie osiągnięcie równowagi sorpcyjnej. Wyniki te potwierdzają potencjał n-BC jako efektywnego narzędzia ograniczającego ryzyko ekologiczne związane z obecnością WWA w glebach.
8. Pomimo że n-BC wykazywał wyższą reaktywność powierzchniową i w niektórych przypadkach podwyższoną toksyczność w testach z organizmami modelowymi, jego zastosowanie jako czynnika immobilizującego zanieczyszczenia w glebie lub osadzie ściekowym przyczyniało się jednak do ograniczenia toksyczności zanieczyszczonej matrycy. Wynika to z wysokiego potencjału sorpcyjnego n-BC, który skutecznie immobilizuje zanieczyszczenia odpowiedzialne za efekt toksyczny lub/i ogólnie pozytywnie wpływa na właściwości fizyko–chemiczne matrycy. Zastosowanie n-BC w środowisku pozwala zatem wykorzystać jego wysoką reaktywność do neutralizacji zanieczyszczeń, tworząc skuteczne narzędzie służące do poprawy jakości środowiska.

7. Życiorys i dorobek naukowy

W 2016 roku ukończyłam II Liceum Ogólnokształcącego im. Gustawa Orlicz-Dreszera w Chełmie. W latach 2016–2019 realizowałam studia I stopnia na kierunku chemia, specjalność: chemia podstawowa i stosowana, na Uniwersytecie Marii Curie-Skłodowskiej w Lublinie. Studia ukończyłam z wynikiem bardzo dobrym, uzyskując tytuł licencjata chemii na podstawie pracy pt. *„Potencjalne substancje rakotwórcze obecne w żywności”*, przygotowanej pod opieką dr Marioli Iwan. W latach 2019–2021 kontynuowałam edukację na studiach II stopnia na kierunku chemia, specjalność: chemia środków bioaktywnych i kosmetyków. Pracę magisterską pt. *„Zastosowanie naturalnych odpadów organicznych do syntezy efektywnych adsorbentów węglowych”* zrealizowałam pod kierunkiem dr hab. Barbary Charnas, prof. UMCS. Za osiągnięcia naukowe i wyniki w nauce wielokrotnie otrzymywałam stypendium Rektora UMCS dla najlepszych studentów. W październiku 2021 roku rozpoczęłam kształcenie w Szkole Doktorskiej Nauk Ścisłych i Przyrodniczych Uniwersytetu Marii Curie-Skłodowskiej w Lublinie, realizując rozprawę doktorską w Katedrze Radiochemii i Chemii Środowiskowej pod opieką naukową prof. dr. hab. Patryka Oleszczuka, w ramach projektu badawczego finansowanego przez Narodowe Centrum Nauki (konkurs MAESTRO), pt. *„Tworzenie się i losy nano-biowęgla w kontekście ochrony zasobów glebowych i wodnych”* (kierownik projektu prof. dr. hab. Patryk Oleszczuk). Od lutego 2024 roku jestem zatrudniona na stanowisku asystenta naukowo-dydaktycznego w Katedrze Radiochemii i Chemii Środowiskowej Uniwersytetu Marii Curie-Skłodowskiej w Lublinie. W tym samym roku zostałam również powołana w skład Rady Młodych Naukowców UMCS, gdzie aktywnie działam na rzecz wspierania rozwoju naukowego środowiska doktorantów i młodych badaczy. Na dzień złożenia rozprawy doktorskiej na mój dorobek naukowy składa się 7 opublikowanych artykułów naukowych w czasopiśmie o wysokim współczynniku wpływu (z ang. *impact factor*) oraz 4 artykuły naukowe w trakcie recenzji, tym 2 artykuły po pierwszych recenzjach, 4 rozdziały w monografiach naukowych oraz 1 zgłoszenie patentowe. Aktywnie uczestniczyłam w konferencjach naukowych, zarówno krajowych, jak i – przede wszystkim – międzynarodowych, prezentując wyniki prowadzonych badań w formie referatów oraz posterów. Brałam również udział jako wykonawczyni w kilku projektach badawczych, w tym finansowanych ze środków Narodowego Centrum Nauki (NCN). Równocześnie aktywnie aplikowałam o finansowanie zewnętrzne na własne badania – w wyniku czego

zostałam kierownikiem projektu badawczego finansowanego przez NCN w ramach konkursu PRELUDIUM. Szczegóły dotyczące publikacji, wystąpień konferencyjnych oraz udziału w projektach znajdują się poniżej.

Dorobek naukowy

Publikacje naukowe

1. M. Hawryluk–Sidoruk, **M. Raczkiewicz**, P. Krasucka, W. Duan, O. Mašek, R. Zarzycki, R. Kobylecki, B. Pan, P. Oleszczuk
"Effect of biochar chemical modification (acid, base, and hydrogen peroxide) on contaminants content depending on feedstock and pyrolysis conditions"
Chemical Engineering Journal, 481, 148329
<https://doi.org/10.1016/j.cej.2023.148329>
IF_{5-letni} = 13.2 MEiN: 200 pkt
2. **M. Raczkiewicz**, I. Ostolska, O. Mašek, P. Oleszczuk
"Effect of the pyrolysis conditions and type of feedstock on nano–biochars obtained as a result of ball milling"
Journal of Cleaner Production, 458, 142456,
<https://doi.org/10.1016/j.jclepro.2024.142456>
IF_{5-letni} = 10.2 MEiN: 140 pkt
3. **M. Raczkiewicz**, O. Mašek, Y. S. Ok, P. Oleszczuk
"Size reduction of biochar to nanoscale decrease polycyclic aromatic hydrocarbons and trace metals content and bioavailability in nano–biochars"
Science of The Total Environment, 937, 173372,
<https://doi.org/10.1016/j.scitotenv.2024.173372>
IF_{5-letni} = 8.2 MEiN: 200 pkt
4. **M. Raczkiewicz**, A. Bogusz, B. Pan, B. Xing, P. Oleszczuk
"Contrasting environmental impacts of nano–biochar and conventional biochar on various organisms"
Science of The Total Environment, 957, 177629,
<https://doi.org/10.1016/j.scitotenv.2024.177629>
IF_{5-letni} = 8.2 MEiN: 200 pkt
5. **M. Raczkiewicz**, P. Oleszczuk
"Enhanced heavy metal removal using nano–engineered biochars synthesized under variable pyrolysis conditions and feedstocks"
Journal of Environmental Management, 389, 126072
<https://doi.org/10.1016/j.jenvman.2025.126072>
IF_{5-letni} = 7.9 MEiN: 200 pkt
6. **M. Raczkiewicz**, P. Oleszczuk
"Boosting sewage sludge safety with nano–biochar for polycyclic aromatic hydrocarbons immobilization and ecotoxicity reduction"
Journal of Hazardous Materials, 492, 138224
<https://doi.org/10.1016/j.jhazmat.2025.138224>

IF_{5-letni} = 11.9

MEiN: 200 pkt

7. **M. Raczekiewicz**, D. Akachukwu, P. Oleszczuk

“Sustainable soil remediation using nano-biochar for improved food safety and resource recovery”

Journal of Hazardous Materials, 494, 138537

<https://doi.org/10.1016/j.jhazmat.2025.138537>

IF_{5-letni} = 11.9

MEiN: 200 pkt

8. **M. Raczekiewicz**, D. Akachukwu, P. Oleszczuk

“Nanoscale biochar outperforms conventional biochar in enhancing soil health and reducing risks from polycyclic aromatic hydrocarbon-contaminated soil”

Environmental Research, po pierwszym etapie recenzji, w trakcie decyzji

IF_{5-letni} = 7.7

MEiN: 100 pkt

Wyslane

9. A. Rombel, **M. Raczekiewicz**, P. Oleszczuk

“Dose-dependent impact of biochar during composting with sewage sludge on metal mobility and ecotoxicity of compost”

Journal of Hazardous Materials, w trakcie recenzji

10. W. Błaszczuk, K. Fila, **M. Raczekiewicz**, T. Paszko, D. C. W. Tsang, P. Oleszczuk, A. Siatecka

“Protic ionic liquid-based extraction of potentially toxic elements from digested sewage sludges with ecotoxicological and bioavailability assessment of residues”

Chemical Engineering Journal, w trakcie recenzji

11. **M. Raczekiewicz**, A. Tkacz, J. Madej, P. Oleszczuk,

“Toxicity and polycyclic aromatic hydrocarbons bioavailability in recycled tire rubber granulate of varying particle sizes”

Environmental and Biogeochemical Processes, po pierwszym etapie recenzji

Monografie naukowe

1. **M. Raczekiewicz**, B. Charnas, M. Zięzio

„Badanie chemii powierzchni węgla aktywnych z szyszek świerku (Picea abies) metodą miareczkowania potencjometrycznego”

Nauka i Przemysł-lubelskie spotkania studenckie, Wydawnictwo UMCS, Lublin, 2021, ISBN 978-83-227-9503-3, str. 225-229.

2. M. Zięzio, B. Charnas, **M. Raczekiewicz**

„Wpływ atmosfery procesu pirolizy na kreowanie struktury porowatej węgla aktywnych”

Nauka i Przemysł-lubelskie spotkania studenckie, Wydawnictwo UMCS, Lublin, 2021, ISBN 978-83-227-9503-3, str. 221-225.

3. **M. Raczekiewicz**, B. Charnas, B. Wawrzaszek

„Charakterystyka struktury porowatej materiałów węglowych otrzymanych z odpadów drzewnych – wpływ temperatury i sposobu modyfikacji”

Nauka i Przemysł–lubelskie spotkania studenckie, Wydawnictwo UMCS, Lublin, 2022, ISBN ISBN 978–83–227–9603–0, str. 329–333.

4. B. Wawrzaszek, B. Charmas, **M. Racziewicz**

„Biowęgle z trocin – proekologiczny sposób na odpady”

Nauka i Przemysł–lubelskie spotkania studenckie, Wydawnictwo UMCS, Lublin, 2022, ISBN ISBN 978–83–227–9603–0, str. 325–329.

Konferencje naukowe

Krajowe

1. Instytut Nauk Chemicznych UMCS, IX Ogólnopolskie Sympozjum „Nauka i przemysł – Lubelskie Spotkania Studentów”, 28 czerwca 2021, Lublin, Polska

Wykład: *„Ocena wpływu atmosfery i warunków modyfikacji hydrotermalnej na właściwości strukturalne i termiczne węgla aktywnych”*, **Monika Racziewicz**, Magdalena Zięzio, Barbara Charmas.

Prezentacja posterowa: *„Wpływ atmosfery procesu pirolizy na kształtowanie się struktury porowatej węgla aktywnych”*, Magdalena Zięzio, **Monika Racziewicz**, Barbara Charmas

2. Polska Akademia Nauk, Oddział w Lublinie oraz Centrum Innowacji Naukowo–Edukacyjnych, VIII Ogólnopolska Konferencja Naukowa „Innowacje w praktyce”, 14 października 2021, Lublin, Polska

Prezentacja posterowa: *„Wpływ promieniowania mikrofalowego na tworzenie się struktury porowatej węgla aktywnych (ACs)”*, **Monika Racziewicz**, Barbara Charmas, Magdalena Zięzio.

3. Instytut Nauk Chemicznych UMCS, X Ogólnopolskie Sympozjum „Nauka i przemysł – Lubelskie Spotkania Studentów”, 27 czerwca 2022, Lublin, Polska

Prezentacja posterowa: *„Charakterystyka struktury porowatej materiałów węglowych otrzymanych z odpadów drzewnych – wpływ temperatury i metody modyfikacji”*, **Monika Racziewicz**, Barbara Wawrzaszek, Barbara Charmas.

4. XIV Konferencja Naukowo–Techniczna „Materiały węglowe i kompozyty polimerowe. Nauka–Przemysł”, 18–21 października 2022, Ustroń, Polska

Prezentacja posterowa: *„Biowęgiel z odpadów drzewnych jako zamiennik cementu w materiałach budowlanych”*, **Monika Racziewicz**, Patryk Oleszczuk.

5. 65. Zjazd Naukowy Polskiego Towarzystwa Chemicznego, 18–22 września 2023, Toruń, Polska

Wykład: *„Charakterystyka ekotoksykologiczna nanobiowęgla otrzymanych metodą rozdrabniania kulowego”*, **Monika Racziewicz**, Aleksandra Bogusz, Patryk Oleszczuk.

Prezentacja posterowa: „*Wpływ warunków pirolizy i rodzaju surowca na właściwości fizykochemiczne nanobiowęgli otrzymanych metodą rozdrabniania kulowego*”, **Monika Racziewicz**, Iwona Ostolska, Karolina Karpińska-Wlizło, Patryk Oleszczuk.

Międzynarodowe

6. Science Week in Montpellier, 12–15 czerwca, 2023, Montpellier, Francja
7. The World Conference on Carbon 2023, 16–21 lipca 2023, Cancún, Meksyk

Prezentacja posterowa: "Physicochemical properties of nano–biochars produced by ball–milling from various feedstocks, temperatures, and gases." **Monika Racziewicz**, Iwona Ostolska, Patryk Oleszczuk

8. ESG Management and Sustainability and the JCK Forum 2023, 8–30 listopada 2023, Seul, Korea Południowa

Wykład: "Ecotoxicological characterization of nano–biochar obtained by ball milling," **Monika Racziewicz**, Aleksandra Bogusz, Patryk Oleszczuk.

9. SETAC Europe 34th Annual Meeting 5–9 maja 2024, Sewilla, Hiszpania

Prezentacja posterowa: "Ecotoxicological characterization of nano–biochar obtained by ball–milling", **Monika Racziewicz**, Aleksandra Bogusz, Patryk Oleszczuk.

10. The 2nd International Conference on Biochar Research and Application & The 7th Asia Pacific Biochar Conference (ICBRA&APBC”, 20–22 września 2024, Shenyang, Chiny

Wykład: “Size Matters: evaluating ecotoxicity–nano–biochar vs. bulk biochar”, **Monika Racziewicz**, Bo Pan, Baoshan Xing, Patryk Oleszczuk

11. International Symposium on Soil and Environmental Health (ISSEH), 12–15 października 2024, Nanjing, Chiny

Wykład: “Comparative ecotoxicological aspects of nano–biochar and bulk biochar: A comprehensive analysis of environmental impact”, **Monika Racziewicz**, Bo Pan, Baoshan Xing, Patryk Oleszczuk

12. Międzynarodowy Kongres Marii Curie–Sklodowskiej „Innowacja jest Kobietą. Nauka–Biznes–Edukacja”, 21–22 października 2024, Lublin, Polska

Prezentacja posterowa: “Beyond the Bulk: The effects of nano–biochar on ecological systems”, **Monika Racziewicz**, Aleksandra Bogusz, Bo Pan, Baoshan Xing, Patryk Oleszczuk

13. Szkoła letnia TwinSubDyn project “Summer School on Sustainable Organic Amendment Applications from a Soil and Groundwater Management Perspective”, 2–6 czerwca, Novi Sad, Serbia

Wykład: “Nano–biochar for sustainable remediation of potentially toxic elements–contaminated soils: a focus on food safety and circular economy”, **Monika Raczekiewicz**, Patryk Oleszczuk

14. International Conference on Solid Waste 2025: Waste Management for Carbon Neutrality and Circular Economy (ICSWDG2025), 9–12 czerwca, Dongguan, Chiny

Wykład: “Boosting sewage sludge safety with nano–biochar for polycyclic aromatic hydrocarbons immobilization and ecotoxicity reduction”, **Monika Raczekiewicz**, Patryk Oleszczuk

15. ICOBTE & ICHMET 2025. 22–26 września, Busan, Korea Południowa

Wykład: “Efficient potentially toxic elements removal from digested sewage sludges using protic ionic liquids”, **Monika Raczekiewicz**, Wiktoria Błaszczuk, Karolina Fila, Tadeusz Paszko, Daniel C.W. Tsang, Patryk Oleszczuk, Anna Siatecka

Projekty badawcze

Poza realizacją rozprawy doktorskiej jako wykonawca (doktorant) w ramach projektu MAESTRO pt. „*Tworzenie się i losy nano–biowęgla w kontekście ochrony zasobów glebowych i wodnych*” (kierownik: prof. dr hab. Patryk Oleszczuk), aktywnie uczestniczę również w innych przedsięwzięciach naukowo–badawczych. Współpracuję przy projekcie finansowanym przez NCN w ramach konkursu SONATA pt. „*Demetalizacja osadów ściekowych w celu uzyskania bezpiecznych surowców do produkcji pofermentu i kompostu*” (kierownik: dr Anna Siatecka). Ponadto jako kierownik realizuję także własny projekt badawczy finansowany przez NCN w ramach konkursu PRELUDIUM, pt. „*Innowacyjne podejścia gospodarki o obiegu zamkniętym z wykorzystaniem zaawansowanego biowęgla do zrównoważonego zagospodarowania pofermentu i ograniczania ryzyka środowiskowego*”. Ponadto uczestniczyłam w międzynarodowym projekcie Agropolis Foundation (nr OPFP22–027), gdzie współpracowałam z dr Doris Akachuwu (czerwiec–grudzień 2023).

Zgłoszenia patentowe

Patent na wynalazek nr P.450246 pt. „Sposób otrzymywania biowęgla z trocin drzewnych o powolnym uwalnianiu składników mineralnych z jego odcieków”, Karol Osipiuk, **Monika Raczekiewicz**, Iwona Ostolska, Patryk Oleszczuk

Nagrody, wyróżnienia i staże

W trakcie kształcenia w Szkole Doktorskiej Nauk Ścisłych i Przyrodniczych UMCS zostałam uhonorowana następującymi nagrodami i wyróżnieniami:

1. Stypendium dla najlepszych doktorantów z Własnego Funduszu Stypendialnego UMCS w Lublinie na rok akademicki 2021/2022.
2. Srebrny medal za najlepszą prezentację posterową podczas konferencji naukowej „Innowacje w praktyce”, Polska Akademia Nauk, Oddział w Lublinie oraz Centrum Innowacji Naukowo-Edukacyjnych, VIII Ogólnopolska Konferencja Naukowa, 14 października 2021, Lublin, Polska
3. Stypendium African Women in Agricultural Research and Development (AWARD) – krótki wyjazd badawczy, Michael Okpara University of Agriculture, 26 czerwca–7 lipca 2024, Umudike, Abia State, Nigeria
4. Nagroda za najlepszą prezentację ustną podczas konferencji naukowej the 2nd International Conference on Biochar Research and Application & The 7th Asia Pacific Biochar Conference (ICBRA&APBC), 20–22 września 2024, Shenyang, Chiny
5. Staż naukowy w Stockbridge School of Agriculture, University of Massachusetts, 10–18 czerwca 2024, Amherst, USA,
6. Staż naukowy w Institute of Organic Contaminant Control and Soil Remediation, Nanjing Agricultural University, 30 września –11 października.2024, Nanjing, Chiny
7. Stypendium wyjazdowe na udział w letniej szkole w ramach projektu TwinSubDyn: „Summer School on Sustainable Organic Amendment Applications from a Soil and Groundwater Management Perspective”, 2–6 czerwca, Nowy Sad, Serbia
8. Laureatka programu „Mentoring” Uniwersytetu Marii Curie-Skłodowskiej w Lublinie
9. Stypendium NAWA STER “Międzynarodowa Sieć Naukowo – Badawcza” – krótki wyjazd badawczy (1 miesiąc), University of Massachusetts, 11 sierpnia–9 września 2025, Amherst, USA

Szkolenia:

1. Webinarium Fundacja Science Watch Polska, „*Publikowanie artykułu naukowego w prestiżowym czasopiśmie: wybór czasopisma i komunikacja*”, 6 kwietnia 2022 r., formuła on–line
2. Szkolenie z chromatografii gazowej, „*Chromatografia gazowa– wybrane aspekty sposobu dozowania próbek oraz prace konserwacyjno–naprawcze. Podstawy teoretyczne i analiza praktyczna*”, 9 grudnia 2022 r., Warszawa, Polska
3. Warsztaty: „*Nowoczesne rozwiązania w pracy z hodowlami komórkowymi*”, 30 listopada 2022 r., formuła on–line
4. Szkolenie z obsługi systemu Field Flow Fractionation (FFF), 7–8 lutego 2023 r., Landsberg, Niemcy
5. Webinarium ScienceDirect: „*Wyszukiwanie literatury naukowej w pełnotekstowej bazie danych – prosto do celu*”, 14 listopada 2023 r., formuła on–line
6. Szkolenie z metody spektroskopii fotoelektronów rentgenowskich (XPS), 5–14 grudnia 2023 r., Lublin, Polska
7. Szkolenie z prowadzenia testów ekotoksykologicznych (*Daphnia magna* i *Lemna minor*), 19–23 sierpnia 2024 r., Warszawa, Polska
8. Szkolenie „*Aplikacyjne wykorzystanie metod GC–MS oraz spektrometrii mas*”, 29 kwietnia 2025 r., formuła on–line

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- feedstock sources. *Sci Total Environ* 661, 685–695.
<https://doi.org/10.1016/j.scitotenv.2019.01.193>
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<https://doi.org/10.1016/j.jhazmat.2019.121980>
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9. Załączniki

9.1. Streszczenie w języku polskim

Rozprawa doktorska pt.: „*Wpływ warunków pirolizy na formowanie, właściwości i oddziaływanie z zanieczyszczeniami nano-biowęgla otrzymanych z różnych surowców*” składa się z cyklu powiązanych ze sobą tematycznie siedmiu artykułów opublikowanych w recenzowanych czasopismach naukowych posiadających wskaźnik wpływu (IF, *ang. impact factor*).

Badania przeprowadzone w ramach pracy doktorskiej składały się z czterech głównych etapów. W pierwszym etapie przeprowadzono syntezę biowęgla oraz jego nano-odpowiedników (nano-biowęgla) i oceniono wpływ procesu nanostrukturyzacji (mielenia kulowego) na właściwości fizykochemiczne materiałów wytwarzanych z różnych surowców (biomasa roślinna, osady ściekowe) w zmiennych warunkach pirolizy (temperatura, gaz nośny). Wykazano, że nanostrukturyzacja znacząco zwiększała powierzchnię właściwą, porowatość oraz liczbę aktywnych miejsc sorpcyjnych, przy jednoczesnym obniżeniu pH i zmianie morfologii cząstek (*Publikacja D1*). W drugim etapie oceniono wpływ nanostrukturyzacji na zawartość i biodostępność zanieczyszczeń natywnych biowęgla, w tym wielopierścieniowych węglowodorów aromatycznych (WWA) oraz pierwiastków potencjalnie toksycznych (PTEs), wskazując na obniżenie ich całkowitej i biodostępnej zawartości, co zwiększa bezpieczeństwo środowiskowe materiału (*Publikacja D2*). W trzecim etapie przeprowadzono ocenę ekotoksyczności biowęgla i nano-biowęgla wobec organizmów modelowych z różnych poziomów troficznych, takich jak bakterie (*Aliivibrio fischeri*), rośliny (*Lemna minor*, *Lepidium sativum*) i bezkręgowce (*Daphnia magna* oraz *Folsomia candida*). Wyniki wykazały, że nano-biowęgiel może wykazywać wyższą toksyczność w stosunku do organizmów wodnych i glebowych w porównaniu do biowęgla w formie makro, szczególnie przy wyższych zastosowanych dawkach (*Publikacja D3*). W czwartym etapie przeprowadzono ocenę właściwości aplikacyjnych nano-biowęgla w zakresie usuwania i immobilizacji zanieczyszczeń w różnych matrycach środowiskowych, takich jak woda, gleba i osady ściekowe (*Publikacje D4–D7*). Badania wykazały, że rodzaj surowca, temperatura pirolizy oraz rodzaj gazu nośnego decydują o efektywności uzyskiwanego nano-biowęgla, co pozwala na świadome projektowanie materiałów dedykowanych do oczyszczania wód i gleb, przy jednoczesnym ograniczeniu ryzyka wtórnej emisji

zanieczyszczeń. Nanostrukturyzacja biowęgla, poprzez zwiększenie powierzchni właściwej i liczby miejsc aktywnych, zwiększała jego potencjał sorpcyjny, a dzięki ograniczeniu biodostępności i toksyczności zanieczyszczeń oraz zmniejszeniu zawartości natywnych zanieczyszczeń (WWA i PTEs), przyczyniała się do poprawy jakości środowiska, wspierając realizację koncepcji zrównoważonego rozwoju oraz gospodarki o obiegu zamkniętym.

9.2. Streszczenie w języku angielskim

The doctoral dissertation entitled “*The effect of pyrolysis conditions on the formation, properties and interactions with contaminants of nano–biochars derived from various feedstocks*” consists of a series of seven thematically related articles published in peer-reviewed scientific journals with an impact factor (IF).

The research carried out as part of the doctoral dissertation consisted of four main stages. In the first stage, biochar and its nano-counterparts (nano-biochar) were synthesized, and the effect of nanostructurization (ball milling) on the physicochemical properties of materials produced from different feedstocks (plant biomass, sewage sludge) under varying pyrolysis conditions (temperature, carrier gas) was evaluated. It was demonstrated that nanostructurization significantly increased the specific surface area, porosity, and number of active sorption sites, while simultaneously decreasing the pH and altering the particle morphology (*Publication D1*). In the second stage, the impact of nanostructurization on the content and bioavailability of native contaminants in biochar, including polycyclic aromatic hydrocarbons (PAHs) and potentially toxic elements (PTEs), was assessed. The results indicated a reduction in both total and bioavailable concentrations, thereby increasing the environmental safety of the material (*Publication D2*). The third stage involved the evaluation of the ecotoxicity of biochar and nano-biochar toward model organisms representing different trophic levels, such as bacteria (*Aliivibrio fischeri*), plants (*Lemna minor*, *Lepidium sativum*), and invertebrates (*Daphnia magna* and *Folsomia candida*). The findings revealed that nano-biochar may exhibit higher toxicity toward aquatic and soil organisms compared to bulk biochar, particularly at higher application doses (*Publication D3*). In the fourth stage, the application properties of nano-biochar in the removal and immobilization of contaminants in different environmental matrices, such as water, soil, and sewage sludge, were investigated (*Publications D4–D7*). The research demonstrated that the feedstock type, pyrolysis temperature, and carrier gas determine the efficiency of the obtained nano-biochar, enabling the deliberate design of materials dedicated to water and soil remediation, while simultaneously reducing the risk of secondary pollutant release. Nanostructurization of biochar, by increasing the specific surface area and number of active sites, enhanced its sorption potential, and through the reduction of contaminant bioavailability and toxicity, as well as the decrease of native pollutant content (PAHs and

PTEs), contributed to environmental quality improvement, supporting the implementation of the concepts of sustainable development and circular economy.

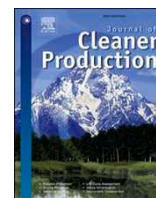
9.3. Oryginalne przedruki opublikowanych artykułów stanowiących integralną część rozprawy doktorskiej

Publikacja D1

M. Racziewicz, I. Ostolska, O. Mašek, P. Oleszczuk

*"Effect of the pyrolysis conditions and type of feedstock on nano–biochars obtained
as a result of ball milling"*

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Effect of the pyrolysis conditions and type of feedstock on nanobiochars obtained as a result of ball milling

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ABSTRACT

Currently, the production of nanobiochar (n-BC) using ball milling is a promising and cost-effective method. Studies concerning the physicochemical properties of bulk biochar (b-BC) in relation to pyrolysis temperature, various feedstocks or types of carrier gas have been thoroughly investigated. However, there is a lack of information on how the mentioned conditions determine the properties of the obtained n-BCs. In this study, b-BCs produced from different feedstocks (willow (WL), sewage sludge (SSL), rice husk (RH) and oilseed rape (OSR)), temperatures (450–750 °C) and carrier gases (N₂ or CO₂) were ball-milled to produce n-BCs. The specific surface area (S_{BET}), elemental composition (C, H, N, O), morphology (SEM), functional groups (FT-IR), and crystalline and amorphous nature of the carbon (RAMAN) were determined. The size of n-BCs depended on the feedstock used (particles ranged from 30 to 87.9 nm). The type of feedstock mostly determined the properties of n-BCs. The most visible differences between n-BCs and b-BCs were observed for surface properties and C content. For all n-BCs, an S_{BET} increase was observed after ball milling, which may enhance the adsorption potential of biochar. The most intense S_{BET} changes were noted for WL-derived n-BCs obtained at 450 °C (increase from 7.2 to 146.3 m²/g) and at 750 °C (increase from 3.9 to 160.8 m²/g) and for RH-derived n-BCs produced at 550 °C (increase from 3.7 to 158.6 m²/g). The C content decreased for n-BCs produced from plant biomass (WL, RH and OSR) and increased (from 20 to 28%) for n-BCs derived from sewage sludge (SSL). Other physicochemical properties mainly depended on the feedstock used for biochar production.

1. Introduction

Nanobiochar (n-BC) is a form of bulk biochar (b-BC) that has been processed to have a much smaller particle size (especially at the nanoscale) than traditional bulk biochar (b-BC). Biochar is a form of charcoal made from biomass materials, such as wood, agricultural waste, herbaceous residues, animal manure, or sewage sludge, for use in applications that do not result in the release of its carbon into the atmosphere on short to medium timescales (Lee et al., 2019). Biochar can also be a precursor to produce n-BC, which can be created by several methods, including hydrothermal carbonization, ball milling, sonication, etc. (He et al., 2023; Oleszczuk et al., 2016; Song et al., 2022). All established methods are compatible with different biomass feedstocks and share a few crucial steps, such as size reduction or screening. The result is carbonaceous nanoparticles with a small particle size (<100 nm), which can be used for a variety of applications (Song et al., 2022). The n-BC

may exhibit properties different from their well-known macroforms, and these different properties have raised questions about potential environmental risks, including toxicity in aquatic and terrestrial environments, remediation of pollutants and influence on crop yields (Sani et al., 2023).

Currently, the production of n-BCs with high surface properties using ball milling has been developed as a promising and a cost-effective method (Amusat et al., 2021). Ball milling is a mechanical-chemical process (MCP) uses mechanical energy to modify the chemical and structural properties of materials (Xing et al., 2013). It is a new greener and more efficient method for producing biochar-based nanoparticles with enhanced characteristics and a variety of uses compared to other common techniques, i.e., sonification, hydrothermal carbonization, disc milling, and vibratory tube milling (Shi et al., 2023). By decreasing the agglomeration and altering the ball milling settings, such as the ball to sample ratio and milling time, the desired particle size can be obtained

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in this procedure (Shah et al., 2015; Zhang et al., 2021). The use of the ball milling method eliminated the need for dangerous chemically assisted operations, increased economic efficiency, waste reduction, product biodegradability and the utilization of renewable energy sources (Pratap et al., 2021, 2022). The wide range of biochar characteristics allows for numerous applications in diverse fields (Jiang et al., 2024; Singh Yadav et al., 2023; Wang et al., 2022). The properties of n-BC depend on the feedstock material and the process conditions utilized in the preparation of b-BC (Naghdi et al., 2019). The n-BC prepared via the reduction in the particle size of b-BC, may demonstrate characteristics like those of b-BC. However, in many cases, n-BC shows characteristics different from those at the macroscale, especially regarding surface area (Ramanayaka et al., 2020).

Studies concerning the physicochemical properties of b-BCs in relation to pyrolysis temperature, various feedstocks, or types of carrier gas have been thoroughly investigated (Askeland et al., 2019; Zhao et al., 2013). However, there is a lack of information on how these properties and conditions determine the properties of n-BCs. Only a few studies have been published regarding the physicochemical properties of n-BCs (Kumar et al., 2020; Naghdi et al., 2017). Nonetheless, most of these studies concentrate on examining n-BC produced from a single type of biomass, under a limited range of temperatures or in the atmosphere of a single gas (mainly N_2). There is a lack of a broad comparison of the main pyrolysis parameters affecting the properties of n-BCs compared to b-BCs. As a result, these comparative studies between n-BCs and b-BCs are incomplete. Furthermore, there is a lack of research on n-BCs obtained from biowaste materials such as sewage sludge (SSL) (He et al., 2023). Additionally, the study of n-BCs from SSL is a relatively new area of research, and most of the previous studies have focused on SSL-derived b-BCs (Raj et al., 2021; Siatecka et al., 2021a). SSL is a significant source of biomass that is widely available and inexpensive, like other well-known feedstocks, and it is a very good material for pyrolysis (Kończak et al., 2019; Zhao et al., 2023; Zielińska and Oleszczuk, 2015). Converting SSL into biochar offers numerous ecological benefits and is a more sustainable solution than traditional disposal methods. It allows for long-term organic matter stabilization and reducing CO_2 emissions. Furthermore, it facilitates the closed-loop cycling of nutrients and eliminates organic pollutants and pathogens. Because of the diverse organic materials and different compositions of SSL compared to biomass-derived biochar, SSL-derived n-BCs may differ compared to biomass-derived n-BCs. To date, no research has investigated this issue.

The n-BCs usually have a larger specific surface area (S_{BET}) and smaller pore volume (V_P) compared to b-BCs (Oleszczuk et al., 2016), leading to an increased number of smaller pores with a higher S_{BET} . As a result, n-BCs exhibit a higher number of sites for adsorption and potential chemical reactions (Chausali et al., 2021; He et al., 2023; Rajput et al., 2022). Moreover, n-BCs may have a different chemical composition and porous structure, influencing their behavior and application (Lyu et al., 2017). Therefore, it is important to examine the properties of both n-BCs and b-BCs considering key factors, such as feedstock, pyrolysis temperature, and carrier gas, which impact biochar properties.

In the present study, b-BCs obtained from different feedstocks at various temperatures and carrier gasses were ball milled to produce different n-BCs. Based on the structural and physicochemical properties of b-BCs, we attempted to identify a trend for predicting the properties of n-BCs. It is possible to partially predict the properties of the resulting n-BCs by conducting a comprehensive characterization of b-BCs with different properties both before and post ball milling.

2. Materials and methods

2.1. Preparation of bulk biochar (b-BC)

A total of ten b-BCs were obtained from four types of feedstocks through a pyrolysis process. These types of feedstocks included woody

biomass (willow), agricultural residues (rice husk and oilseed rape) and anthropogenic biowaste (SSL). The details of the pyrolysis conditions were described in our previous research (Gul et al., 2021; Siatecka et al., 2021b; Stefaniuk and Oleszczuk, 2015; Yang et al., 2022). In our laboratory, we obtained b-BCs from willow (WL) and sewage sludge (SSL2-3), while b-BCs from other feedstocks (sewage sludge (SSL1), rice husk (RH) and oilseed rape (OSR)) were obtained from the UK Biochar Research Centre in collaboration with another research group. Briefly, feedstock, i.e., WL and SSL2-3, were dried and crushed (<2 mm) and then pyrolyzed at 450–750 °C under a nitrogen (N_2) or carbon dioxide (CO_2) atmosphere for 3 h (heating rate 10 °C·min⁻¹).

SSL1, RH, and OSR biochar were produced using a pilot-scale pyrolysis unit at the UK Biochar Research Centre. The pyrolysis unit consists of a rotary kiln with a heat tube length of 2.8 m, an inner diameter of 244 mm, an angle of 0.5°, and a 1–7 rpm rotational speed. Previous studies provide further details on the production conditions used (Mašek et al., 2018; UKBRC, 2019).

A pyrolysis temperature of 500–550 °C is the most widely used for biochar formation (Chen et al., 2017). Thus, this temperature was selected for comparisons between feedstocks and carrier gases. Finally, the obtained b-BCs were stored and frozen in a laboratory freezer (−80 °C) in sealed plastic jars before ball milling. The major pyrolysis conditions and labeling of the obtained materials are presented in Supplementary Information (SI).

2.2. Preparation of nanobiochar (n-BC)

The n-BCs were obtained following a previous method (Naghdi et al., 2017). Six grams of each b-BC was placed in a planetary ball mill (PM100, Retsch Corporation, German) in a 125 mL corundum jar with 7 corundum balls (ball diameter was 20 mm) (biochar-to-ball mass ratio was 1:100). The planetary ball mill was operated at room temperature with a speed of 440 rpm for 1.5 h, and the rotation direction was altered every 5 min with short intervals to control the temperature boost.

2.3. Characterization of bulk (b-BC) and nanobiochar (n-BC)

2.3.1. Physicochemical properties

Detailed information about n-BC and b-BC characterization is presented in the Supporting Information (SI). Briefly, the porous structure of n- and b-BCs was determined using low-temperature (−195.8 °C) nitrogen adsorption-desorption isotherms (ASAP 2420 V2.09, Micromeritics, USA). The specific and micropore surface area (S_{BET}/S_{micro}), total pore and micropore volume and average pore width were calculated based on the nitrogen desorption data (Michot and Villieras, 2013). The pH and electrical conductivity (EC) were determined using a previously described procedure (Kończak et al., 2020). One gram of each BC was dispersed into 10 mL of deionized water (solid/liquid ratio 1:10 (w/v)), and the pH/EC of the suspensions was measured using a digital pH and EC meter (HQ430d Benchtop Single Input, HACH, USA or CP-401, ELMETRON, Poland). The ash content of n- and b-BC samples was determined using the ASTM standard technique by burning 1 g of each sample at 760 °C for 6 h and weighing the residue after ashing (Abdel-Fattah et al., 2015). The ash content was calculated using the following equation:

$$\% \text{ Ash} = \frac{\text{weight after ashing} - \text{weight of crucible}}{\text{dry weight of BC sample}} \cdot 100\% \quad (1)$$

The structural properties and morphology of n- and b-BCs were characterized by a high-scanning electron microscope (FEI Quanta 3D FEG, USA). The SEM images were obtained with a resolution of 1–500 μm at 5 kV. The composition of elements (C, H, N) was determined using an elemental analyzer (CHN Analyzer EuroEA3000, EuroVector, Italy). The average results of each triplicate sample were reported. The total content of O was determined by the following equation:

$$\% O = 100\% - \% Ash - \% C - \% H - \% N \quad (2)$$

The functional groups of BCs were determined by FTIR-ATR spectra in the region of 400–4000 cm^{-1} with a resolution of 1 cm^{-1} (Nicolet 8700A, Thermo Scientific, USA). Additionally, the crystalline and amorphous nature of the carbon in the samples was determined by a Raman microscope equipped with a 514 nm laser diode (nVia Reflex, Renishaw, UK).

2.3.2. Hydrodynamic size and electrokinetic properties (zeta potential)

The hydrodynamic diameter of n-BCs was determined by dynamic light scattering (DLS) using a Zetasizer Nano ZS90 analyzer (Malvern Instrument Inc., UK) equipped with a universal dip cell. The intensity-weighted hydrodynamic particle size was determined assuming the Stokes-Einstein equation for spherical particles (Chen and Elimelech, 2008). The zeta potential of the n-BC suspensions was calculated from the electrophoretic mobility measurements and the Smoluchowski equation (Zetasizer Nano ZS, Malvern Instruments) (Yang et al., 2019). Briefly, 100 mg/L n-BC suspensions were prepared from the background electrolyte solution (10^{-3} M NaCl or deionized water) and treated with ultrasound for 1 min. Then, the zeta potential was measured in the pH range from 2 to 10, adjusted by adding 0.1 M HCl or NaOH solution. All measurements were performed 10 times, and the average values are reported.

3. Results and discussion

3.1. Surface area and porosity

The physicochemical properties of n-BCs and b-BCs are shown in Table 1. The b-BCs produced from agriculture wastes (RH and OSR) at 550 °C and woody biomass (WL) at 450 °C have total surface areas (S_{BET}) ranging from 2.90 to 7.20 m^2/g and were low compared to previous works regarding the same feedstocks (Hyväluoma et al., 2018; Palansooriya et al., 2022). For example, Zhang et al. (2020) and Siatecka et al. (2021) observed significantly higher S_{BET} values for RH- and WL-derived b-BCs (S_{BET} ranged from 182 to 297 m^2/g and 229 m^2/g ,

respectively) produced at temperatures of 500–600 °C. We observed a comparable S_{BET} but only for WL-derived b-BCs obtained at temperatures of 550–650 °C (Table 1). The S_{BET} values for the previously mentioned b-BCs were higher compared to the WL-derived b-BCs at 450 °C and ranged from 130.1 to 195.2 m^2/g . However, the WL-derived b-BC produced at the higher pyrolysis temperature (750 °C) again has a low S_{BET} value ($S_{\text{BET}} = 3.90 \text{ m}^2/\text{g}$). This may be due to the crumbly structure of the starting material, as suggested before (Yu et al., 2023). The b-BCs derived from SSL also have low S_{BET} (from 13.6 to 41.6 m^2/g), which is typical for this feedstock (Zhao et al., 2023; Zielińska et al., 2015).

The ball milling affected the S_{BET} of all tested b-BCs (Table 1). The observed higher porosity in n-BC may be attributed to the aggregation of n-BC rather than the development of pores in the n-BC structure. The type of feedstock used has the greatest influence on the S_{BET} value of n-BCs, followed by the pyrolysis temperature, and finally, the carrier gas during the pyrolysis process (feedstock > temperature > carrier gas). Generally, the n-BCs derived from woody and agricultural residues, which have a low S_{BET} in the bulk phase, had a high increase in S_{BET} (from 658 to 4160%). For b-BCs that were characterized by a high S_{BET} (b-BC-WL-550/650), ball milling had the least effect on S_{BET} . Moreover, for these materials, n-BCs exhibited similar surface charges, as indicated in Fig. 5a, along with a comparable average particle size in 1M NaCl (Table 3). After ball milling, the n-BCs received from SSL had a small S_{BET} (ranging from 15.0 to 98.4 m^2/g) compared to woody and agricultural residues-derived b-BCs, probably due to the high content of ash and inorganic matter, which is negatively correlated with S_{BET} ($|r| = 0.53$). For most woody and agricultural residue-derived n-BCs, the increase in S_{BET} was significantly higher (from 16 to 4160%) compared to SSL-derived n-BCs, where the increase in S_{BET} after ball milling ranged from 10 to 487%.

In terms of the carrier gas, it was observed that despite the lower initial S_{BET} values for b-BC-SSLs, using CO_2 as the carrier gas resulted in a greater enhancement of S_{BET} after ball milling compared to N_2 (Table 1). This may be attributed to the better release of volatile matter (e.g., CO, CO_2) from the carbon skeleton from BC produced in the CO_2

Table 1

Physicochemical properties of bulk (b-BC) and nanobiochars (n-BC) produced at various temperatures and conditions by ball milling.

Sample	S_{BET} [m^2/g]	S_{micro} [m^2/g]	S_{ext} [m^2/g]	V_p [cm^3/g]	V_{micro} [cm^3/g]	Pore width [nm]	pH	EC [$\mu\text{S}/\text{cm}$]	I_D/I_G	L_{α} [nm]
b-BC-WL-450	7.2	5.0	2.8	0.020	0.002	10.95	8.92	299	0.55	30.5
n-BC-WL-450	146.3	88.1	58.2	0.109	0.037	2.99	7.35	1157	0.50	33.5
b-BC-WL-550	195.2	170.5	25.3	0.091	0.075	1.85	9.02	270	0.60	27.9
n-BC-WL-550	195.8	142.9	52.4	0.143	0.060	2.92	7.43	1089	0.57	29.4
b-BC-WL-650	130.1	123.6	6.5	0.062	0.055	1.91	10.6	966	0.67	25.0
n-BC-WL-650	150.9	112.5	38.5	0.132	0.047	3.49	7.76	1253	0.62	27.0
b-BC-WL-750	3.8	16.4	1.6	0.024	0.008	2.48	10.98	486	0.77	21.8
n-BC-WL-750	160.7	120.3	40.5	0.136	0.050	3.39	7.98	1265	0.77	21.8
b-BC-RH-550	3.7	1.1	2.6	0.014	0.003	14.52	9.71	970	0.75	22.8
n-BC-RH-550	158.6	99.8	58.8	0.139	0.042	3.49	9.13	1328	0.65	25.3
b-BC-OSR-550	2.9	2.1	0.9	0.010	0.001	14.40	10.20	4840	0.73	22.9
n-BC-OSR-550	21.9	9.7	12.3	0.045	0.004	8.15	10.01	6880	0.71	23.6
b-BC-SSL1-550	13.6	2.6	11.1	0.055	0.084	22.11	8.18	535	0.67	20.2
n-BC-SSL1-550	15.0	1.2	13.8	0.075	0.025	14.69	6.82	2350	0.53	25.0
b-BC-SSL2-500	16.7	–	–	0.078	–	18.65	9.56	1573	0.78	21.5
n-BC-SSL2-500	98.3	46.7	51.7	0.135	0.020	5.50	7.66	2450	0.77	21.8
b-BC-SSL3-500	41.6	29.1	–	0.055	0.012	5.27	9.55	552	1.22	13.7
n-BC-SSL3-500	51.2	22.0	29.2	0.090	0.010	7.03	7.45	1358	0.79	21.2
b-BC-SSL3-CO2-500	31.5	21.9	–	0.055	0.009	6.26	8.72	352	1.10	15.2
n-BC-SSL3-CO2-500	83.5	40.3	43.2	0.119	0.017	5.70	7.44	1685	0.82	20.4

Where, b-BC = bulk biochar; n-BC = nanobiochar; WL = willow; SSL 1–3 = different sewage sludges; RH = rice husk; OSR = oilseed rape; 450,500,550,650,750 = temperature of pyrolysis; $\text{CO}_2 = \text{CO}_2$ as carrier gas; S_{BET} = specific surface area of biochar; S_{micro} = specific surface area of micropores; S_{ext} = external surface area of biochars; V_p = pore volume; V_{micro} = micropore volume; pH = in H_2O ; EC = electrical conductivity; I_D/I_G = the ratio of the D and G band intensities; L_{α} = crystallite sizes; The values are mean of three repetitions. Detailed information about sample origin and pyrolysis condition is provided in SI.

atmosphere. The zeta potential values obtained for n-BC-SSL (Fig. 5c) are consistently more positive, which can confer a negative charge to its surface, in comparison to plant-derived n-BC. This can lead to the formation of smaller agglomerates of BC nanoparticles. Consequently, for SSL-derived n-BCs, the increase in S_{BET} values was not as significant as observed for some WL-derived n-BCs. This outcome implies that the higher aggregation behavior influencing the higher S_{BET} values of n-BC is determined by both particle size and surface charge.

Slight differences ($p > 0.05$) in external surface area (S_{ext}) were observed for n-BC-WL-550 and n-BC-WL-650 (increase from 2 to 6 times), and the increase in S_{ext} was not significant for these BCs, as for n-BC-WL-450 and n-BC-WL-750, where a 26-fold increase was observed (Table 1). According to a recent study (Lyu et al., 2019), the S_{ext} expanded throughout the ball milling process, with a small effect on the internal surface area (S_{int}) because the fine biochar particles may clog the pores during ball milling. Thus, the milling process may influence the S_{ext} , resulting in a low increase in surface area. In our research, however, we noted the opposite trend, where significant differences in surface area (including S_{ext}) after ball milling were exhibited under different pyrolysis conditions and feedstocks. Swaren et al. (2022) observed that ball milling increases the S_{ext} of low-temperature biochar, enhancing surface adsorption. At higher pyrolysis temperatures (700 °C), S_{int} , S_{ext} , pore volume, and aromaticity increased, improving pore filling and π - π interaction removal mechanisms. The surface area is mainly determined by the amount of hemicellulose, lignin, mineral and organic components in the feedstock (Pasangulapati et al., 2012). After ball milling, biochar particles shrink, leading to an increase in the number of nano- and micropores and a decrease in pore diameter, leading to an increase in S_{BET} .

Fig. 1 presents low-temperature nitrogen adsorption/desorption isotherms determined for all obtained BCs. According to IUPAC classification, the isotherms were classified as type IV (Ertl et al., 1997) despite the kind of biochar (nano- or bulk-). Type IV isotherms are characteristic of mesoporous materials with a sharp increase in adsorption at high relative pressures (Calzaferri et al., 2022). Adsorption occurs in the mesopores of the material, and the hysteresis loop is typical of the H3 type. The type IV isotherm is often observed for materials with a narrow pore size distribution and a relatively uniform pore size (Thommes, 2010). The analysis of the isotherm curves indicated that the porosity of the b-BCs was poorly developed, and there was only slight adsorption observed across the entire range of relative pressures, except for b-BC-WL-550 and b-BC-WL-650 (Fig. 1). Conversely, the isotherms for n-BCs showed low adsorption values in the low range of relative pressures ($p/p_0 = 0-0.02$), indicating a significant content of nano- and micropores in the obtained n-BCs, which further confirmed the conclusions drawn based on structural parameters. The analysis of the structural data indicates porous materials with a considerable proportion of micropores (from 8.4% to 88.1% for b-BCs and from 9.3% to 45.6% for n-BCs, depending on the feedstock and conditions used). The n-BCs have pore volumes (V_p) that are 2–10 times higher than those of the b-BCs for most investigated biochar and vary depending on the feedstock,

temperature, and carrier gas used (Table 1).

The lowest values of V_p were noted for b-BCs (Table 1). Depending on the pyrolysis temperature, the highest increase in V_p after ball milling was observed for n-BC-WL-450 (5.5 times) and n-BC-WL-750 (5.7 times). Additionally, the highest increase in V_p was observed for n-BC-SSL3-CO2-500 (2 times) for these kinds of feedstock. Converting of b-BC to n-BC for SSL-derived BCs did not affect V_p as much as observed for plant-derived BCs because of the decomposition of the cellulose or hemicellulose backbone, the appearance of amorphous carbon and the compaction and agglomeration effects. When the pyrolysis temperature raised above 500 °C, the breakdown of hemicellulose and other organic compounds leads to the formation of an increased number of micropores within the BC (Amalina et al., 2022; Wang and Wang, 2019). The degradation of lignin and the rapid release of H_2 and CH_4 contribute to a significant augmentation in both S_{BET} and V_p (Chatterjee et al., 2020). The highest increase in V_p depending on feedstock was noted for n-BC-RH-550 (10 times). A notable increase in V_p was observed after ball milling for all b-BCs with initially low V_p values (Table 1). Conversely, b-BCs with initially high V_p did not show a significant increase. This similar pattern of changes was also observed for S_{BET} . This observation is crucial because the initial values of these parameters (S_{BET} and V_p) in b-BCs determine the alterations in these parameters after ball milling in n-BCs.

3.2. The pH and electrical conductivity (EC)

All tested b-BCs were strongly alkaline ($pH > 8$), and their conversion to n-BCs significantly decreased the pH values from 2 to 27% (Table 1), which could be related to the gradual loss of alkaline functional groups on the surface and/or exposure as a result of ball milling (Lyu et al., 2018). After ball milling, all investigated n-BCs had a neutral ($pH < 7$) or conserved alkaline pH (from 7.35 to 10.01). It was observed that RH- and OSR-derived b-BCs were the most resistant to pH changes after ball milling (no change or slight change of approximately 6%). The highest decrease in pH was observed from WL- and SSL-derived b-BCs, which behaved similarly (a decrease of approximately 20% for both feedstocks). Using CO_2 instead of N_2 resulted in greater resistance to pH changes in n-BCs after ball milling. Regarding the pyrolysis temperature, an increase in the temperature led to a higher decrease in pH values (Table 1).

The ball milling significantly increased the electrical conductivity (EC) for all b-BCs. The increase in the EC value indicates a better exchange of inorganic ions, such as Na^+ , K^+ , Ca^{2+} , and Mg^{2+} , which became more accessible after ball milling (Table 1) (Qu et al., 2016). The most significant changes in EC values were observed for n-BC-SSLs, where an increase ranged from 55.7 to 378.7%. The trend regarding feedstock dependency was as follows: sewage sludge > woody biomass > agricultural residues. There was no clear trend in the changes in EC values depending on pyrolysis temperature, as the values changed randomly. Considering the combined dependencies of feedstock and temperature, it can be concluded that the optimal temperature for

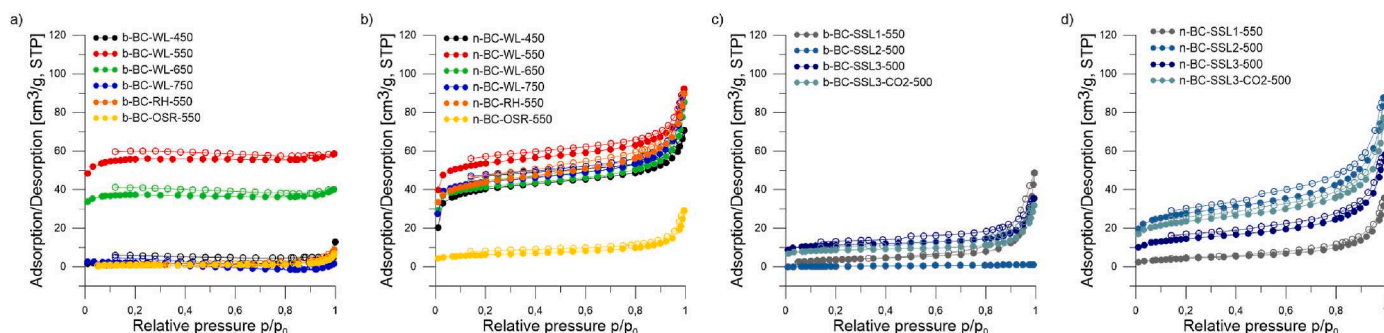


Fig. 1. Adsorption/desorption isotherms of obtained bulk- (a and c) and nanobiochars (b and d).

obtaining n-BCs with high EC values is 550 °C. Notably, a considerable change was observed in the choice of carrier gas, with CO₂ providing a 2-fold higher increase in the EC value of n-BCs compared to N₂. EC is one of the parameters proposed for assessing the quality of biochar. As observed, the reduction in particle size significantly increases this value, which may disqualify certain types of n-BCs as soil amendments. This is most likely due to an increased ash content. When ash enters the water, it raises the measured EC value of the biochar-water suspension. The decrease in particle size often leads to an increase in S_{BET}, which in turn enhances the number of active sites available for chemical reactions. Consequently, this increased surface area can facilitate higher EC in the biochar material (Hoffmann et al., 2019). It is essential to consider that higher EC in biochar may not always be desirable for soil amendment purposes. This is because elevated EC can increase soil salinity levels and alter the soil's ability to hold onto water and nutrients. Excessive salt content from high EC levels can lead to soil degradation, hinder plant growth, and negatively affect overall soil health (Li et al., 2018; Wang et al., 2017).

3.3. Elemental composition and ash content

The elemental composition of b-BCs was influenced by pyrolysis temperature and feedstock, as previously observed for biochars produced from similar feedstocks (Table 2). The C content increased with increasing pyrolysis temperature, correlated with O and H losses for WL-derived b-BCs. In contrast, previous studies (Song and Guo, 2012) indicated a reduction in the N content of BCs with increasing pyrolysis temperature. Among the feedstocks, b-BCs from SSL had significantly lower C content due to the high content of mineral components and lower values of N, H, and O compared to b-BCs from woody biomass (WL) and agriculture wastes (RH and OSR), which is also typical for this kind of biochar (Wang et al., 2022; Wijitkosum, 2022). Additionally, no significant changes in C, N, H, and O content after using different carrier gases were observed for b-BC-SSLs, as noted before (Stefaniuk and Oleszczuk, 2015).

In general, the elemental composition was influenced by ball milling (Table 2). For n-BCs produced from woody biomass (WL) at different

Table 3
The nanobiochar particle size in the analyzed background electrolytes.

Nanobiochar	Average particle size [nm]	
	Doubly distilled water	1M NaCl
n-BC-WL-450	280.0 ± 28.9	54.3 ± 4.8
n-BC-WL-550	310.4 ± 31.3	48.1 ± 3.9
n-BC-WL-650	256.5 ± 23.7	43.5 ± 4.4
n-BC-WL-750	173.0 ± 12.9	38.9 ± 2.8
n-BC-RH-550	252.0 ± 30.7	87.9 ± 6.4
n-BC-OSR-550	176.6 ± 18.0	69.9 ± 7.8
n-BC-SSL1-550	177.9 ± 11.9	59.3 ± 5.9
n-BC-SSL2-500	188.9 ± 15.8	65.3 ± 7.2
n-BC-SSL3-500	226.7 ± 20.8	30.0 ± 3.0
n-BC-SSL3-CO2-500	198.4 ± 14.7	57.6 ± 5.8

Where, n-BC = nanobiochar; W = willow; SSL 1–3 = different sewage sludges; RH = rice husk; OSR = oilseed rape; 450,500,550,650,750 = temperature of pyrolysis; CO₂=CO₂ as carrier gas; Average particle size = by volume mean; The values are mean of ten repetitions. Detailed information about sample origin and pyrolysis condition is provided in SI.

temperatures, the C, N, and H contents were significantly lower than those of their bulk counterparts. Depending on the temperature, the C, N, and H contents in the n-BCs were lower than those in the b-BCs from 17 to 35%, from 13 to 58% and from 29 to 88%, respectively. Ball milling had the least effect on the elemental composition of WL-derived biochar obtained at the lowest temperature. This could be attributed to the more difficult breaking of bonds in the biochar structure, which are weaker with increasing pyrolysis temperature. Depending on the pyrolysis temperature, the O content also varied for WL-derived n-BCs. For n-BC-WL450 and n-BC-WL-750, the O content slightly increased by 10% and 8%, respectively, while for n-BC-WL-550 and n-BC-WL-650, the O content decreased (by 24 and 10%, respectively). For n-BCs produced from agriculture wastes (RH and OSR), the C, H and O contents were significantly lower in n-BCs than their b-BCs from 27 to 55%, 66–84% and 9–10%, respectively. The N content increased from 36 to 55% depending on the agricultural waste used. This can be explained by the occurrence of N in complex structures that are resistant to degradation by ball milling. Moreover, due to the reduction in the particle size, N is

Table 2
Elemental (CHNO) and ash content of bulk- and nanobiochars fabricated by ball milling.

Sample	C [%]	H [%]	N [%]	O [%]	O/C [molar ratio]	N/C [molar ratio]	H/C [molar ratio]	(O + N)/C [molar ratio]	Ash [%]
b-BC-WL-450	78.87	3.46	1.49	11.50	0.1094	0.0162	0.5264	0.1256	4.68
n-BC-WL-450	65.66	2.46	1.29	12.66	0.1446	0.0168	0.4496	0.1614	17.93
b-BC-WL-550	79.23	2.69	2.24	11.51	0.1089	0.0242	0.4074	0.1331	4.34
n-BC-WL-550	54.46	1.53	0.93	8.71	0.1199	0.0146	0.3371	0.1346	34.37
b-BC-WL-650	83.35	1.72	1.46	8.79	0.0791	0.0150	0.2476	0.0941	4.68
n-BC-WL-650	54.36	0.21	1.06	7.90	0.1090	0.0167	0.0464	0.1257	36.47
b-BC-WL-750	85.30	1.53	0	7.42	0.0653	–	0.2152	0.0653	5.75
n-BC-WL-750	59.73	0.31	0.93	7.98	0.1002	0.0133	0.0623	0.1136	31.05
b-BC-RH-550	47.97	2.07	0.62	13.71	0.2143	0.0111	0.5178	0.2254	35.63
n-BC-RH-550	34.99	0.71	0.96	12.52	0.2683	0.0235	0.2435	0.2919	50.82
b-BC-OSR-550	69.95	2.38	1.19	17.43	0.1869	0.0146	0.4083	0.2015	9.05
n-BC-OSR-550	31.52	0.38	1.62	15.64	0.3722	0.0441	0.1447	0.4163	50.83
b-BC-SSL1-550	26.22	2.00	3.33	20.63	0.5901	0.1089	0.9153	0.6990	47.82
n-BC-SSL1-550	28.51	1.23	3.75	–	–	0.1127	0.5177	0.1127	72.78
b-BC-SSL2-500	25.61	1.16	2.95	19.55	0.5725	0.0987	0.5435	0.6713	61.53
n-BC-SSL2-500	26.41	0.53	3.24	8.22	0.2333	0.1052	0.2408	0.3385	61.60
b-BC-SSL3-500	20.53	0.74	2.52	3.69	0.1348	0.1052	0.4325	0.2400	72.31
n-BC-SSL3-500	23.06	0.53	3.04	7.83	0.2548	0.1130	0.2758	0.3677	65.54
b-BC-SSL3-CO2-500	20.82	0.60	2.47	4.15	0.1495	0.1017	0.3458	0.2512	71.60
n-BC-SSL3-CO2-500	23.93	0.37	3.14	–	–	0.1125	0.1855	0.1125	76.63

Where, b-BC = bulk biochar; n-BC = nanobiochar; WL = willow; SSL 1–3 = different sewage sludges; RH = rice husk; OSR = oilseed rape; 450,500,550,650,750 = temperature of pyrolysis; CO₂=CO₂ as carrier gas; C, H, N, O [%] = total content; O [%] = total content calculated by difference; O/C, N/C, H/C, (O + N)/C = molar ratios; Ash [%] = total content; The values are mean of three repetitions. Detailed information about sample origin and pyrolysis condition is provided in SI.

more exposed on the n-BC surface. In this case, some of C could have undergone oxidation during ball milling, as the newly forming biochar nanoparticles can be highly reactive and interact with oxygen from the air. A different trend compared to woody biomass (WL) and agriculture wastes (RH and OSR) was observed for SSL-derived n-BCs. The C and N contents were higher (increased from 9 to 15% and 10–27%, respectively), and the H content was lower in the n-BCs (decreased from 28 to 54%) than in the b-BCs. The O content was higher for b-BCs (from 100 to 112% - except for n-BC-SSL2-500) than for n-BCs. Ball milling entirely reduced the O content in n-BC-SSL1-550 and n-BC-SSL3-CO2-500. The different behaviors of SSL-derived biochar produced at CO₂ and N₂ may show that applying different gases can significantly affect the O content during ball milling. In biochar produced in N₂, an increase was observed, while in biochar produced in CO₂, a reduction in O content occurred.

The pyrolysis temperature and feedstock influenced the ash content of the b-BCs. The pyrolysis temperature can lead to increased degradation and combustion of organic components, resulting in more non-combustible minerals and ash in the WL-derived b-BCs. In terms of feedstock, b-BCs produced from SSL exhibited higher ash content compared to b-BCs derived from woody biomass (WL) and agricultural wastes (RH and OSR). This is because SSL-derived b-BCs contain higher amounts of inorganic mineral substances such as phosphorus, nitrogen and sulfur compounds and heavy metals (Zhao et al., 2023). These inorganic substances contribute to the higher ash content in SSL-derived b-BCs. Moreover, no significant changes in the ash content after using different carrier gases were observed for b-BCs.

After ball milling, the ash content increased, which was confirmed by previous research (Qu et al., 2016). This phenomenon was more apparent for BCs produced from the woody biomass (WL) and agriculture residues (RH and OSR) (increase from 43 to 692%) than that of the SSL-derived n-BCs. For SSL-derived n-BCs, the ash content after milling mostly increased but was only slightly higher in n-BCs than in b-BCs (from 0.1 to 52%). The SSL-derived b-BCs were rich in ash (>47%), which is related to the composition of the feedstock reached in minerals and heavy metals (Cao et al., 2018). A significantly lower ($p < 0.05$) increase in ash after milling SSL-derived n-BCs compared to plant-based n-BCs was probably related to the crumbling of the different components during ball milling. SSL-derived b-BCs might have a higher proportion of mineral-rich or ash-rich components compared to plant-based b-BCs (Yin et al., 2021). During ball milling, the mechanical forces applied can cause these mineral-rich components to crumble or break down into smaller particles, resulting in a less pronounced increase in ash content. Moreover, the ash content in SSL b-BCs is significantly higher than in plant-based b-BCs; it is anticipated that the relative change will be much smaller. A collision between metallic balls and b-BC may reduce the size of biomass and generate some high energy, which increases the temperature because some amorphous carbon becomes volatile and leaves some inorganic components (Zhuang et al., 2021). Because plant-derived b-BCs contained more C than SSL-derived b-BCs, the effect was more visible for plant-derived b-BCs. The previous literature also confirmed that the ash content in n-BC strictly depends on feedstock. For example, Anupama and Khare (Anupama, 2021) observed that n-BC prepared from manure was characterized by a higher ash content than plant residue-derived BC.

3.4. Molar ratio

The elemental molar ratios were calculated to assess the aromaticity (H/C ratio) and polarity (O/C, (N + O)/C ratio) of the BCs (Elnour et al., 2019). The higher the aromaticity and degree of carbonization of a specific substance, the lower the calculated H/C value. Greater ratios of O/C and (N + O)/C suggest the material has more polar functional groups on the surface (increased polarity). Moreover, a H/C ratio greater than 1.0 indicates that the material may include a significant quantity of organic residues such as polymeric -CH₂, lignin, cellulose, and hemicellulose (Chen and Elimelech, 2008; Sevilla and Fuertes, 2009).

The change trends in H/C and O/C ratios were quite different among the b-BCs obtained from woody biomass (WL) and agriculture wastes (RH and OSR) and SSL-derived BCs (Fig. 2). The b-BCs derived from SSL (except for b-BC-SSL3-500 and b-BC-SSL3-CO2-500) had from 2 to 5 times higher polarity compared to b-BCs from agriculture waste (RH and OSR) and woody biomass (WL). Regarding the pyrolysis temperature, a slight decreasing trend for polarity and an increasing trend for aromaticity with increasing temperature were observed for WL-derived b-BCs. Slight changes were also noted regarding the carrier gas, where an increase in polarity and in aromaticity were observed for SSL-derived b-BCs (Fig. 2).

Based on the van Krevelen diagram (Fig. 2), it can be concluded that the changes in the H/C and O/C ratios after ball milling were not significant enough to change the biogeochemical character of n-BCs, which can be classified as the coal/black carbon class, such as b-BCs. All observed trends in H/C and O/C ratios were the same for both b-BCs and n-BCs regarding the type of feedstock, pyrolysis temperature and carrier gas. Additionally, after ball milling, as the particle size decreased, the H/C ratio decreased and O/C increased (except for n-BC-SSL1-550, n-BS-SSL2-500 and n-BS-SSL3-CO2-500), indicating more aromaticity and more O-containing functional groups (increased polarity) in the n-BCs. Certainly, a notable change in character from hydrophobic to hydrophilic can have a profound impact on the behavior of n-BCs in soils compared to b-BCs. Furthermore, the change in character from hydrophobic to hydrophilic can influence the interactions of n-BCs with organic and mineral substances in the soil and with microorganisms. These interactions are crucial in nutrient cycling and organic matter decomposition, impacting soil fertility.

3.5. Morphology and chemical structure

Fig. S1 shows the SEM images of the b-BCs and corresponding n-BC particles. For plant-derived n-BCs, noticeable changes in morphology are evident. The b-BCs exhibit elongated, fibrous particles with visible pores, while after ball milling, these particles become more rounded and compact. In contrast, no distinct differences are observed in the shapes and forms between SSL-derived n-BCs and b-BCs. However, it is evident that for n-BCs, aggregates composed of smaller, more rounded particles are visible. In all n-BCs, angular particles were observed, which formed concentrated aggregates (Fig. S1). The SEM images of n-BC showed that the dominant materials were almost uniform in size and shape with an aggregate diameter of approximately 2 µm. For all n-BCs, primary small particles (<100 nm) with flat and smooth structures were observed (Fig. S1). Gnaneshwar and Sabarikirishwaran (2015) also reported the polygonal morphologies of nanoparticles of carbon obtained by crushing burnt coconut shells. The elemental analysis indicated that the surface of all n-BCs was composed of carbon and oxygen.

3.6. Raman spectroscopy

Raman spectroscopy can be used to analyze the structure of graphene, and the identification of selected peaks (peak D 1350 cm⁻¹, peak G 1575 cm⁻¹, and peak 2D 2710 cm⁻¹) may provide valuable information about the graphene structure. The ID/IG ratio was also calculated (Table 1) to evaluate the number of layers in graphene. The Raman spectra of both the b-BC and n-BCs demonstrated the characteristic two peaks D and G (at 1350 cm⁻¹ and 1575 cm⁻¹), which can be assigned to sp³ and sp² carbon configuration, respectively. The band at 1350 cm⁻¹ corresponds to disordered carbon or defective graphitic structures, while the band at 1575 cm⁻¹ corresponds to the allowed Raman transition for large graphite crystals and is thus indicative of graphitic layers. An increase in pyrolysis temperature resulted in a decrease in the peak intensity of the Raman spectra and an increase in intensity ratios of D to G bands (I_D/I_G), which indicates a higher level of structural disorder and defects. This trend was observed for WL-derived b-BCs and n-BCs (Fig. 3a–d). Regarding the carrier gas, no differences were observed in

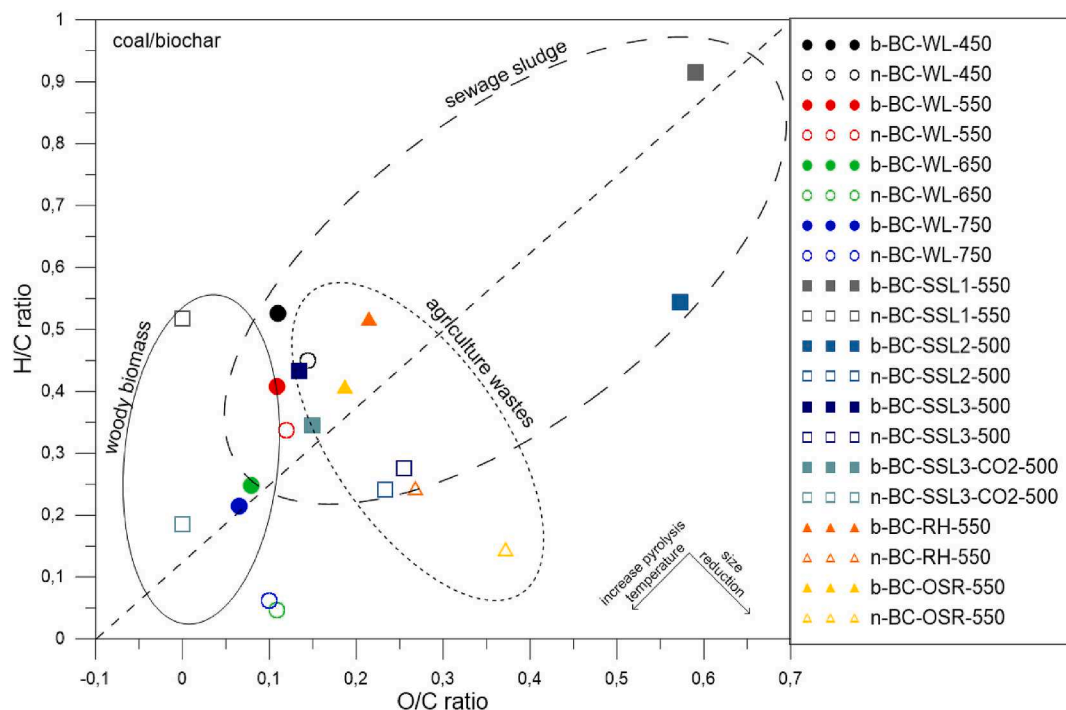


Fig. 2. The van Krevelen plot for elemental data of bulk- and nanobiochars.

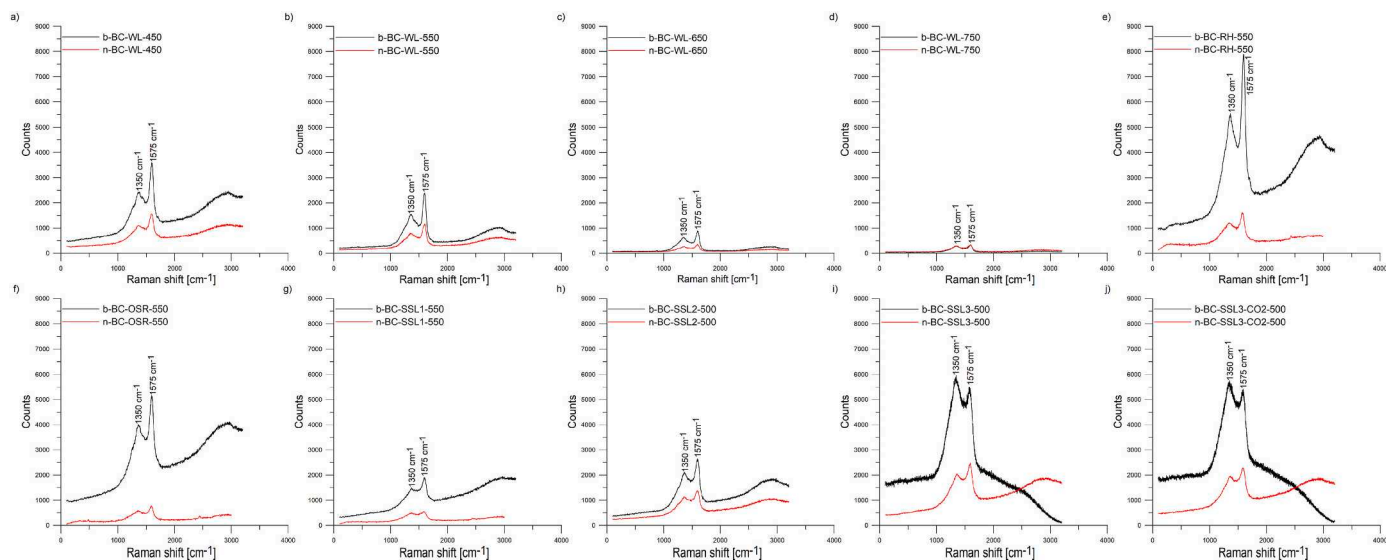


Fig. 3. Raman spectra of obtained bulk- and nanobiochars.

the Raman spectra for both the b-BCs and n-BCs (Fig. 3i and j). The intensity ratios of D to G bands (I_D/I_G) are close to 1.0 for both b-BC-SSL3-500 (1.22) and b-BC-SSL3CO2-500 (1.10), suggesting that they have comparable contents of amorphous and graphitic carbon. Regarding the feedstock, changes in Raman spectrum intensity for b- and n-BCs were observed following the trend of agriculture wastes > woody biomass > sewage sludge (Fig. 3). The highest intensity ratios of D to G bands (I_D/I_G) were observed for SSL-derived b-BCs and agriculture wastes-derived b-BCs. The noticeable difference in the spectra between the b- and n-BCs was in the intensity of the peaks, except for the spectra between b-BC-WL-750 and n-BC-WL-750. In general, the crystalline structure (Fig. 3 and Table 1) of materials after ball milling changed significantly under the influence of high energy input during ball milling, affecting the amorphization of n-BCs. This is clear from the

decreasing intensity ratios of D to G bands (I_D/I_G), indicating fewer defects and a more ordered structure, along with a higher content of amorphous carbon. Amorphization occurs because of the build-up of flaws in milled crystals, resulting in a severely defective crystalline phase (Deguchi et al., 2006; Shah et al., 2015). An increase in the size of graphite crystallites (increase in L_w) after ball milling was also observed. This could be attributed to the agglomeration (or sticking) of individual graphene layers at higher temperatures during the planetary movement of balls during milling.

3.7. FT-IR analysis

The FT-IR spectra of the b- and n-BCs are shown in Fig. 4. The FT-IR spectra of the examined BCs are complex due to multiple bands

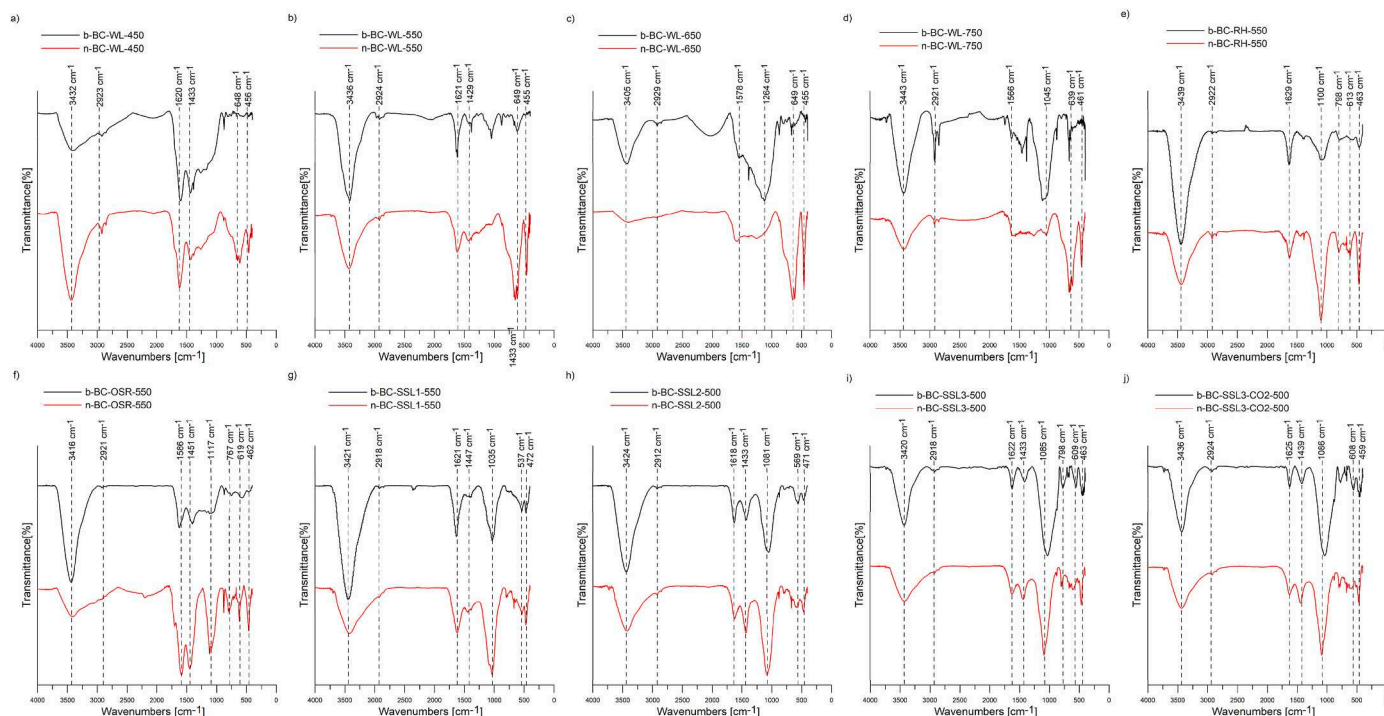


Fig. 4. FT-IR spectra of obtained bulk- and nanobiochars.

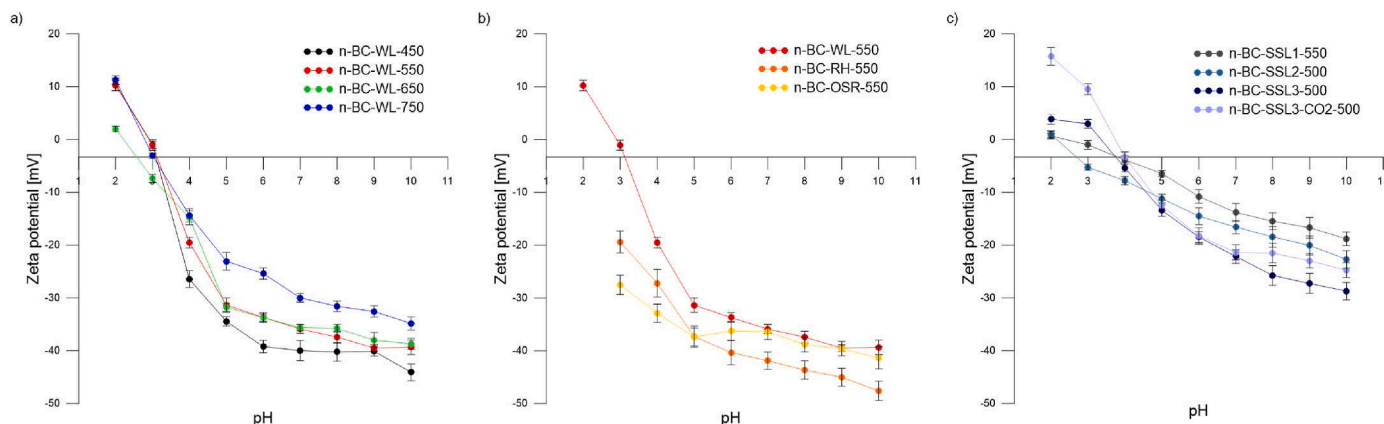


Fig. 5. Comparison of the zeta potential curves for nanobiochars (10^{-3} M NaCl as a background electrolyte) obtained from willow (a), rice husk and oilseed rape* (b) and sewage sludge (c). *Except at pH = 2 because measurements were not feasible.

corresponding to functional groups on the biochar surface at various wavenumbers (Ilić et al., 2022). There is no clear difference in the spectra between the b-BCs and n-BCs. The spectra differ only in the intensity of the peaks. Stronger differences can be noticed between the FT-IR spectra of the b- and n-BCs obtained from different feedstocks (Fig. 4). In all b- and n-BCs, the broad peak located at 3400 cm^{-1} (attributed to -OH stretching vibrations of the carboxylic groups or water adsorbed in pores) (Ternero-Hidalgo et al., 2016), weak peak at 2920 cm^{-1} (the presence of aliphatic -C-H bands and = C-H stretching in aromatic compounds) (Gromov et al., 2017) and the strong peaks at approximately $1430\text{--}1621\text{ cm}^{-1}$ (represented the -C=C and -C=O stretching vibration in aromatic ring supported the existence of carboxylic and phenol groups) (Cordero-Lanzac et al., 2018) can be distinguished. The peaks of -C=C and -C=O stretching vibrations in the aromatic ring gently shifted to a higher wavenumber due to the weakening of the conjugation effect after ball milling (Gromov et al., 2017). The bands at $1200\text{--}1000\text{ cm}^{-1}$ correspond to -C-H deformation vibrations in the aromatic structures. The bands in the $1060\text{--}783\text{ cm}^{-1}$ range

are related to -Si-O vibrations of inorganic materials in BCs. Finally, the bands at 950-600 cm^{-1} in BCs are attributed to -C-H bending in aromatic compounds (Barroso-Bogeat et al., 2015). For WL-derived b-BCs produced at different temperatures, the greatest changes in the FT-IR spectra after ball milling were noticeable in the range 1750-500 cm^{-1} (Fig. 4a-d). In the range 1750-500 cm^{-1} , the predominant vibrations originate from aromatic rings (-C=C and -C=O stretching vibrations, -C-H deformation vibrations etc.). Changes in this range may indicate increased aromaticity, confirmed by the reduction in the H/C ratio values for n-BCs. No changes in the FT-IR spectra were observed for SSL-derived and RH- and OSR-derived b- and n-BCs, despite the different origins of the feedstocks and applied gasses (Fig. 4e-j). These results showed that the n-BCs have broad functional groups and aliphatic chains like those of b-BCs, implying their high environmental reactivity.

3.8. Size of the n -BCs

The background electrolyte significantly affected the values of the

particle size potential (Table 3). A broad range of particle sizes characterized the n-BCs obtained during ball milling. For n-BCs suspended in deionized water, the particle size ranged from 2.5 (for n-BC-OSR-550) to 7 times larger (for n-BC-SSL3-500) compared to the values obtained in 1 M NaCl. Differences resulted from particle aggregation occurring, especially in distilled water. An increase in the solution ionic strength prevented the particles from merging into larger aggregates due to electrostatic repulsion forces.

Comparing the particle size (in 1 M NaCl) of the n-BCs obtained from different feedstock materials at 500 or 550 °C, the smallest aggregates were observed for n-BC-SSL3-500, whereas the largest particles occurred in the n-BC-RH-550 suspension. The n-BCs obtained from the agricultural waste (RH and OSR) were characterized by a high elemental carbon and ash content (Table 2). Including the presence of numerous surface oxygen-containing groups (confirmed by CHNO and FT-IR measurements), it could be concluded that the surface of biochar particles is filled with oppositely charged active sites. Their mutual attraction causes the particles to clump together into larger aggregates. For n-BC-SSL2-500, an increase in the inorganic ash content and a simultaneous decrease in the number of oxygen groups (usually acquiring a negative charge in the solution) contributed to the appearance of electrostatic repulsion forces, which in turn resulted in a smaller particle size. The mentioned parameter analysis for n-BC-WL-550 indicated greater participation of π - π type interactions in aggregate formation. Comparing the results measured for the n-BCs obtained from the same feedstock but in the atmosphere of different gases showed that inert N₂ positively affected particle size reduction. Despite the similar contents of carbon and ash, the differences observed for n-BC-SSL3-500 and n-BC-SSL3-CO2-500 were a consequence of the oxygen content changes in the biochar. The introduction of oxygen-containing groups on the n-BC-SSL3-500 particle surface increased the repulsive forces between similarly charged active sites found on the different particles and, as a result, the formation of smaller aggregates. An interesting relationship was observed for the WL-derived n-BCs. The data showed that the higher the pyrolysis temperature, the smaller the particles formed after ball milling. This could be related to the greater thermal degradation of the feedstock at higher temperatures, which facilitated the formation of nanoparticles. Cracks and damage to the cellulose backbone occurring during pyrolysis make the subsequent processes of freezing and ball milling more effective in obtaining nanoparticles (Gustafsson et al., 2000; Yang et al., 2020).

3.9. Zeta potential

At an environmentally relevant pH range (from 4 to 10), all the analyzed n-BCs were negatively charged (Fig. 5). The shape of the zeta potential curves depends on the surface functional group number and reflects their ionization behavior (Dougherty et al., 2008). In the case of the WL-derived n-BCs, the FT-IR spectra indicated that increasing the pyrolysis temperature tends to decrease the polar surface functional group intensity (Fig. 4a–d). Dehydration of the cellulose and ligneous components of the feedstock is reflected in the zeta potential curve course. The zeta potential values obtained for n-BC-WL-750 are more positive, irrespective of the background electrolyte used, than for n-BC-WL-450. This is attributed to the higher presence of oxygen surface groups in n-BC-WL-450, which can confer a negative charge to its surface. Comparing the FT-IR spectra of both n-BCs, a strong band attributed to the out-of-plane aromatic -C-H bending vibrations (with a maximum of approximately 625 cm⁻¹) could also be seen. This indicated that the appearance of more aromatic clusters directly affected the smaller number of charged active sites located on the surface of the biochar particles. A similar temperature-dependent relationship was noted previously (Hong et al., 2019). The comparison of the zeta potential measured for the three plant-derived n-BCs is presented in Fig. 5b. The course of the zeta potential curves varied in the pH range from 2 to 4. The positive charge of n-BC particles was observed only for

n-BC-WL-550 at pH 2. At pH 3, the n-BC-RH-550 and n-BC-OSR-550 suspensions showed negative values. As the solution pH increased, many negative charges were present on the particle surface. Above pH 5, the electrokinetic potential curves were similar and partially overlapped (especially for n-BC-WL-550 and n-BC-OSR-550). Under basic pH conditions, the lowest values were obtained for n-BC-RH-550. The reason was the differences in the structure and composition of the feedstock used for biochar production.

The positive zeta potential values for n-BC-WL-550 resulted from the presence of oxygen surface groups, including hydroxyl groups (Fig. 4b), capable of obtaining a positive charge under these conditions. Although this biochar was characterized by the lowest nitrogen and oxygen content (compared to the n-BC produced from RH and OSR), the developed porous structure provided more surface groups at the interface. Silica and other inorganic substances associated with the ash content, as well as the oxygen-containing groups, were responsible for the negative zeta potential of the n-BC-RH-550 and n-BC-OSR-550 samples. The former was characteristic of biochar obtained from the risk husks. In the analyzed case, this fact could be confirmed in the FT-IR spectrum (Fig. 4e and f). An intense band visible in the wavelength range of 1250–1000 cm⁻¹ could also be assigned to the silanol moiety stretching and bending vibrations. Furthermore, in the case of the n-BC-RH-550 and n-BC-OSR-550 n-BCs, the strong bands related to the vibrations of -OH or C=O groups belonging to cellulose structures can be identified in the FT-IR spectra.

Data showing changes in the zeta potential as a function of the solution pH (in 0.001 M NaCl) for SSL-derived n-BCs are presented in Fig. 5c. At pH 2 and 3, positively charged particles were observed, whereas the highest value was noted for n-BC-SSL3-CO2-500 ($\zeta = 15.75$ mV). This could be attributed to more positively charged surface groups located on the solid-liquid interface. An increase in the solution pH contributed to the gradual decrease in the electrokinetic potential associated with surface oxygen group dissociation above pH 4 and negative charge accumulation in the diffusion layer of the nanocarbon particles. At pH 10, the lowest values were obtained for the samples in the following order: n-BC-SSL3-500 < n-BC-SSL3-CO2-500 < n-BC-SSL2-500 < n-BC-SSL1-550. The zeta potential curve course could be explained by quantitative and qualitative changes in the surface functional groups and the content of inorganic substances. It can also be concluded that the positively charged active sites could be in the n-BC-SSL3-CO2-500 structure as a result of the structural defects related to the presence of inorganic impurities, e.g., divalent, and trivalent metal ions. These conclusions correlated with the presence of inorganic substances confirmed in the FT-IR spectra – a band with a maximum of approximately 1100–1000 cm⁻¹ could be attributed to the inorganic compound vibrations as silica, carbonates, phosphates, and nitrates in the biochar structure (Fig. 4g–j) (Zielińska et al., 2015). In the case of n-BC-SSL3-500 and n-BC-SSL2-500, characterized by lower nitrogen and ash contents, made the contribution of the above mentioned factors to the zeta potential smaller. On the other hand, the lack of a large number of positively charged groups at pH 2–3 for n-BC-SSL1-550 (with similar ash and nitrogen contents as n-BC-SSL3-CO2-500) followed by the lower availability of nitrogen-containing groups and crystal lattice defects caused by a less developed porous structure. The S_{BET} for the n-BC-SSL3-CO2-500 sample was 5.5 times larger than that for n-BC-SSL1-550, whereas the micropore surface area was already 33 times larger. The inorganic compound content and the nitrogen and oxygen surface groups significantly determined the zeta potential curve course under alkaline conditions. Above pH 8, the curves' order changed compared to the acidic solutions. The lowest electrokinetic potential values were observed for the n-BC-SSL3-500 suspension and the highest for the n-BC-SSL1-550. It should be noted that under these conditions, the electrokinetic potential values resulted from two opposite phenomena: dissociation of the oxygen functional groups and deprotonation of the nitrogen-containing groups. Additionally, a significant contribution was made by the presence of impurities in the form of inorganic

substances. It is known that the presence of divalent and trivalent cations caused diffusion layer compression, and hence the higher zeta potential measured for the n-BC-SSL3-CO2-500 sample in comparison to n-BC-SSL3-500 characterized by the lower ash content (Torrie et al., 1984). Moreover, in the case of n-BC produced in CO₂, the nitrogen functional groups on the BC' surface were gradually deprotonated at pH > 8. This process was reflected in higher zeta potential values compared to the n-BC-SSL3-500 suspension. For the other two SSL-derived n-BCs, the electrokinetic potential corresponds mainly to the oxygen groups receiving the negative charge under these conditions. Lower values of the n-BC-SSL3-500 zeta potential (relative to the n-BC-SSL1-550 sample) could be attributed to more surface hydroxyl groups. This was confirmed by the peak assigned to the lignin macromolecule skeletal vibration with a maximum of 1508–1445 cm⁻¹. As in acidic pH, the small specific surface area and the lack of a developed porous structure of n-BC-SSL1-550 allowed only a limited number of surface functional groups to participate in forming charges at the solid–liquid interface.

4. Conclusion

Feedstock, pyrolysis conditions, and properties of b-BC influence the properties of n-BCs. This study found that ball milling increased specific and external surface area, enhanced aromaticity, and reduced polarity of n-BCs. Additionally, pH, electrical conductivity, and elemental composition changes were observed. The study demonstrated that the properties of n-BCs depend significantly on those of b-BCs, with initial b-BC characteristics predicting resulting n-BC properties. Higher pyrolysis temperatures produced n-BCs with smaller particle sizes, higher aromaticity, and lower polarity.

These findings have significant practical implications for various n-BCs applications, particularly in environmental contexts. Tailoring the production n-BCs to possess specific attributes holds the potential to enhance their effectiveness in addressing environmental challenges, such as improving soil nutrient retention and water-holding capacity, as well as enhancing pollutant adsorption in water treatment systems.

CRediT authorship contribution statement

Monika Raczkiewicz: Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Iwona Ostolska:** Methodology, Investigation. **Ondřej Mašek:** Writing – review & editing, Methodology. **Patryk Oleszczuk:** Writing – review & editing, Supervision, Resources, Conceptualization.

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.jclepro.2024.142456>.

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Supplementary material for:

Effect of the pyrolysis conditions and type of feedstock on nano-biochars obtained as a result of ball milling

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This file includes:

Table S1

Figure S1

Physicochemical analysis

The analysis of the content of total elemental carbon (C), hydrogen (H) and nitrogen (N) as well as sulfur (S), oxygen (O) was determined with an elemental analyzer (MicroCube, Elementar, Germany), where 2 mg of samples were placed in a sheet metal boat and heated in an incineration furnace at a temperature range of 25-1150°C. In the carbon mode, the pressure He was 0.12MPa and the flow rate was 200 ml / min; The O₂ pressure was 0.2MPa and the flow rate was 11 ml/min. The temperature of the reflux furnace was 850°C. In the oxygen mode, the pressure He was 0.12MPa and the flow rate was 130 ml / min; in this mode, there is no need for a reduction furnace.

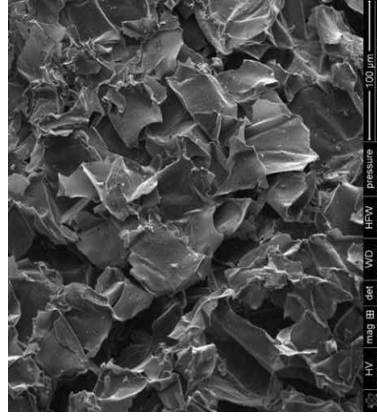
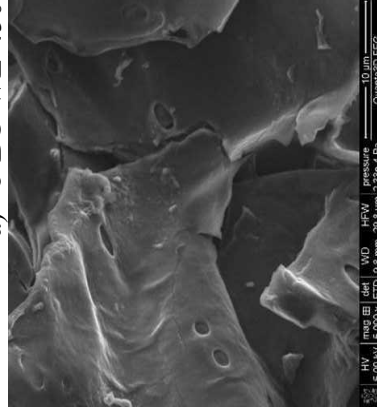
The specific surface area (S_{BET}) and the volume of biochar micropores were determined by the one-point Barrett-Brunauer-Emmett-Teller (BET) method using a BET analyzer (ASAP 2020, American Mike, USA). 100 mg of biochar powder was placed in a test tube at 120 °C overnight for degassing, and then the isothermal N₂ adsorption at 77K was measured. Pore volume was calculated by the Barrett-Joyner-Halend (BJH) method.

Fourier transform infrared (FTIR) spectra of biochar were obtained from powdered samples mixed with potassium bromide in a mass ratio of 1: 100 using FTIR spectroscopy (Variance 640-IR, USA). The spectra of all samples were recorded with a resolution of 4 cm⁻¹ in the area of 400-4000 cm⁻¹ and for each sample it was scanned 32 times. Additionally, the crystalline and amorphous nature of the carbon in the samples was determined by a Raman microscope equipped with a 514 nm laser diode (nVia Reflex, Renishaw, UK).

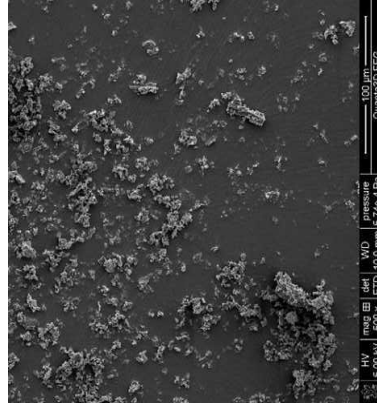
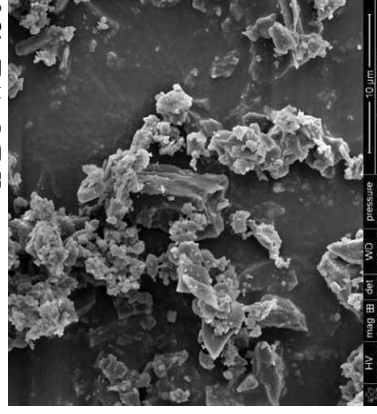
The morphology of the samples were analysed with a scanning electron microscope (SEM, VEGA3 TESCAN, Czech Republic) at an accelerating voltage of 20 kV, biochar samples were coated with Au prior to measurement.

Supplementary Figure

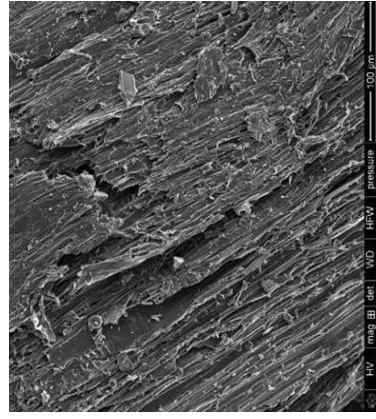
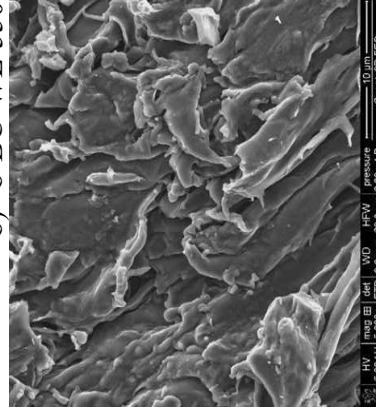
a) b-BC-WL-450



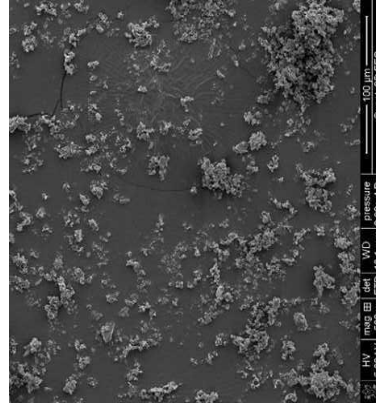
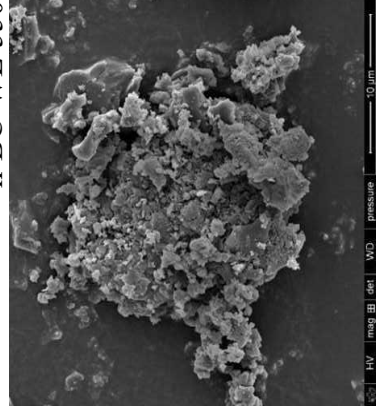
n-BC-WL-450



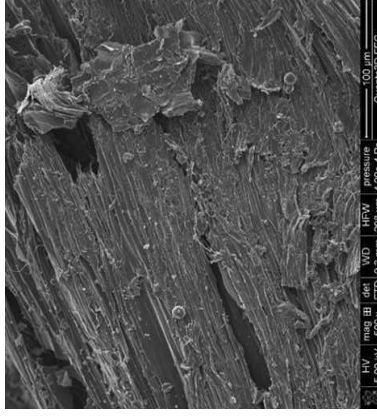
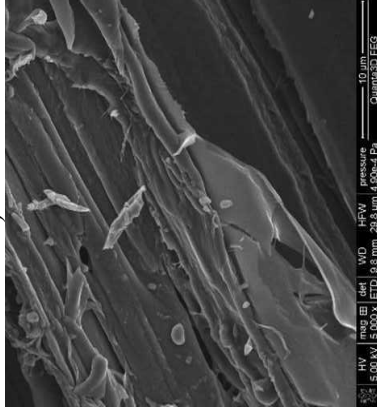
b) b-BC-WL-550



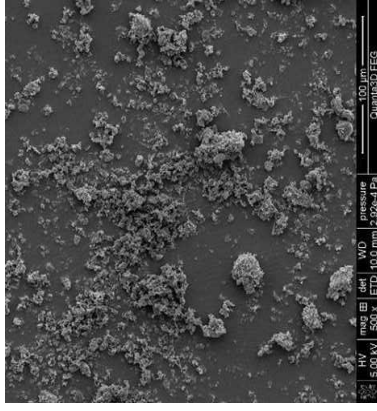
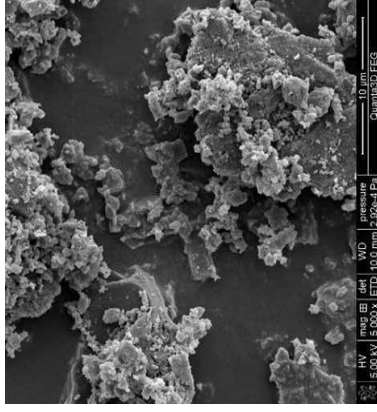
n-BC-WL-550



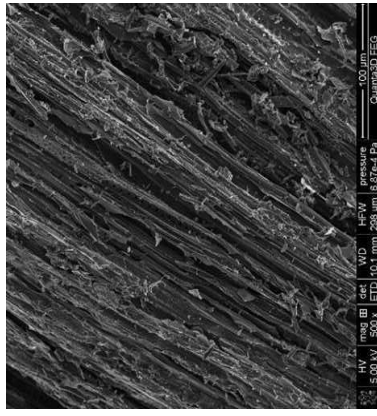
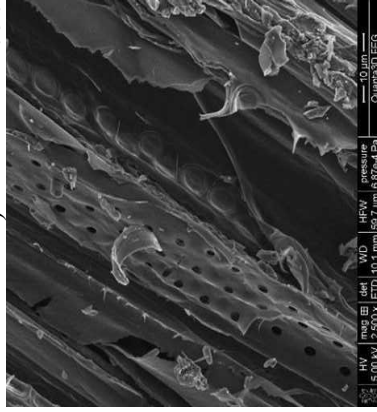
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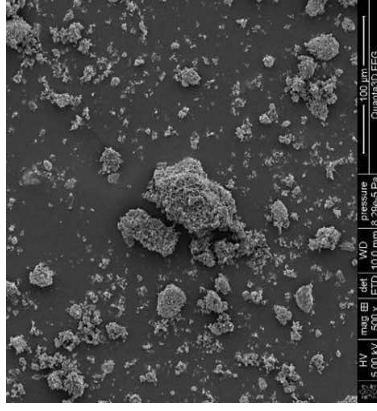
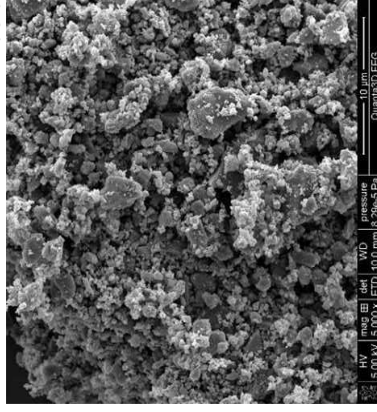
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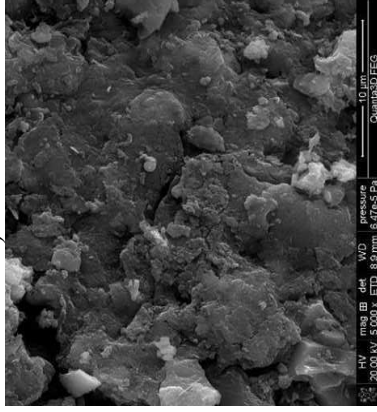
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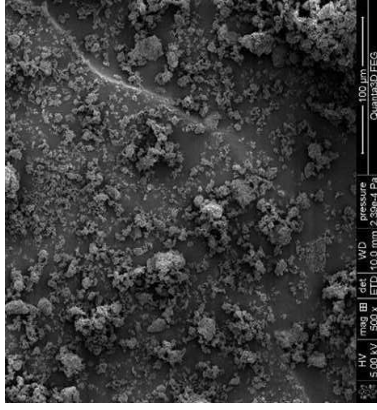
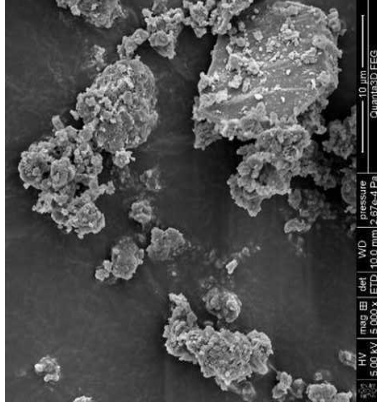
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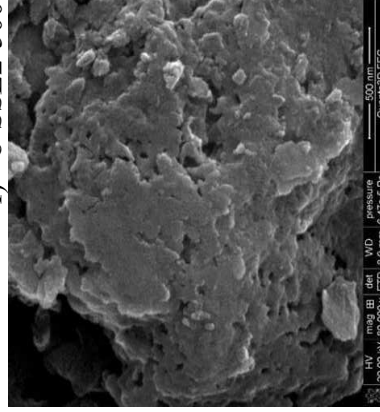
e) b-BC-SSL1-550



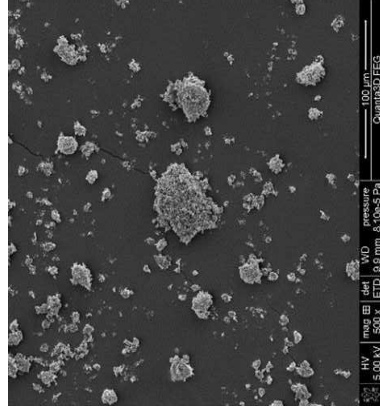
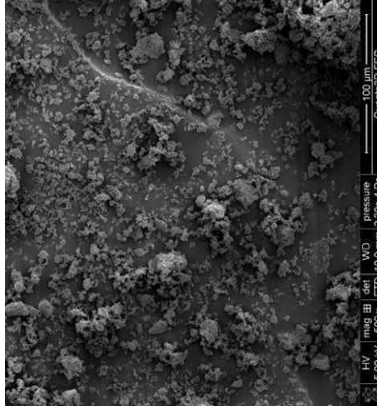
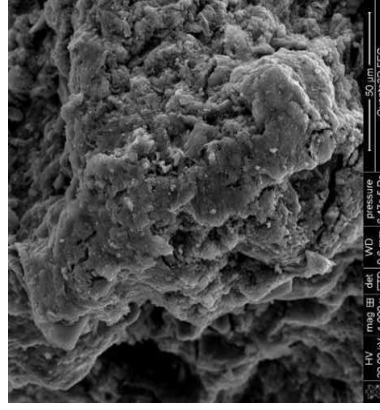
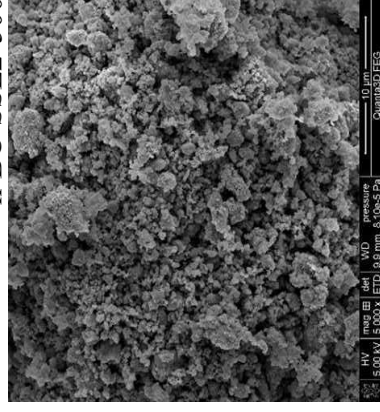
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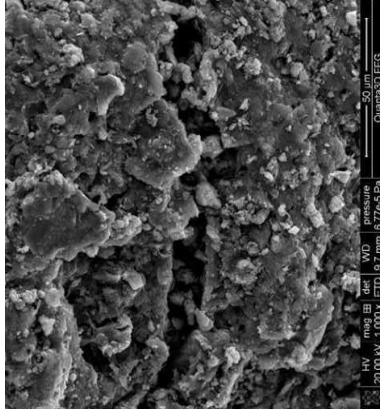
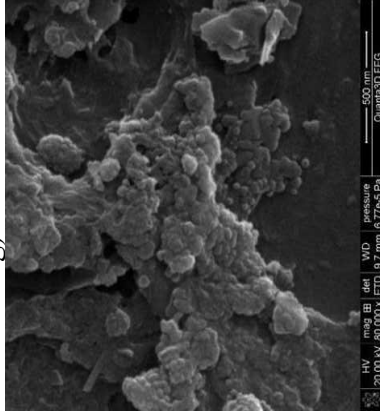
f) b-SSL2-500



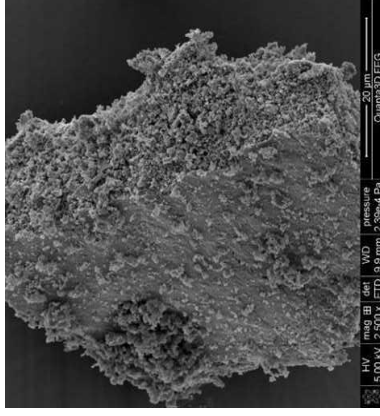
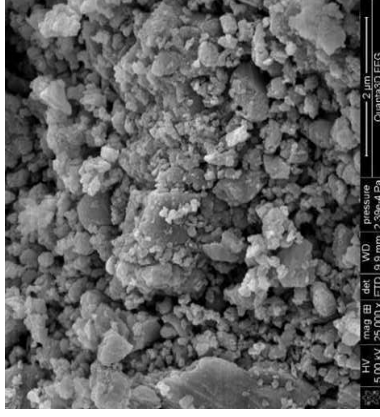
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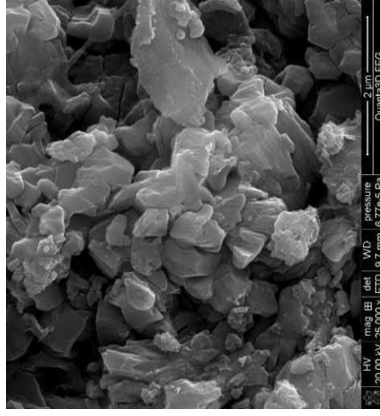
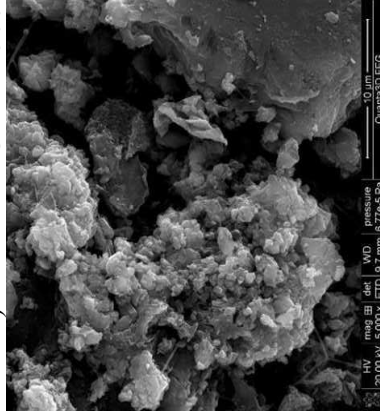
g) b-BC-SSL3-500



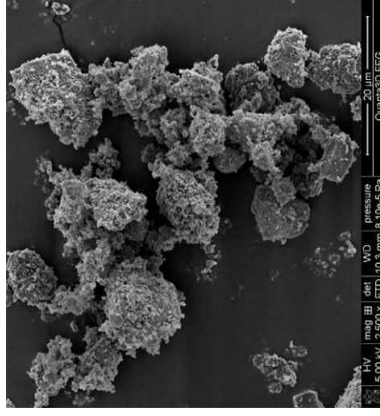
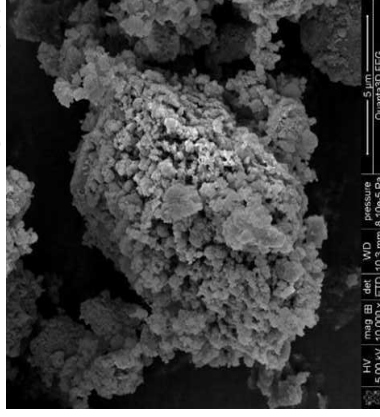
n-BC-SSL3-500



h) b-BC-SSL3-CO2-500



n-BC-SSL3-CO2-500



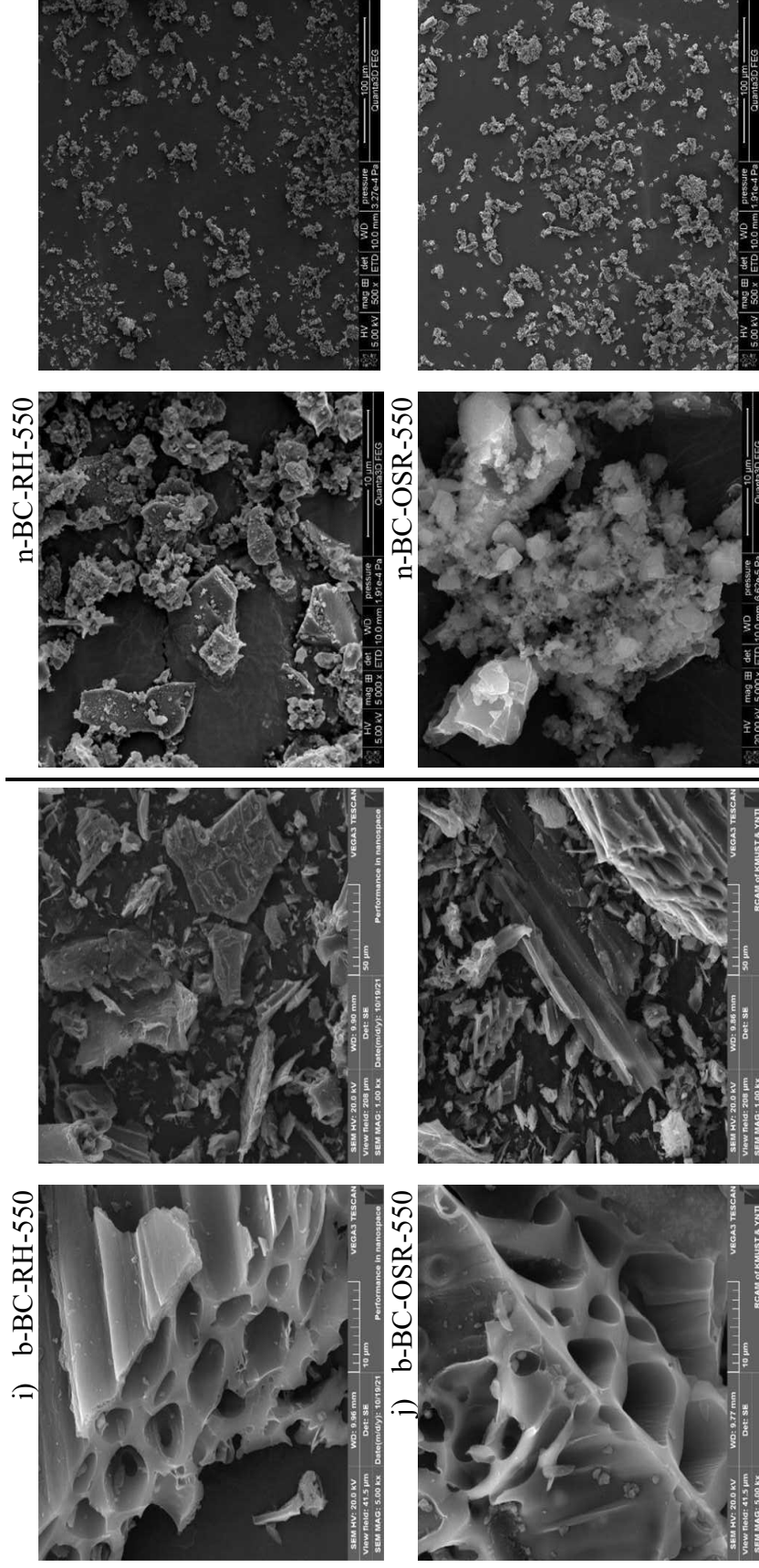


Figure S1. SEM images of bulk- and nanobiochars.

Where, b-BC= bulk biochar; n-BC= nanobiochar; WL=willow; SSL 1-3=different sewage sludges; RH=rice husk; OSR=oilseed rape; 450,500,550,650,750=temperature of pyrolysis; CO₂= CO₂ as carrier gas.

Publikacja D2

M. Raczkiewicz, O. Mašek, Y. S. Ok, P. Oleszczuk

*"Size reduction of biochar to nanoscale decrease polycyclic aromatic hydrocarbons
and trace metals content and bioavailability in nano-biochars"*

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Size reduction of biochar to nanoscale decrease polycyclic aromatic hydrocarbons (PAHs) and metals content and bioavailability in nanobiochar

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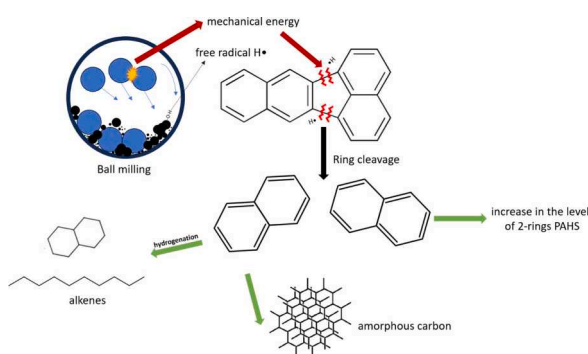
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HIGHLIGHTS

- Metal content in nanobiochar (n-BCs) obtained during ball milling was lower than those in bulk biochar (b-BCs).
- PAHs content in n-BCs strongly correlated with PAHs content b-BCs.
- The aromaticity of b-BCs determined the bioavailability of PAHs.
- Alteration of biochar surface (from hydrophobic to hydrophilic) weakens its interaction with PAHs.

GRAPHICAL ABSTRACT



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ABSTRACT

Objective of this research was to investigate ball milling of biochar from various feedstocks (willow, rice husk, oilseed rape, sewage sludge) under different pyrolysis conditions (500 and 750 °C, N₂ and CO₂ atmosphere) on polycyclic aromatic hydrocarbons (PAHs) and metals content. A significant reduction in both total (C_{total} Σ16) (1.1 to 2.4-fold decrease) and freely available (C_{free} Σ16) (1.1 to 2.9-fold decrease) PAHs in nanobiochar (n-BC), particularly in sewage sludge (SSL)-derived n-BC was observed. The changes in biochar surface characteristics induced by ball milling, such as surface area, pore volume, and hydrophilicity, contribute to the decreased PAHs content. Mechanisms involving mechanochemical degradation, free-radical reactions, and defects on the biochar surface may explain the decrease of PAHs. The n-BC showed lower log K_d values for PAHs (reduced by 14 to 41 %) compared to bulk biochar (b-BC), indicating reduced effectiveness in immobilizing PAHs. The ball milling significantly reduced also metal contents by 37 to 55 %, especially in willow pellets and agricultural residue (rice husk and oilseed rape straw) with optimal reduction (by 55 %) observed for WL-derived n-BC produced at 650 °C. Importantly, no metals were detected in the aqueous phase after ball milling, ensuring environmental

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safety of these materials. Reduction mechanisms of metals during ball milling include stable aggregate formation, enhanced defects in crystalline structures, and irreversible adsorption onto new surfaces. The results demonstrate that the application of an eco-friendly ball milling method allows for the production of materials characterized by reduced contamination levels, which is crucial from an environmental protection viewpoint.

1. Introduction

Biochar (BC) is a carbon-rich product obtained from the pyrolysis of biomass and wastes such as wood, agricultural and herbaceous residues, animal waste or sewage sludge (Lee et al., 2019). Under limited oxygen conditions and relatively higher temperatures (Buss et al., 2016), biomass undergoes a transformation releasing volatile components and forming a solid carbonized structure (Bridgwater, 2003). During pyrolysis polycyclic aromatic hydrocarbons (PAHs) are also created, which are a significant group of organic pollutants linked to environmental concerns (Abdel-Shafy and Mansour, 2016). BC can also contain metals (Rinklebe et al., 2017), and their presence is primarily linked to feedstock contamination (Lian et al., 2023). Numerous studies have explored the impact of feedstock and pyrolysis conditions on PAHs and metal content in BC (Kończak et al., 2019; Marcińczyk et al., 2023; Zielińska and Oleszczuk, 2016a). Hale et al. (2012) observed a decrease in PAHs content with an increase of pyrolysis temperature, with the highest content typically observed at 350–550 °C. In contrast, Keiluweit et al. (2012) found varying optimal temperatures for wood and grass-based BCs, with wood-based BC reaching the highest Σ16 PAHs content at 400 °C (2-fold higher than grass-based BC at the same temperature). On the other hand, grass-based BC exhibited the highest content at 500 °C (approximately 3.7-fold) than wood-based BCs. Kończak et al. (2019) explored the influence of carrier gas on PAHs content, noting that BCs produced in the presence of CO₂ from sewage sludge had lower Σ16 PAHs compared to N₂, with reductions ranging from 35 % to 51 %, depending on pyrolysis temperature. This reduction was attributed to pyrolysis reactions in a CO₂-rich environment hindering PAHs formation (Igalavithana et al., 2019). Regarding metals in b-BCs, their presence, including lead, cadmium, and mercury, is a significant concern. Reports suggest that metal content in BCs is closely linked to their presence in the feedstock (Mullen et al., 2010; Singh et al., 2010; Zielińska and Oleszczuk, 2015a). Qiu et al. (2015) observed variations in metal content in b-BCs based on feedstock type, with higher content produced from animal manures compared to other plant-derived feedstocks. They noted that initial feedstock metals contents were lower than in resulting b-BCs as a result of mass loss due to release of volatiles and preferential retention of metals, at least at lower to moderate pyrolysis temperatures (Sonoyama et al., 2006).

In recent years, nanobiochar (n-BC) has become an attractive and useful material that has gained significant attention in natural sciences and engineering (Ramanayaka et al., 2020). The n-BC is a derivative of bulk biochar (b-BC) that has undergone processing of significant reduction of its dimensions, particularly to the nanoscale, in contrast to the conventional b-BC. The production of n-BC involves diverse techniques like ball milling and sonication, among others (Oleszczuk et al., 2016; Song et al., 2019). However, ball milling is the most common and cost-effective method of n-BC production (Kumar et al., 2020; Naghdi et al., 2017; Yang et al., 2020). The outcome n-BC is composed of carbonaceous nanoparticles featuring tiny particle sizes (<100 nm), suitable for various applications (Song et al., 2022). These nanoparticles can exhibit distinct properties from their macro-counterparts, which has consequently prompted inquiries about potential ecological implications (Pratap et al., 2021).

The phenomenon associated with the presence of PAHs and metals in b-BC has already been well-understood, encompassing both their overall content and bioavailability and mobility. However, there remains a significant knowledge gap pertaining to the occurrence of PAHs and metals in n-BC, which has been gaining prominence in recent years.

Notably, the impact of ball milling can present a dual perspective. On the one hand, grinding may cause a decrease in the content of PAHs due to the increase in the specific surface area of BCs (Liu et al., 2018), while on the other hand, grinding of the BC may lead to the release of previously inaccessible PAHs, which were trapped inside the BCs matrix before grinding increasing their bioavailability. With an increase in the hydrophilicity of BCs, indicated by an elevated O/C ratio resulting from ball milling (Zhang et al., 2019), the interaction between PAHs and the BCs surface may weaken. However, simultaneously, the interaction between metals and the BCs surface may strengthen due to enhanced interactions between functional groups created on the BCs surface and metal cations. However, the literature lacks information in this domain, which is extremely important from the environmental point of view and risk associated with the use of BCs. By quantifying the presence of PAHs and metals in n-BCs derived after ball milling, we can avoid unintended environmental release during n-BC deployment, ensuring that these materials can safely contribute to waste management and carbon sequestration without causing harm to ecosystems and human health.

The aim of this study was to investigate the impact of BCs size reduction using the ball milling technique to the nano-scale on polycyclic aromatic hydrocarbons and trace metals content and bioavailability. The potential changes were studied in context of feedstock and the pyrolysis conditions (temperature and carrier gas), which may provide insights into the factors affecting PAHs and metals bioavailability and mobility. These insights have significant implications for the application and environmental impact assessment of BC as a potential remediation agent or soil amendment.

2. Materials and methods

2.1. Bulk and nanobiochar

Twenty biochar (b-BC) with different physicochemical properties were selected for the study (Table S3). The b-BC were obtained under controlled conditions at temperatures ranging from 450 °C to 750 °C under either a nitrogen (N₂) or carbon dioxide (CO₂) atmosphere, with a different heating rates and pyrolysis times (Table S1).

The b-BCs underwent ball milling using a planetary ball mill (PM100, Retsch Corporation, Germany). Before milling, b-BCs were placed in plastic jars and frozen in a laboratory freezer at –80 °C for 24 h. The ball milling technique effectively reduced the particle size of the b-BC, resulting in the formation of n-BCs with particle sizes <100 nm (Naghdi et al., 2017). Detailed information about b-BC and n-BC production is presented in Supporting Information and in our previous study (Raczkiewicz et al., 2024).

2.2. Analysis of freely dissolved PAHs content (C_{free})

The content of freely dissolved PAHs (C_{free} PAHs) in b-BC and n-BC was determined according to the method previously described by Cornelissen and Gustafsson (2006). Briefly, 1 g of each b-BC/n-BC was placed in a 50 mL flask with two polyoxymethylene polymer (POM) passive samplers (40 × 40 mm). A 40 mL solution of sodium azide (200 mg L^{–1}) was added to each flask as a biocide. Subsequently, the flasks were shaken for 30 days using a rotary shaker (Rotax 6.8. VELP, Usmate Velate, Italy). After shaking, the POM strips were removed from the flasks and any solid residues present on the strips were removed using Milli-Q-water, and then dried. POM samplers were extracted by a mixture of acetone and n-heptane (1:4, v/v) for 48 h. The extract was

concentrated to 1 mL using a rotary vacuum concentrator RVC 2–25CD plus (Martin Christ, Germany). The qualitative and quantitative analysis of the extracted PAHs was performed using a gas chromatograph (GC–MS) (Trace 1300, ThermoFisher Scientific, USA) equipped with a Restek Rxi-5 ms column (Restek, Glaston, France) with dimensions of 30 m length, 0.25 mm inner diameter and 0.25 μm film thickness. Detailed information about the procedure is presented elsewhere (Zielińska and Oleszczuk, 2016a, 2016b).

2.3. Analysis of the organic solvent extractable PAHs (C_{total})

Organic-solvent extractable PAHs (C_{total} PAHs) were extracted using an accelerated solvent extraction system (Dionex ASE 350 Accelerated Solvent Extractor) in accordance with a procedure proposed by Kim et al. (2003). Briefly, a stainless-steel cell (with a volume of 22 mL) was sequentially filled with a cellulose filter, 6 g of powdered activated silica gel mixed with 0.1 g of powdered copper, 0.1 g of EDTA, and 0.5 g of the each b-BC/n-BC. Glass spheres were used to fill the space in the cell. The n-hexane was used for PAHs extraction at a temperature of 150 °C under a pressure of 14 MPa. The extract was concentrated to 1 mL and analyzed by GC–MS as previously mentioned. Detailed information about GC–MS conditions and applied procedures, recovery and limit of detection is presented in Supporting Information..

2.4. Total metal (metal) contents and metals leachability

Determination of the pseudo-total content of metals was carried out after digestion of 200 mg of b-BC/n-BC using a mixture of $\text{HNO}_3 + \text{HCl}$ (1:3, v/v) for 1.5 h at 120 °C in a microwave oven (Start D, Microwave Digestion System, Milestone, ETHOS EASY, Italy). After the digestion, the samples were cooled down, and the digested solution was passed through a paper filter with a pore diameter 22 μm and quantitatively transferred to a flask. The content of metals was determined using Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (iCAP 7400 Duo Thermo Scientific, USA). Argon (99.9999 %, Air-Liquide, Poland) was used for the plasma generation and sample nebulization at flow rates of 12 L min^{-1} and 0.5 L min^{-1} , respectively. The quantification of heavy metals was performed based on a six-point calibration curve prepared for all metals in the range from 0.02 to 5 mg L^{-1} .

To determine the available fraction of metals in b-BC/n-BC, 1.5 g of

each sample were dispersed into 15 mL of Milli-Q water or 15 mL 0.01 mol L^{-1} CaCl_2 solution (solid/liquid ratio 1:10, w/v). The samples were shaken on a horizontal shaker (Model 358 A, ELPINSC+, Poland) for 24 h according to the procedures presented elsewhere (Joško et al., 2019). Then, the resulting solution was filtered through a 0.45 μm syringe filter, acidified using HNO_3 (1 %, v/v) and the trace metals content was determined using the ICP-OES technique as previously described.

3. Results and discussion

3.1. C_{total} PAHs in bulk biochar

The content of sum of 16 C_{total} PAHs (C_{total} $\Sigma 16$ PAHs) is presented in Fig. 1a. The SSL-derived b-BCs produced at N_2 atmosphere (b-BC-SSL1–3) showed the highest C_{total} $\Sigma 16$ PAHs content (from 3590 to 6156 $\mu\text{g kg}^{-1}$) compared to other feedstock and BC produced at CO_2 atmosphere (Lee et al., 2017). Notably, for SSL-derived b-BCs a clear trend between higher surface area (S_{BET}) and increasing C_{total} $\Sigma 16$ PAHs content was observed, indicating an increased presence of potential active sites for PAHs binding (Fig. S2). This indicates that organic anthropogenic-derived biosolids differ in structural characteristic and physicochemical properties from plant-derived materials, for which, such a relationship does not occur. The content of C_{total} PAHs in b-BCs produced from willow (WL) and agricultural residues (such as RH and OSR) was on average 3-, 8-, and 24-fold lower than in SSL-derived b-BCs, respectively. This tendency was also observed by others (Hale et al., 2012; Krzyszczyk et al., 2021) and may indicate a specific pattern in the content of C_{total} PAHs in SSL-derived b-BCs. The higher C_{total} $\Sigma 16$ PAHs in SSL-derived b-BCs can be attributed to the elevated and diverse type organic matter found in sewage sludge compared to other feedstocks (Rorat et al., 2019). With a greater amount of organic matter in bulk sewage sludge, there is a higher quantity of precursors (including different organic compounds) available for the formation of PAHs during pyrolysis (Sharma et al., 2022). The next group of b-BCs regarding PAHs content was WL-derived b-BC (1784 $\mu\text{g kg}^{-1}$). During pyrolysis, organic constituents of biomass serve as crucial precursors for the formation of PAHs and their levels can vary depending on the quantity and type of organic components in the feedstock (Odinga et al., 2021). The b-BCs obtained from agricultural residues (RH and OSR) exhibited the lowest C_{total} $\Sigma 16$ PAHs content, ranging from 200 to 476 $\mu\text{g kg}^{-1}$, compared to other investigated b-BCs (Fig. 1a). This value is consistent

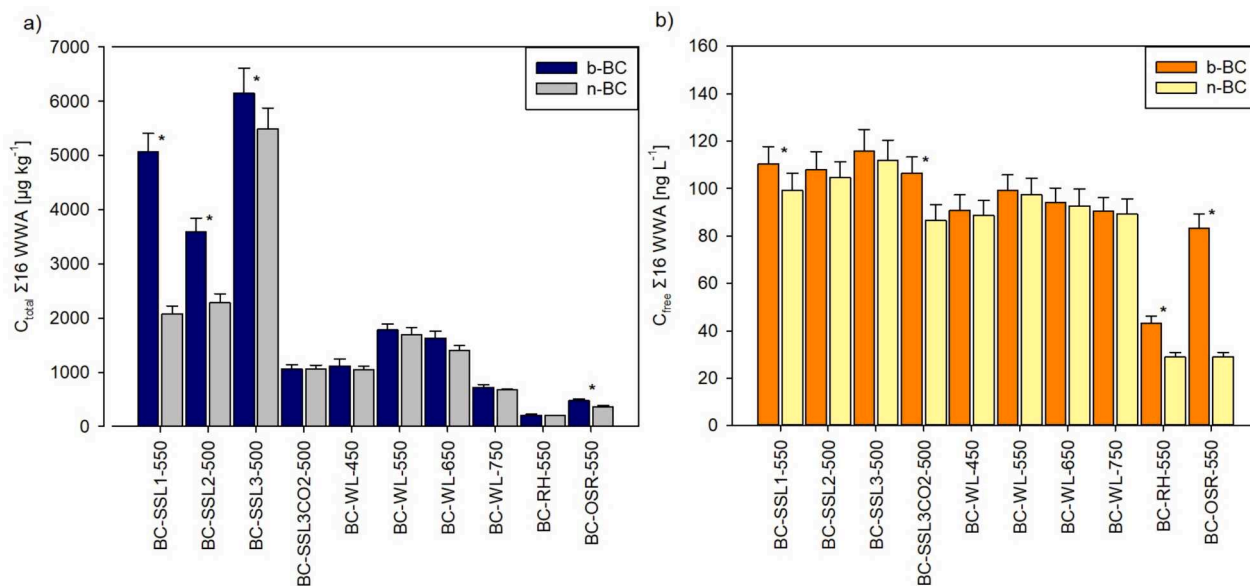


Fig. 1. C_{total} $\Sigma 16$ PAHs (a) and C_{free} $\Sigma 16$ PAHs (b) content in bulk and nanobiochar, where * means statistically significant change ($p \leq 0.05$). Error bars represent the standard deviation of the mean ($n = 3$).

with typical levels found in b-BCs derived from agricultural waste materials (Devi and Dalai, 2023). Generally, plant-derived b-BCs with a lower contribution of C_{total} $\Sigma 16$ PAHs typically contain more unconverted lignin and cellulose, which do not transform into PAHs (Ghani et al., 2013). These substances transform into organic matter during pyrolysis, which can attract aromatic compounds like PAHs (Zielińska and Oleszczuk, 2016a). However, there is no definitive confirmation in the literature that the content of PAHs present in the BC can be directly correlated with their content in feedstock. Nevertheless, it is important to acknowledge that this possibility cannot be definitively excluded, given the limited extent of research in this area.

The type of feedstock also influences the distribution of C_{total} $\Sigma 16$ PAHs in terms of the number of aromatic rings. The 4- and 5-rings PAHs were predominant in SSL-derived b-BCs (b-BC-SSL1–3) constituting 23 to 25 % and 32 to 43 %, respectively (Fig. 2a). Chrysene (CHR) and benzo[a]pyrene (BaP) were predominant compounds (Table S4). In the case of b-BC-WL-550, the highest contribution of 5-rings PAH was observed (30 %), with benzo[a]pyrene (BaP) being the predominant compound. For b-BCs obtained from agricultural residues, 2-ring PAH (NAP) was predominant constituting 55 % in b-BC-RH-550 and 59 % in b-BC-OSR-550. Fig. 3a presents the distribution of C_{total} $\Sigma 16$ PAHs based on the number of rings in b-BCs. Green shades indicate a dominance of particular group of PAHs regarding the number of rings (2–6 rings), while red shades indicate a low contribution of particular group. This allows for a quick and easy comparison of the analyzed materials. The obtained results reveal a notable similarity among b-BCs derived from SSL (designated as SSL1–3), particularly concerning the distribution of PAHs based on their ring numbers. It is evident that SSL-derived b-BCs differ compared to b-BCs originating from plant sources.

Depending on the pyrolysis temperature (450 °C to 750 °C), the C_{total} $\Sigma 16$ PAHs content ranged from 724.3 to 1784 $\mu\text{g kg}^{-1}$ (Fig. 1a). The increase in pyrolysis temperature from 450 °C to 550 °C resulted in an increase in the content of C_{total} $\Sigma 16$ PAHs. However, a noticeable decrease in C_{total} $\Sigma 16$ PAHs content was observed in the pyrolysis temperature from 650 °C to 750 °C.

A similar trend of increasing temperature with increase PAHs content was observed previously (Greco et al., 2021; Hung et al., 2023). The decrease in C_{total} $\Sigma 16$ PAHs with increase of the temperature can be due to the evaporation of the non-crystalline phase, the condensation of aromatic compounds into non-extractable layered formations, and the solid adsorption of PAHs from processing gases within the biochar

framework (Chen et al., 2019; Freddo et al., 2012). There were clear trend between C_{total} $\Sigma 16$ PAHs content in WL-derived b-BCs and the S_{BET} of biochar, which aligns with the prior research (Hale et al., 2012) (Fig. S2). This is due to the higher affinity of PAHs to biochar with a larger surface area (Li et al., 2014). Biochar with highest surface area have higher capacity to PAHs (Shen et al., 2022), thus the adsorption of PAHs from syngas is more intensive and efficient.

Contribution of groups of PAHs depended on pyrolysis temperature. Generally, the contribution of 2-ring PAHs increased with the increasing of pyrolysis temperature as was previously observed by Kloss et al. (2012). When analyzing the distribution of PAHs concerning the number of rings in the studied b-BCs, it can be concluded that b-BC-WL-450 and b-BC-WL-550, in terms of the proportion of specific PAHs groups based on the number of rings, are more similar to SSL-derived b-BCs than to agricultural residues-derived b-BCs, what is supported by a dendrogram generated through cluster analysis based on the ring contribution in C_{total} $\Sigma 16$ PAHs for all obtained b-BCs (Fig. 3a and 4a).

It was observed that using CO_2 as the carrier gas reduced almost 6-fold the C_{total} $\Sigma 16$ PAHs content (b-BC-SSL3–500 versus b-BC-SSL3- CO_2 –500) (Fig. 1a). The change in the carrier gas also influenced the distribution of PAHs in terms of number of rings (Fig. 2a). The 2-ring PAH dominated accounting almost 2-times higher contribution of these compounds (55 %) than SSL-derived b-BC produced in N_2 atmosphere (22 %) (Fig. 2a and 3a). Moreover, the SSL-derived biochar produced in CO_2 atmosphere exhibited higher contribution of 6-ring PAHs (dominated by BghiP) than SSL-derived b-BCs produced at N_2 (Fig. 2a). Analyzing the dendrogram (Fig. 4a), it can be observed that b-BC-SSL3 CO_2 –500 in terms of ring contribution in C_{total} $\Sigma 16$ PAHs is similar to b-BC-W-650 than to other SSL-derived b-BCs. This observation is crucial and suggests that the change in the carrier gas during pyrolysis has a significant influence on its chemical composition. The alteration in the carrier gas, from CO_2 in this case, introduces different chemical reactions and conditions during pyrolysis. The use of CO_2 as a carrier gas during the pyrolysis process may affect the thermal decomposition of organic matter in the SSL, potentially leading to the formation of different types and amounts of PAHs. The CO_2 -rich environment may influence the pyrolysis reactions, hindering the formation or facilitating the breakdown of certain PAHs. Moreover, additional carbon from CO_2 can also influence the chemical structure of b-BC as well as the quantity and types of by-products, such as PAHs. During the pyrolysis process, condensation and polymerization reactions can occur, leading to the

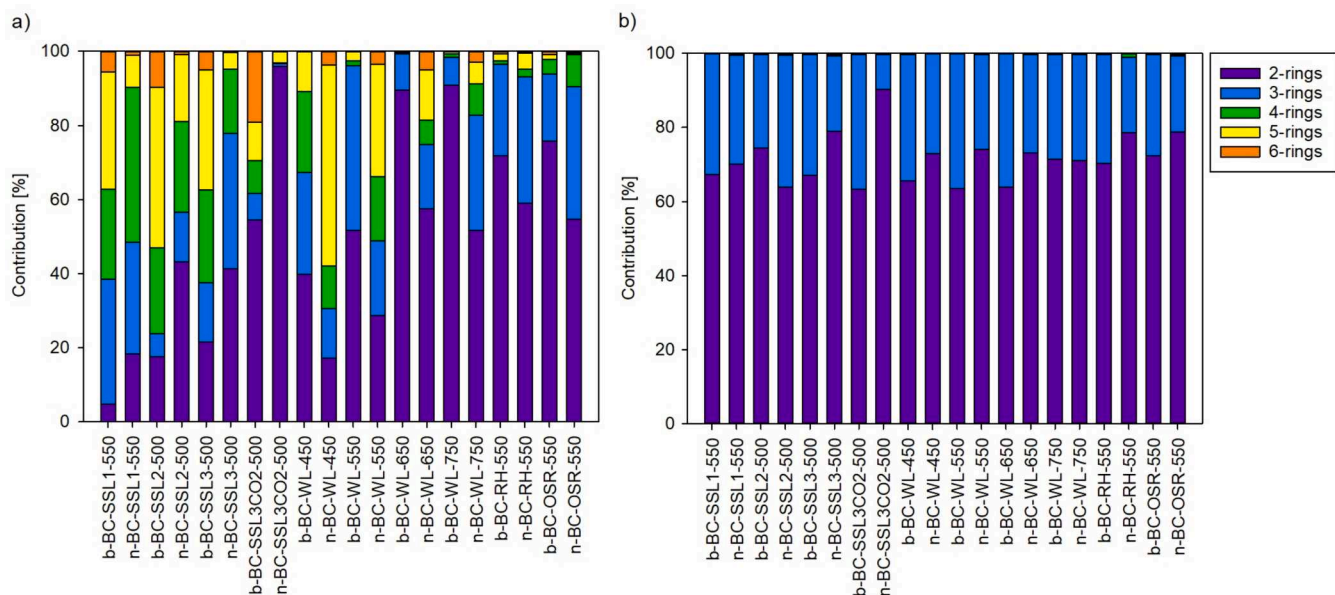


Fig. 2. Ring contribution in C_{total} $\Sigma 16$ PAHs (a) and C_{free} $\Sigma 16$ PAHs (b) content in in bulk- and nanobiochars.

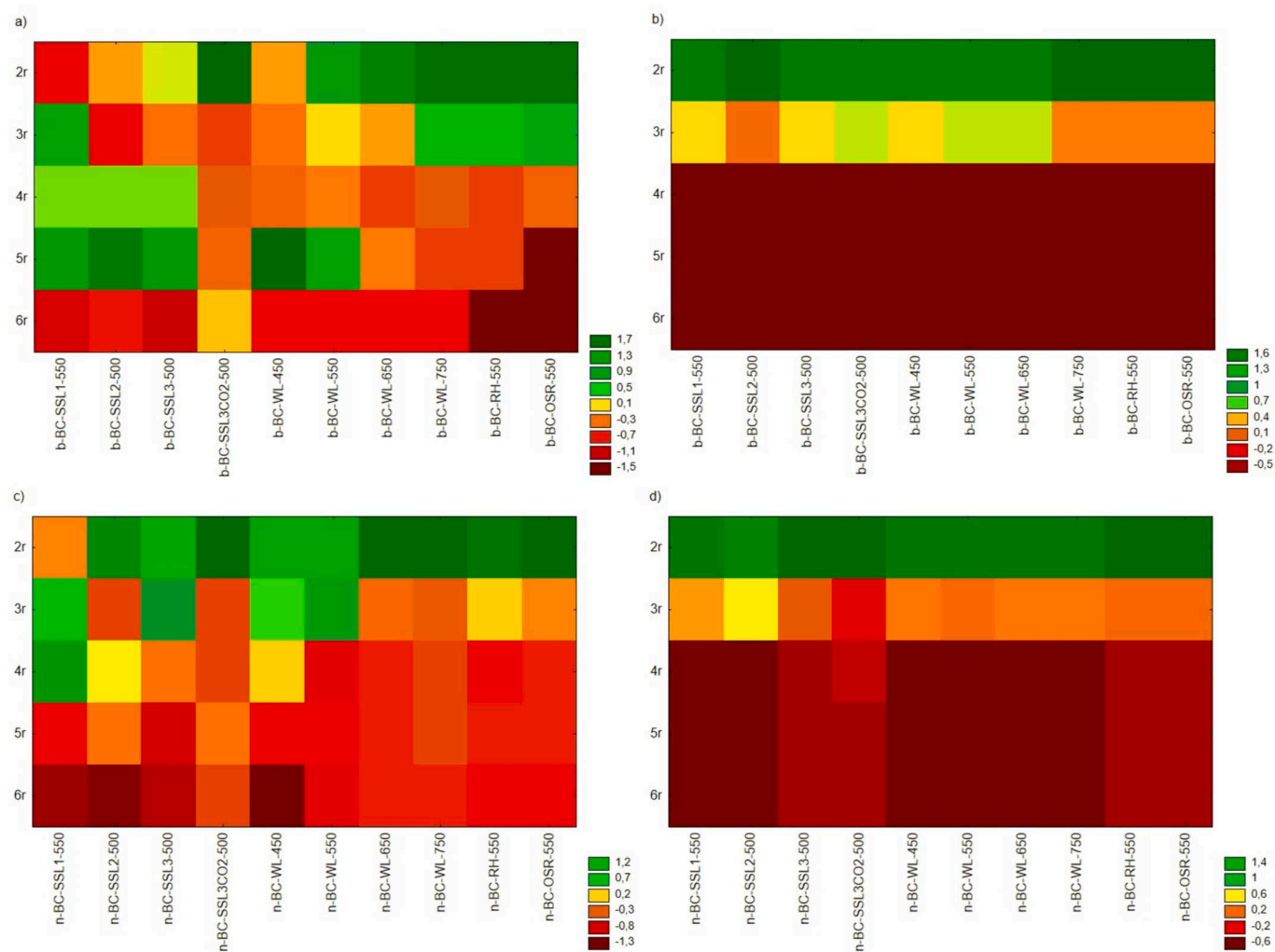


Fig. 3. Heatmap with relative changes in the distribution of C_{total} $\Sigma 16$ PAHs (a) and C_{free} $\Sigma 16$ PAHs (b) in b-BCs and C_{total} $\Sigma 16$ PAHs (c) and C_{free} $\Sigma 16$ PAHs (d) in n-BCs based on the number of rings.

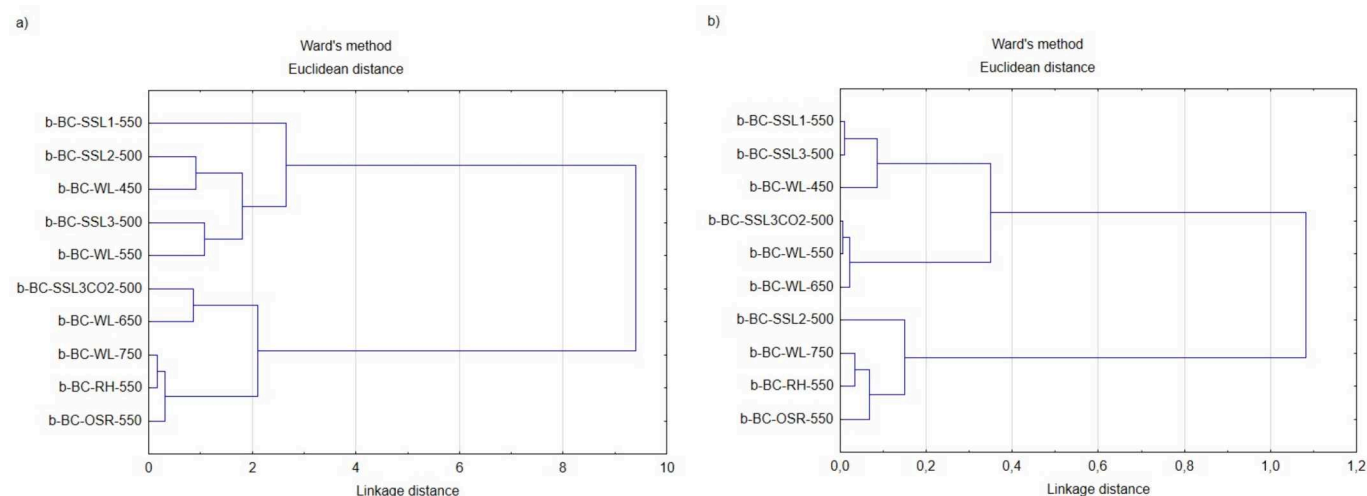


Fig. 4. Dendrogram of the distribution of C_{total} $\Sigma 16$ PAHs (a) and C_{free} $\Sigma 16$ PAHs (b) based on the number of rings for the examined b-BCs.

formation of complex aromatic compounds like PAHs. The extra carbon supplied by CO_2 can significantly impact the amount of carbon available for these reactions. In our previous study, we observed an increase in the

O/C ratio (Table S3) induced by a CO_2 atmosphere (Raczkiewicz et al., 2024). This modification can lead to the formation of BC with a more oxidized surface, which in turn potentially reduces its efficacy in binding

PAHs within the BC's matrix. We noted that such chemical modification of the BC surface significantly impacts the C_{total} $\Sigma 16$ PAHs content, as evidenced by the markedly lower levels of PAHs in b-BC-SSL3CO₂-500 compared to b-BC-SSL3-500. Consequently, this affects the quantity, and types of PAHs present in the resulting biochar. This alteration in the PAHs composition could result in a biochar more similar to those derived from biomass, such as b-BC-WL-650. A similar trend was previously observed by Kończak et al. (2019). They observed C_{total} $\Sigma 16$ PAHs content decrease within the biochar is linked to pyrolysis processes that either delay PAHs formation or accelerate their decomposition in a CO₂-rich environment. This phenomenon is supported by previous studies (Gao et al., 2013; Kwon et al., 2015), which suggest that the presence of CO₂ as a carrier gas hinders addition reactions in the gas phase, while expediting the thermal cracking of volatile compounds resulting from feedstock thermal degradation (Kwon et al., 2015). This process leads to the fragmentation of benzene rings and discourages the formation of polycyclic aromatic compounds (Gao et al., 2013).

3.2. C_{free} PAHs in bulk biochar

The content of sum of 16 C_{free} PAHs (C_{free} $\Sigma 16$ PAHs) is presented in Fig. 1b. The SSL-derived b-BCs also exhibited the highest content of C_{free} $\Sigma 16$ PAHs among all investigated b-BCs obtained from different feedstocks at 500–550 °C. However, the difference in C_{free} $\Sigma 16$ PAHs content in b-BC from other feedstocks was not so immense as it was observed for C_{total} $\Sigma 16$ PAHs (Fig. 1a). The C_{free} $\Sigma 16$ PAHs content was at the similar level as it was observed previously (Kończak et al., 2023; Tomczyk et al., 2020). The content of the C_{free} $\Sigma 16$ PAHs in WL- and agriculture waste-derived biochar was from 1.1 to 2.7-fold lower compared to SSL-derived biochar. The specific surface area (S_{BET}) of n-BCs determined C_{free} $\Sigma 16$ PAHs content, which increased with the increase in specific surface area (S_{BET}) (Fig. S2b).

The content of C_{free} $\Sigma 16$ PAHs, was very similar between different temperature of pyrolysis and ranged from 90.4 to 99.3 ng L⁻¹ for WL-derived b-BCs (Fig. 1b). A decreasing trend in their content was observed after reaching a temperature of 550 °C, however, these changes were less pronounced compared to C_{total} $\Sigma 16$ PAHs (Fig. 1a). It was also observed that a temperature of 550 °C was critical for this material, as the highest content of C_{free} $\Sigma 16$ PAHs (99.3 ng L⁻¹) was observed at this temperature. On the other hand, pyrolysis temperatures above 550 °C, i.e., 650 °C and 750 °C, resulted in a decrease in the content of C_{free} $\Sigma 16$ PAHs compared to b-BC-WL-550. Hilber et al. (2017) and Marciniak et al. (2023) reported lower content of C_{free} $\Sigma 16$ PAHs in biochar obtained from woody feedstock than in presented studies, which ranged from 12 to 85 ng L⁻¹ and from 10.7 to 12.7 ng L⁻¹, respectively.

When comparing the impact of the carrier gas on the C_{free} $\Sigma 16$ PAHs content (b-BC-SSL3-500 and b-BC-SSL3-CO₂-500), it was noted that CO₂ used as the carrier gas resulted in a 1.1-fold reduction in the C_{free} $\Sigma 16$ PAHs content.

Among the C_{free} $\Sigma 16$ PAHs, 2- and 3-ring compounds were predominant regardless of the type of feedstock and pyrolysis conditions constituting from 25.2 % to 74.4 %, 28.4 % to 71.3 % and 27.3 % to 72.4 % for SSL-, WL- and agriculture waste-derived b-BCs, respectively (Fig. 2b and 3b). Naphthalene (NAP) and phenanthrene (PHE) were predominant individual PAHs (Table S5). Regarding to the ring distribution in C_{free} $\Sigma 16$ PAHs, there was no clear trend of similarity in the proportion of different PAHs depending on the feedstock used. Briefly, SSL-derived b-BCs and b-BCs derived from plant materials (WL, RH, and OSR) do not exhibit close similarities in the dendrogram (Fig. 4b). Based on the data presented above, it can be assumed that for C_{free} PAHs, the PAHs with lower molecular weight dominate. This characteristic makes them more volatile and more soluble in water compared to larger PAHs, which may contribute to their predominance in the freely dissolved phase.

3.3. Ball milling effect on C_{total} PAHs content

The content of C_{total} $\Sigma 16$ PAHs in investigated BCs after ball milling is presented in Fig. 1a. The ball milling led to a decrease in the content of C_{total} $\Sigma 16$ PAHs for selected n-BCs. In relation to the feedstock, the most significant reduction ($p \leq 0.05$) in C_{total} $\Sigma 16$ PAHs after ball milling was observed for SSL-derived n-BCs, which ranged from 11 % to 59 % (Fig. 1a). The decrease in C_{total} $\Sigma 16$ PAHs in SSL-derived n-BCs depended on kind of SSL. For n-BCs obtained from plant materials (such as WL, RH, and OSR), the significant reduction in C_{total} $\Sigma 16$ PAHs was observed only for n-BC-OSR-550 (24 %) and no significant changes ($p > 0.05$) were noted after ball milling for WL- and RH-derived n-BCs.

Regarding the pyrolysis temperature, no statistically significant ($p > 0.05$) differences in C_{total} $\Sigma 16$ PAHs content were observed for n-BCs produced at lowest temperature (450–550 °C). However, for n-BCs produced in higher temperatures (650–750 °C), a more noticeable decrease in C_{total} $\Sigma 16$ PAHs was observed (7 % and 14 %). It can be emphasized that after ball milling, the trend in content in C_{total} $\Sigma 16$ PAHs concerning the pyrolysis temperature was analogous to that observed for initial b-BCs. Thus, this observation may provide prediction in the PAHs content in similar materials obtained in different temperatures after ball milling based on the initial content in b-BCs (Fig. 1a).

The content of C_{total} $\Sigma 16$ PAHs did not change significantly ($p > 0.05$) after ball milling for n-BCs produced in CO₂ used as a carrier gas (Fig. 1a). It appears that application of CO₂ as a carrier gas is effective enough in reducing C_{total} $\Sigma 16$ PAHs, thus, ball milling did not provide any noticeable additional effects. No change in the PAHs after ball milling in this case can also be explained by internal interaction between PAHs and BC's matrix. The PAHs in n-BC-SSL3CO₂ are more strongly bound by the BC's matrix and are situated deeper within the pores than in n-BC-SSL3 (produced under N₂) because of higher hydrophobicity (lower O/C ratio) and larger surface area. These conditions make PAHs more resistance to degradation in n-BC-SSL3-CO₂ than n-BC-SSL3 during ball milling.

The size reduction also led to changes in the distribution of C_{total} $\Sigma 16$ PAHs regards to the number of rings (Fig. 2a and 3c). In the context of feedstock, the content of 2–4-rings compounds in SSL-derived n-BCs increased by 1.1 to 3.9-fold with a simultaneous decrease by 2.4 to 17.5-fold in the presence of 5–6-ring PAHs (Fig. 2a). However, for WL-derived n-BCs, there was an increase by 1.8 to 2.2-fold in the contribution of only 2- and 3-ring PAHs, however the contribution of 4-, 5- and 6-rings PAHs decreased (from 12.0 to 45.6-fold). For n-BCs obtained from agricultural residues (RH and OSR), the contribution of 2-ring PAH increased by 1.2 to 1.4-fold, with naphthalene (NAP) being predominant comprising 72 % and 76 % respectively.

The increase in pyrolysis temperature for ball milled n-BCs did not result significantly ($p > 0.05$) in an increase in the contribution of 2-ring PAHs. For all WL-derived n-BCs in the temperature range of 450–750 °C, the increase in the contribution of 2-ring PAHs was similar, approximately 1.6 to 2.3-fold increase. However, for 3-ring PAHs, a trend was observed where with an increase of pyrolysis temperature, the increase in the contribution of 3-ring PAHs decreased from 2.2 to 0.2-fold. Overall, for 4–6-ring PAHs, a decrease in contribution to the C_{total} $\Sigma 16$ PAHs was observed, although no specific trend was noted concerning temperature.

The carrier gas change from N₂ to CO₂ had a noticeable effect on the C_{total} $\Sigma 16$ PAHs distribution regarding the number of rings. For n-BC-SSL3-CO₂-500, there was a 1.8-fold increase in the contribution of 2-ring PAH, constituting 96 % of the C_{total} $\Sigma 16$ PAHs and was lower than in b-BC-SSL3-CO₂-500 (1.9-fold increase). Increase of the contribution of 2-ring PAHs was mainly related to the mechanical degradation of 3–6-rings PAHs (Fig. 2a and 3c) as was mentioned earlier.

The reduction in the C_{total} $\Sigma 16$ PAHs in n-BCs was closely related to the initial content of C_{total} $\Sigma 16$ PAHs in their bulk counterparts (b-BCs) regarding to feedstock used and pyrolysis conditions (Fig. S3a). Similar differences in C_{total} $\Sigma 16$ PAHs content persists after size reduction to the

nanoscale between particular n-BCs, which may provide the valuable insights into the impact of particle size on the behavior of the PAHs in biochar. This observation may suggest that the PAHs changes after ball milling maintain continuity across different materials and conditions for obtaining b-BCs, which may have significant practical implications, like optimization of BC properties and risk assessment. The relationships show (Fig. S3a) that the C_{total} $\Sigma 16$ PAHs is equally reduced under the influence of ball milling. However, a discernible grouping emerges based on the feedstock, while no such distinctive pattern is observed in relation to pyrolysis conditions (temperature, carrier gas). This categorization implies that the decrease in C_{total} $\Sigma 16$ PAHs is influenced by the properties of the biochar. Furthermore, the decrease in C_{total} $\Sigma 16$ PAHs content in n-BCs is linked to several factors such as the increase in total pore volume (V_p) (Fig. S4a). Greater porosity (reflected in higher S_{BET} and V_p) supports the volatilization of PAHs during the carbonization process and can be connected to a lower content of C_{total} $\Sigma 16$ PAHs in n-BCs (Luo et al., 2019). Furthermore, for SSL- and WL-derived n-BCs (besides of n-BC-WL-450), the average particle size of n-BCs corresponds with reduction of the $\Sigma 16$ PAHs (Fig. S5a).

3.4. Ball milling effect on C_{free} PAHs content

The content of C_{free} $\Sigma 16$ PAHs in selected n-BCs after ball milling decreased (Fig. 1b) as it was also observed for the C_{total} $\Sigma 16$ PAHs (Fig. 1a). However, these changes did not mostly affect the same BCs (except BC-SSL1–500) as it was observed for C_{total} $\Sigma 16$ PAHs. Comparing the content of C_{free} $\Sigma 16$ PAHs in n-BCs obtained from various feedstocks, ball milling led to a statistically significant decrease ($p \leq 0.05$) of C_{free} PAHs for n-BC-SSL1–550, n-BC-RH-550, and n-BC-OSR-550 by 10.1 %, 32.8 %, and 65.1 %, respectively.

No significant changes ($p > 0.05$) were observed in the content of C_{free} $\Sigma 16$ PAHs in relation to the pyrolysis temperature for WL-derived n-BCs. However, the gas changing from N_2 to CO_2 caused significant reduction ($p \leq 0.05$) of C_{free} $\Sigma 16$ PAHs during ball milling by 19 %. The ball milling of SSL-derived n-BC produced at N_2 (n-BC-SSL3–500) does not affect C_{free} $\Sigma 16$ PAHs content (Fig. 1b), which means that production of biochar in CO_2 creates not only biochar with different properties than biochar produced in N_2 , but also affect the PAHs behavior and persistence in obtained BCs.

Certainly, decrease in C_{free} $\Sigma 16$ PAHs in n-BCs was correlated ($r = 0.661$) with the decrease in C_{free} $\Sigma 16$ PAHs in b-BCs (Fig. S2b). Furthermore, by comparing the change profiles between C_{total} $\Sigma 16$ PAHs and C_{free} $\Sigma 16$ PAHs, a consistent pattern of variations in C_{total} $\Sigma 16$ PAHs alongside C_{free} $\Sigma 16$ PAHs in n-BCs is confirmed and similar to that observed for b-BCs (Fig. S1). The reduction in C_{free} $\Sigma 16$ PAHs content was associated with a decrease in C_{total} $\Sigma 16$ PAHs in n-BCs after ball milling. The decrease in C_{total} $\Sigma 16$ PAHs, and thus C_{free} $\Sigma 16$ PAHs could be linked to several mechanisms of PAHs degradation and a weakening of the bond between PAHs and the biochar surface, which is associated with the increased hydrophilicity of n-BCs after ball milling (Table S3). As was mentioned before (Yang et al., 2021), high BC's aromaticity may be linked to strong PAHs sorption affinity because of large electron-accepting capacity.

The trend between the reduction of C_{free} $\Sigma 16$ PAHs and an increase O/C ratio in n-BCs was observed (Fig. S1 others). Higher O/C values indicate a less aromatic material, which, in turn, bounds organic pollutants less effectively (Ahmad et al., 2012; Qiu et al., 2014). The content C_{free} $\Sigma 16$ PAHs decreased as the O/C ratio increased (Table S3). As the surface of the biochar became more hydrophilic and specific surface area (S_{BET}) increase following ball milling (releasing initially unavailable PAHs), weaker interactions with PAHs occurred on this surface, coinciding with the simultaneous mechanical degradation of C_{total} $\Sigma 16$ PAHs.

The profile of C_{free} $\Sigma 16$ PAHs in n-BCs regarding to the number of rings is presented in Fig. 2b and 3d. After ball milling, there was an increase in the contribution of 2-ring PAH and decrease of 3-ring PAHs

in n-BCs for all investigated n-BCs. No distinct trends were observed regards the feedstock or pyrolysis conditions. Furthermore, the decrease of ≥ 3 -ring PAHs in n-BCs compared to their bulk counterparts suggests that the size reduction through ball milling has led to preferential removal or degradation of these compounds, possibly due to increased surface reactivity and changes in the chemical environment of the biochar matrix.

We can suggest several key mechanisms responsible for reduction of PAHs content during ball milling. The ball milling may trigger degradation reactions, leading to the fragmentation of larger 5- and 6-ring PAHs into more diverse compounds (Borowski et al., 2001), potentially reducing their contents. This phenomenon was previously observed by Magoha (2004), who proposed ball milling for a mechanochemical degradation of PAHs and aliphatic hydrocarbons in soil. The increased availability of active surface sites resulting from ball milling enhanced the degradation of naphthalene, anthracene and phenanthrene with destruction efficiency depending on the milling time from 9 to 24 %, from 36 to 95 %, and from 28 to 68 %, respectively. Discrepancies in the adsorption capabilities of individual PAHs observed in our research may be related to specific chemical structures of particular compounds and thus different interactions with the biochar surface (Lyu et al., 2018). We believe that this mechanism was crucial in reducing the C_{total} $\Sigma 16$ PAHs and C_{free} $\Sigma 16$ PAHs content in our samples. This assertion is supported by the FTIR spectra of b-BC n-BC, which showed diminished peak intensities associated with surface functional groups (Raczkiewicz et al., 2024). These reduced intensities suggest significant alterations to the surface groups, which are essential for the stabilization of these organic pollutants. Such changes in the BC's surface chemistry are likely due to the intense physical and chemical interactions occurring during ball milling. This process facilitates the release of bound PAHs from the BC's surface, subsequently leading to the mechanochemical degradation of complex PAHs structures into simpler, less toxic compounds.

The second mechanisms responsible for PAHs degradation during ball milling can be free-radical reactions during a milling process. Pan et al. (2019) previously observed that BC can serve as a source of free radicals due to the thermal conversion of organic matter. Free radicals can also form due to the thermal activity of oxygen atoms and inorganic substances in the feedstock, which can be further enhanced by external mechanical energy. In ball milling, kinetic energy is transferred through collisions, with the rotational speed of corundum balls determining the total energy input (Delogu et al., 2003). Previous study (Joseph-Ezra et al., 2014) has reported that manual grinding can enhance the degradation of PAHs in combination with magnetite. Proposed mechanism of PAHs degradation during ball milling is presented in Graphical Abstract (GA).

The reaction and formation of new defects on the biochar surface after milling has been recognized as a fundamental factor influencing the alterations and redistribution of PAHs in BC (Yao et al., 2023). When biochar is ball-milled in an oxygen atmosphere, various reactions lead to the generation of free radicals (Zhang et al., 2021). Activation of hydroxyl groups on the biochar surface due to mechanical energy initiates the formation of hydroxyl free radicals by releasing a hydrogen atom from the hydroxyl group:



These generated hydroxyl radicals are highly reactive and can participate in oxidation reactions. Another possible mechanism leading to the formation of hydroxyl free radicals is activating hydroxyl groups by water particles (such as moisture) present in the system:



In above reaction, a hydroxyl radical ($\bullet OH$) is formed, and the hydroxyl group is transformed into a R-OH type compound resulting in free radical attack on the carbon skeleton (Zhang et al., 2014). Formation of the naphthalene (NAP) indicates successful destruction of the

heterocyclic 5- and 6-ring PAHs. The observed increase in naphthalene content in presented studies may be associated with the fragmentation of heavier PAHs (4–6 rings) due to the formation of free radicals and mechanochemical degradation. Additionally, the formation of free radicals (especially carbon-centered radicals) can occur through the breaking of chemical bonds within biochar macromolecules also during ball milling (GA). The reduction in the content of C_{total} $\Sigma 16$ PAHs, and consequently C_{free} $\Sigma 16$ PAHs in n-BCs, may be associated with the conversion of some 2–3-ring PAHs in the presence of free radicals into non-aromatic compounds or saturated forms (Moldoveanu, 2019).

3.5. Log K_d values for b-BCs and n-BCs

The distribution coefficient (K_d) was determined as the ratio of C_{total} $\Sigma 16$ PAHs to C_{free} $\Sigma 16$ PAHs (Table S6). The log K_d values determined for individual PAHs and particular BC depending on feedstock, temperature of pyrolysis and carrier gasses used are presented in Table S6. Here, we selected the PAHs representing compounds with different number of rings: naphthalene (NAP), phenanthrene (PHE), fluoranthene (FLA) and benzo[a]pyrene (BaP) (Fig. 5). The 6-ring C_{free} PAHs were not detected in all BC, thus the calculation of the K_d values was impossible for these compounds.

Comparing the impact of different feedstocks, in the case of naphthalene (NAP), a reduction in size increased the log K_d values for most BC in the range of 4 to 12 %. The greatest increase in log K_d values was observed for n-BC-OSR-550, indicating an enhanced affinity of this compound to the investigated n-BCs. For phenanthrene (PHE) and fluoranthene (FLA), the change in log K_d values primarily depended on the

type of feedstock. A significant increase in log K_d values for phenanthrene (PHE) was observed only for n-BC-SSL2–500 and n-BC-SSL3–500, by 29 % and 10 %, respectively. Regarding fluoranthene (FLA), an increase in log K_d values of 1 to 9 % was observed for SSL-derived n-BCs, while a decrease (ranging from 13 to 23 %) was noted for plant-derived n-BCs. This suggests that ball milling increase affinity of SSL-derived n-BCs to fluoranthene (FLA) and decrease this capacity for n-BCs obtained from plant materials (WL, RH, and OSR). In the case of benzo[a]pyrene (BaP), a significant decrease in log K_d values was observed, ranging from 10 to 39 %, suggests reduced affinity of more hydrophilic surface of n-BCs towards this strongly aromatic compound. This observation aligns with the well-established understanding that the aromaticity of a compound increases with the number of aromatic rings (Szatylowicz et al., 2019). Consequently, the diminished binding of n-BCs surface to benzo[a]pyrene implies a lower tendency for hydrophilic fractions to associate with highly aromatic substances.

3.6. Content of metals and metals leachability

Fig. 6 presents the content of metals in the investigated biochar. Generally, SSL-derived b-BCs contained higher levels of metals than WL-, RH- and OSR-derived b-BCs. This phenomenon is typical and results from the high metals content in SSL (Lu et al., 2016; Zielińska and Oleszczuk, 2015b) and loss of mass during pyrolysis and content of metals in BCs. The composition and content of metals in the feedstock is the primary factor determining the presence of metals in BCs (Zielińska and Oleszczuk, 2015a). Thus, the content of metals in n-BCs was correlated with their content in b-BCs. According to the metals limits

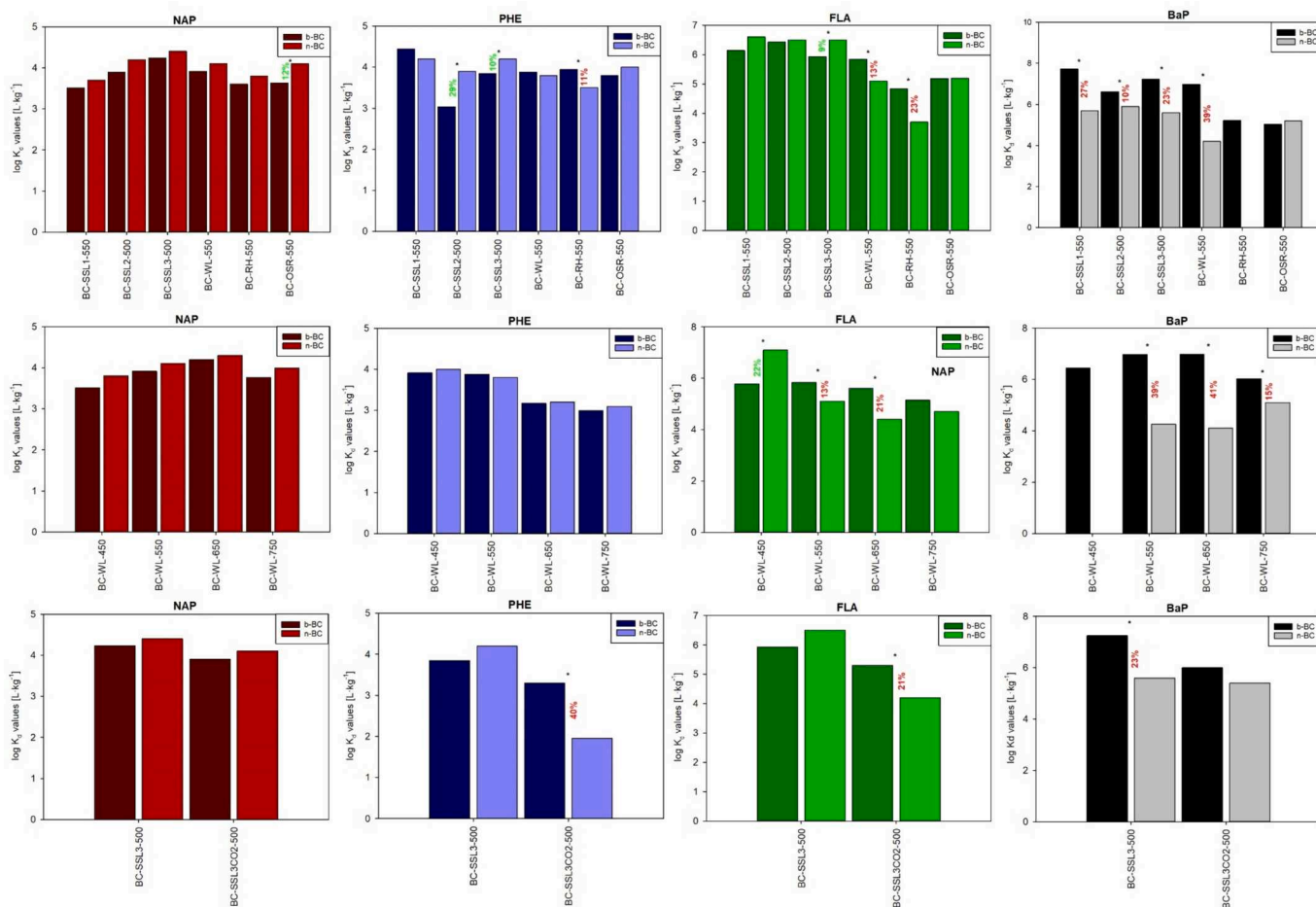


Fig. 5. Log K_d values for 4 representative PAHs, where * means statistically significant change ($p \leq 0.05$) and red or green indicates an decrease or increase in log K_d values.

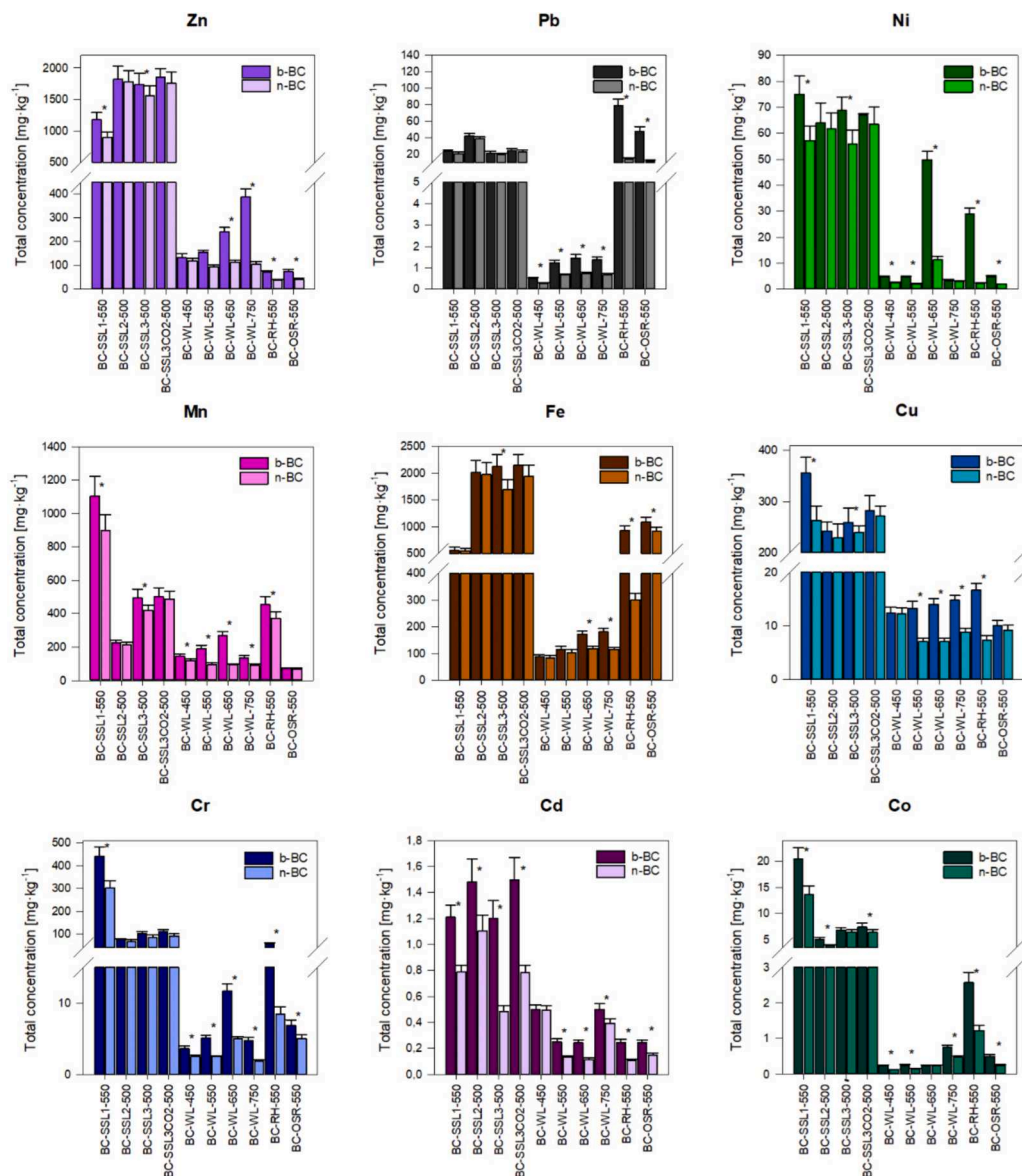


Fig. 6. Total trace metals (TMs) [$\text{mg}\cdot\text{kg}^{-1}$] content in bulk- and nanobiochar, where * means statistically significant change ($p < 0.05$). Error bars represent the standard deviation of the mean ($n = 5$).

specified by the European Biochar Certificate (EBC, 2023) and the International Biochar Initiative (IBI, 2015), the content of Cr in b- and n-BC-SSL1 exceeded the recommended values (100 mg/kg) by 4 and 3-times, respectively. The content of other metals met the permissible standards in all studied biochar (both b-BCs and n-BCs). However, notably higher contents of all Metals were detected in b-BCs and n-BCs derived from SSL compared to other feedstocks (WL, RH and OSR).

It was observed that after ball milling, the contents of all metals mostly decreased. The most significant reductions of metals were generally observed for n-BCs derived from WL and agricultural residues (RH and OSR). This reduction was particularly evident for Cd, Ni, Cu, Co, and Zn content, where plant-derived n-BCs exhibited reductions exceeding 50 % compared to their bulk counterparts. This might be attributed to the easier disintegration of various metal components in plant-based BCs than in SSL-derived BCs during ball milling. Additionally, this could be due to the differing stability of metal compounds and changes in the structure of the biochar (such as porosity) during ball milling, which could facilitate decomposition. It has been previously suggested that the low metal contents can positively affect the degradation of PAHs (Zukauskaitė et al., 2008), which our research

confirmed. Moreover, the ball milling process can trigger oxidation reactions that may lead to the release of metals from the BC, where they were initially bound. Additionally, these released metals may become adsorbed onto the surfaces of the milling balls used during the grinding process. This release could result in decreased metals contents and also contribute to the formation of free radicals. However, the extent of changes in metals contents primarily depended on the raw material used. It was also noted that for n-BC-WL-650, the highest reductions in the content of most Metals were observed compared to other wood lignin (WL)-derived non-aqueous bioavailable fractions (n-BCs). This observation suggests that in this case, the temperature of 650°C during biochar production is optimal.

The superior reduction in metal contents in n-BC-WL-650 may be attributed to specific characteristics associated with this particular temperature. The intricacies of biochar production at 650°C could result in enhanced disintegration of metal components, increased stability of metal compounds, and alterations in the biochar structure, such as improved porosity, during the ball milling process. These factors collectively contribute to the effective decomposition and release of metal compounds, aligning with the observed reductions in metals

contents.

No metals were determined in the aqueous phase of b-BCs and n-BCs, which holds notable significance in terms of environmental perspective (Table S8). This absence of metals leaching suggests that after ball milling do not affect metals mobility and there is no risk that metals can be released into environment. This aspect adds to the overall environmental viability of using n-BCs for various applications, particularly in contexts where minimizing heavy metal release is a critical concern.

Previous research have showed (Yuan et al., 2019) that under acid condition, Pb, Cu, and Zn can be released from soil treated by ball milling in the range from 73.9 % to 90.4 % initial soil content. Similar studies concerning fly ash and metals were carried out by Chen et al. (2016), who observed the decreases in leaching of Cu, Pb and Cr from fly ash after ball milling with reductions of 31 %, 46 %, and 60 %, respectively. Montinaro et al. (Montinaro et al., 2009, 2008, 2007) suggested following reason which can be adapted to this research which explain decrease of metals after mechanical treatment of soils. First of all, after mechanical treatment through ball milling, a stable aggregate with compact structures can be formed, effectively trapping metals within solid particles. The second, the increase in defects, dislocations, and vacancies in the crystalline structure due to ball milling enhances diffusivity, subsequently reducing the leaching toxicity, mobility, and bioavailability of metals. The third explanation suggests the higher irreversible adsorption onto newly formed surfaces resulting from the breakdown of soil particles. The additional mechanism can be metals immobilize on corundum balls and vessel walls (used in present experiment) during collisions with the surface of b-BCs, thereby decreasing metals content in n-BCs. Consequently, comprehensive research into such mechanisms is required.

4. Conclusion

In this research we showed that ball milling is an effective method for substantially reducing the content of PAHs and selected metals. This method not only reduce the particle size of b-BCs to the nanoscale dimensions but also significantly alters their physicochemical properties, thereby augmenting their capacity to bind PAHs and metals. The reduction in PAHs content was influenced by the specific characteristics of the biochar, such as its surface area, pore volume, and hydrophilicity, which were altered during ball milling. Moreover, changes in the distribution of PAHs based on the number of rings were also observed, indicating preferential removal or degradation of higher-ring PAHs. Furthermore, ball milling significantly reduced metals contents, especially in WL and agricultural residue (RH and OSR) n-BCs. WL-derived n-BCs at 650 °C exhibited optimal reduction (55 %). No metals were detected in the aqueous phase post-milling, indicating environmental safety. Overall, the findings suggest that ball milling can be an effective strategy for decreasing PAHs in biochar, with potential implications for environmental applications. The proposed methodology promises significant ecological advantages by mitigating the impact of contaminants encapsulated within biochar, representing a crucial development in the domain of sustainable environmental management.

CRediT authorship contribution statement

Monika Raczkiewicz: Writing – original draft, Visualization, Methodology, Investigation, Conceptualization. **Ondřej Mašek:** Writing – review & editing, Formal analysis. **Yong Sik Ok:** Writing – review & editing, Formal analysis. **Patryk Oleszczuk:** Writing – review & editing, Supervision, Resources, Conceptualization.

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.scitotenv.2024.173372>.

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Size reduction of biochar to nanoscale decrease polycyclic aromatic hydrocarbons and trace metals content and bioavailability in nanobiochars

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Preparation of bulk biochar (b-BC) and nanobiochar (n-BC)

A total of 10 b-BCs were obtained from four types of feedstocks through a pyrolysis process. These types of feedstocks included woody biomass (willow), agricultural residues (rice husk and oilseed rape) and anthropogenic biowaste (SSL). The details of the pyrolysis conditions were described in our previous research (Gul et al., 2021; Siatecka et al., 2021; Stefaniuk and Oleszczuk, 2015; Yang et al., 2022) . In our laboratory, we obtained b-BCs from willow (WL) and sewage sludge (SSL), while b-BCs from other feedstocks (sewage sludge (SSL), rice husk (RH) and oilseed rape (OSR)) were obtained from the UK Biochar Research Centre in collaboration with another research group. Briefly, feedstock, i.e., WL and SSL, were dried and crushed (<2 mm) and then pyrolyzed at $450-750$ °C under a nitrogen (N_2) or carbon dioxide (CO_2) atmosphere for 3 h (heating rate 10 °C·min $^{-1}$).

The SSL, RH and OSR biochar were produced using a pilot-scale pyrolysis unit at the UK Biochar Research Centre. The pyrolysis unit consists of a rotary kiln with a heat-tube length of 2.8 m, an inner diameter of 244 mm, an angle of 0.5° and a rotational speed of 1 – 7 rpm. Further details on the production conditions used can be found in previous studies (Mašek et al., 2018; UKBRC, 2019).

A pyrolysis temperature of $500-550$ °C is the most widely used for biochar formation (Chen et al., 2017) Thus, for comparisons between feedstocks and carried gases, this temperature was selected. Finally, the obtained b-BCs were stored and frozen in a laboratory freezer (-80 °C) in sealed plastic jars before ball milling.

The n-BCs were obtained following a previous method (Naghdi et al., 2017). Six grams of each b-BC was placed in a planetary ball mill (PM100, Retsch Corporation, German) in a 125 mL corundum jar with 7 corundum balls (ball diameter was 20 mm) (biochar-to-ball mass ratio was 1:100). The planetary ball

mill was operated at room temperature with a speed of 440 rpm for 1.5 h, and the rotation direction was altered every 5 min with short intervals to control the temperature boost.

Table S1. Process parameters of obtaining bulk-biochar (b-BC).

Feedstock material	Biochar	Temperature [°C]	Heating time [min]	Heat rate [°C/min]	Gas flow [L/min]	Gas type
willow	b-BC-W-450	450	180	10	1	N ₂
willow	b-BC-W-550	550	180	10	1	N ₂
willow	b-BC-W-650	650	180	10	1	N ₂
willow	b-BC-W-750	750	180	10	1	N ₂
rice husk	b-BC-RH-550	550	15	98	10	N ₂
oilseed rape	b-BC-OSR-550	550	12	78	10	N ₂
sewage sludge SCOTLAND EDINBURGH 55°56' N, 3°14' W	b-BC-SSL1-550	550	13	86	10	N ₂
sewage sludge POLAND ZAMOŚĆ 50°42' N, 23°13' E	b-BC-SSL2-500	500	180	7	1	N ₂
sewage sludge POLAND LUBLIN 51°14' N, 22°35' E	b-BC-SSL3-500	500	180	10	1	N ₂
sewage sludge POLAND LUBLIN 51°14' N, 22°35' E	b-BC-SSL3-CO2-500	500	180	10	1	CO ₂

Where, b-BC= bulk biochar; W=willow; SSL 1-3=ifferent sewage sludges; RH=rice husk; OSR=oilseed rape; 450,500,550,650,750=temperature od pyrolysis; CO2= CO₂ as carrier gas.

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):

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

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

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

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

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} (ng 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 $\alpha = 0.05$ was performed.

Solid-water distribution coefficient calculation (K_d)

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

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

where C_{total} is the PAH concentration in biochar ($\mu\text{g/kg}$) and C_{free} the freely dissolved concentration in water ($\mu\text{g/L}$).

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C_{total} and C_{free} PAHs profiles

The comparison of changes in C_{total} Σ16 PAHs with C_{free} Σ16 PAHs provides valuable insights into whether the observed variations in C_{free} Σ16 PAHs were linked to reductions or increases in C_{total} Σ16 PAHs. It also helps determine if these variations resulted from alterations in the weakened or strengthened interactions between PAHs and the studied material.

Figure 6 represents the shifts in C_{total} Σ16 PAHs and C_{free} Σ16 PAHs content in b-BCs and n-BCs based on several factors, including the feedstock and pyrolysis conditions. The relationships depicted in the figure provide a clear indication that the presence of C_{free} Σ16 PAHs in b-BCs is strongly associated with the total content of C_{total} Σ16 PAHs and changes in the content of C_{free} Σ16 PAHs in b-BCs are associated with a decrease or increase in their C_{total} Σ16 PAHs, which is influenced by factors such as the raw material used, pyrolysis temperature and carrier gas. Therefore, all trends in changes in C_{free} Σ16 PAHs were also observed previously in C_{total} Σ16 PAHs. Moreover, the contents of C_{total} Σ16 PAHs and C_{free} Σ16 PAHs in n-BCs are strongly correlated with their contents in b-BCs, which perfectly illustrates the profile of changes.

Remarkably significant changes are particularly noticeable in b-BCs derived from SSL, underlining the distinct characteristics of this specific type of feedstock. This information contributes to a better understanding of how different factors influence the presence of PAHs in biochar, particularly in relation to their total and freely dissolved forms.

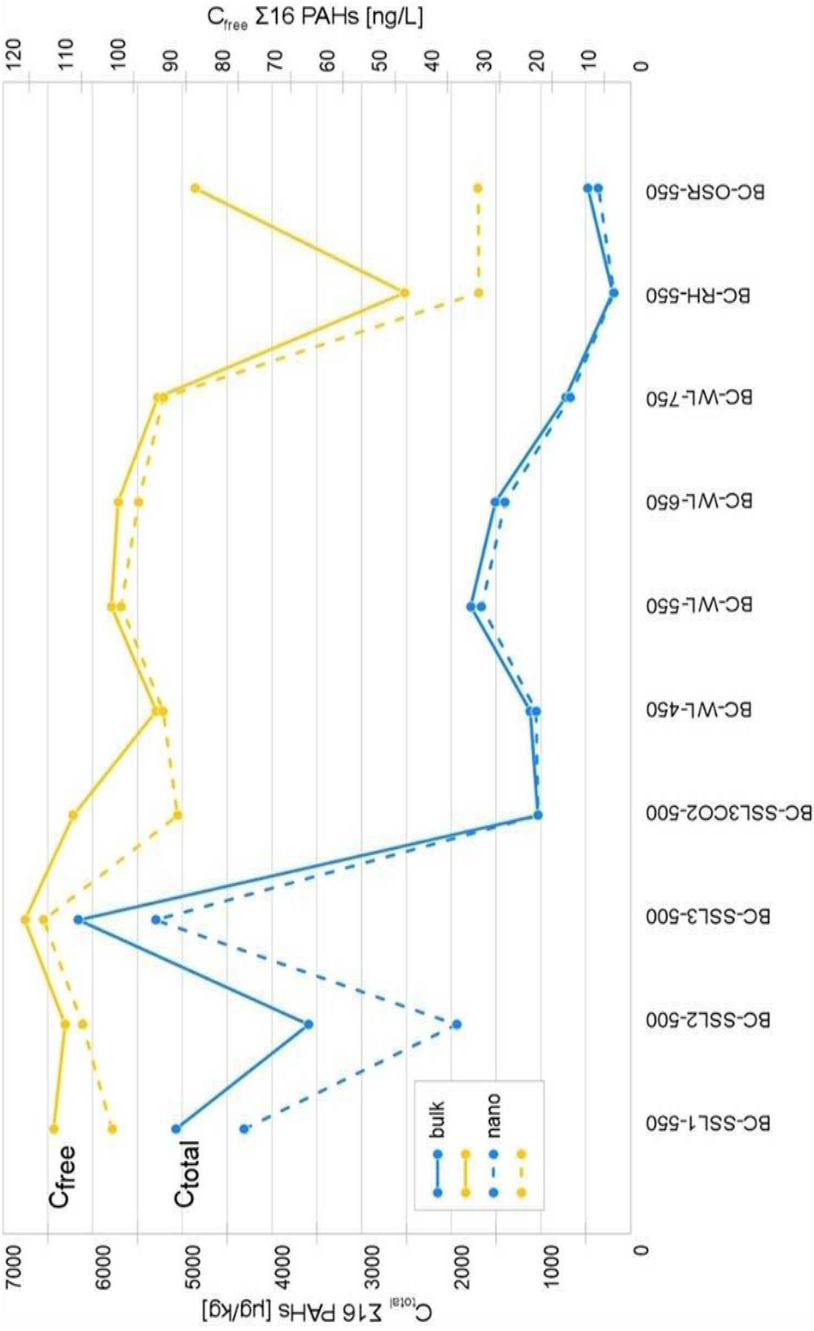


Figure S1. The profile of C_{total} $\Sigma 16$ PAHs and C_{free} $\Sigma 16$ PAHs in bulk- and nanobiochars.

Limit of detection GC-MS

To ensure the accuracy and precision of the PAHs analysis in this investigation, a five-point calibration standard (25 to 250 ng/mL) was prepared in solution, encompassing detection limits, procedural blanks, check standards, sample duplicates, and matrix spike standards. The limit of detection (LOD) for each PAH is presented in Table S1. Extraction blanks (below 3 times LOD) were systematically analyzed within each sample batch and deducted from all sample

measurements. The precision of both the extraction procedure and GC-MS analysis was evaluated by measuring three replicates of tree distinct biochar samples (**b-BC-WL-550**, **b-BC-SSL1-550** and **b-BC-RH-550**), yielding a precision level below 20%.

Table S2. The limit of detection (LOD) of PAHs for GC-MS analysis.

Polycyclic aromatic hydrocarbons (PAHs)	LOD
Naphthalene [NAP]	0.00357
Acenaphthylene [ACY]	0.00242
Acenaphthene [ACE]	0.00256
Fluorene [FLO]	0.00217
Phenanthrene [PHE]	0.00240
Anthracene [ANT]	0.00174
Fluoranthene [FLA]	0.00124
Pyrene [PYR]	0.00073
Benzo[a]anthracene [BaA]	0.00042
Chrysene [CHR]	0.00103
Benzo[b]fluoranthene [BbF]	0.00010
Benzo[k]fluoranthene [BkF]	0.00058
Benzo[a]pyrene [BaP]	0.00192
Indeno[1,2,3-c,d]pyrene [IcdP]	0.00631
Dibenz[a,h]anthracene [DahA]	0.00815
Benzo[g,h,i]perylene [BghiP]	0.00464

Table S3. Physicochemical properties of bulk (b-BC) and nanobiochars (n-BC) produced at various temperatures and conditions by ball milling

Sample	S _{BET} [m ² /g]	S _{micro} [m ² /g]	S _{ext} [m ² /g]	V _p [cm ³ /g]	V _{micro} [cm ³ /g]	Pore width [nm]	pH	EC [μS/cm]	O/C [molar ratio]	H/C [molar ratio]
b-BC-WL-450	7.2	5.0	2.8	0.020	0.002	10.95	8.92	299	0.1094	0.5264
n-BC-WL-450	146.3	88.1	58.2	0.109	0.037	2.99	7.35	1157	0.1446	0.4496
b-BC-WL-550	195.2	170.5	25.3	0.091	0.075	1.85	9.02	270	0.1089	0.4074
n-BC-WL-550	195.8	142.9	52.4	0.143	0.060	2.92	7.43	1089	0.1199	0.3371
b-BC-WL-650	130.1	123.6	6.5	0.062	0.055	1.91	10.6	966	0.0791	0.2476
n-BC-WL-650	150.9	112.5	38.5	0.132	0.047	3.49	7.76	1253	0.1090	0.0464
b-BC-WL-750	3.8	16.4	1.6	0.024	0.008	2.48	10.98	486	0.0653	0.2152
n-BC-WL-750	160.7	120.3	40.5	0.136	0.050	3.39	7.98	1265	0.1002	0.0623
b-BC-RH-550	3.7	1.1	2.6	0.014	0.003	14.52	9.71	970	0.2143	0.5178
n-BC-RH-550	158.6	99.8	58.8	0.139	0.042	3.49	9.13	1328	0.2683	0.2435
b-BC-OSR-550	2.9	2.1	0.9	0.010	0.001	14.40	10.20	4840	0.1869	0.4083
n-BC-OSR-550	21.9	9.7	12.3	0.045	0.004	8.15	10.01	6880	0.3722	0.1447
b-BC-SSL1-550	13.6	2.6	11.1	0.075	0.084	22.11	8.18	535	0.5901	0.9153
n-BC-SSL1-550	15.0	1.2	13.8	0.055	0.025	14.69	6.82	2350	-	0.5177
b-BC-SSL2-500	16.7	-	-	0.078	-	18.65	9.56	1573	0.5725	0.5435
n-BC-SSL2-500	98.3	46.7	51.7	0.135	0.020	5.50	7.66	2450	0.2333	0.2408
b-BC-SSL3-500	41.6	29.1	-	0.055	0.012	5.27	9.55	552	0.1348	0.4325
n-BC-SSL3-500	51.2	22.0	29.2	0.090	0.010	7.03	7.45	1358	0.2548	0.2758
b-BC-SSL3-CO2-500	31.5	21.9	-	0.055	0.009	6.26	8.72	352	0.1495	0.3458
n-BC-SSL3-CO2-500	83.5	40.3	43.2	0.119	0.017	5.70	7.44	1685	-	0.1855

Where, b-BC= bulk biochar; n-BC= nanobiochar; WL=willow; SSL 1-3= different sewage sludges; RH=rice husk; OSR=oilseed rape; 450,500,550,650,750=temperature of pyrolysis; CO2= CO₂ as carrier gas; S_{BET}= specific surface area of biochars; S_{micro}= specific surface area of micropores; S_{ext}= external surface area of biochars; V_p= pore volume; V_{micro}= micropore volume; pH= in H₂O; EC= electrical conductivity; O/C, H/C,= molar ratios The values are mean of three repetitions.

Table S4. The content of individual C_{total} PAHs [$\mu\text{g/kg}$] in b-BCs and n-BCs.

PAHs	n-BC-WL-450	b-BC-WL-450	n-BC-WL-550	b-BC-WL-550	n-BC-WL-650	b-BC-WL-650	n-BC-WL-750	b-BC-WL-750	nS-BC-SSL1-550	b-BC-SSL1-550	n-BC-SSL2-500	b-BC-SSL2-500	n-BC-SSL3-500	b-BC-SSL3-500	n-BC-SSL3CO2-500	b-BC-SSL3CO2-500	n-BC-RH-550	b-BC-RH-550	n-BC-OSR-550	b-BC-OSR-550
NAP	420±31	194±16	878±85	511±53	1252±114	940±98.5	613±54	374±26.3	381±38	239±21	986±109	634±59	2274±221	1332±133	580±34	140±12	122±9.8	273±28	260±16	
ACY	3.05±0.24	5.95±0.72	17.2±1.9	0.30±0.02	20.4±1.9	3.61±0.26	7.55±0.80	44.6±2.9	11.6±0.9	266±29	11.5±1.0	50.1±4.1	15.3±1.1	359±29	0.62±0.03	1.92±0.13	2.92±0.23	5.87±0.61	9.29±0.87	
ACE	10.6±0.68	10.5±0.8	70.6±5.9	120±11	10.9±0.9	101±7.9	9.97±0.87	36.5±2.1	44.3±3.5	434±38	36.2±3.2	42.1±3.6	76.0±4.9	47.9±4.2	2.18±0.17	1.54±0.10	4.14±0.44	8.84±0.57	10.5±0.8	
FLO	30.9±2.5	13.1±1.0	549±45	85.8±5.3	20.8±1.3	102±8.5	6.93±0.55	108±8.8	264±25	384±21	73.1±5.7	44.7±2.6	1584±143	184±15	30.7±2.9	11.8±1.0	5.10±0.36	12.9±0.9	18.8±1.7	
PHE	216±25	118±8	113±9.4	150±15	31.3±1.8	27.9±3.2	24.5±2.6	20.4±2.6	295±21	563±50	170±12	22.8±2.8	315±37	150±15	0.42±0.04	10.5±0.7	57.5±5.8	33.7±3.9	120±12	
ANT	29.5±2.8	1.90±0.19	6.88±0.77	2.14±0.24	53.6±4.4	47.9±3.8	1.95±0.17	17.0±1.2	6.69±0.56	70.9±7.0	11.9±1.0	64.7±6.2	15.1±1.6	237±24	0.35±0.03	23.7±2.6	1.00±0.11	5.00±0.55	11.3±0.9	
FLA	55.1±5.3	31.3±2.7	12.4±1.2	50.7±5.3	2.52±0.26	30.9±3.2	4.11±0.32	15.4±1.8	295±27	113±12	228±25	263±28	235±21	109±8.1	1.26±0.14	0.5±0.03	3.56±0.30	7.20±0.64	14.5±0.9	
PYR	60.3±6.5	50.4±5.1	6.40±0.68	55.2±6.1	1.62±0.19	35.5±3.3	2.14±0.18	25.5±2.6	179±21	171±16	178±14	223±26	165±16	330±39	1.61±0.16	1.39±0.09	0.47±0.05	5.28±0.54	13.8±1.3	
BaA	55.8±5.6	10.9±1.3	1.24±0.13	115±12	0.65±0.05	26.0±2.8	0.57±0.04	9.03±0.70	184±17	203±22	140±10	116±10	111±8.7	520±52	0.05±0.01	0.03±0.01	0.04±0.01	0.76±0.08	6.97±0.67	
CHR	59.1±6.4	33.4±2.9	1.58±0.14	90.1±11.2	0.50±0.05	14.1±1.5	0.10±0.01	11.1±0.9	206±21	739±67	11.2±1.3	224±22	441±46	580±64	0.22±0.01	0.02±0.01	0.13±0.01	0.88±0.10	5.84±0.46	
BbF	40.3±3.1	250±21	40.7±2.6	161±15	0.39±0.04	45.9±4.2	0.60±0.05	10.9±0.9	11.4±1.3	287±30	4.20±0.47	108±10	12.1±1.4	498±49	0.55±0.06	1.39±0.15	0.60±0.05	1.17±0.13	0.36±0.04	
BkF	10.7±0.9	233±21	0.88±0.11	135±16	0.15±0.01	75.2±6.7	0.72±0.06	13.8±1.4	10.0±1.1	330±25	27.1±2.9	311±28	10.4±1.0	561±58	0.82±0.09	1.21±0.12	0.41±0.04	0.25±0.02	0.13±0.01	
BaP	56.8±4.4	125±7.7	1.33±0.09	135±9	1.49±0.10	80.8±5.9	1.35±0.11	9.16±0.58	151±11	754±49	365±26	683±46	210±15	449±27	30.6±2.2	0.88±0.05	8.14±0.56	2.12±0.15	1.04±0.06	
IcdP	0.08±0.01	15.1±1.1	0.82±0.05	31.3±2.2	0.52±0.04	51.7±3.9	0.61±0.04	9.32±0.67	13.1±0.9	79.1±5.4	8.46±0.64	117±8.0	8.28±0.59	149±9.9	0.27±0.01	0.08±0.01	0.07±0.01	1.51±0.11	0.73±0.05	
DahA	5.79±0.4	0.13±0.01	0.22±0.02	110±7.8	0.62±0.04	20.6±1.4	0.48±0.04	8.36±0.59	9.90±0.71	230±14	13.9±0.9	450±32	11.8±0.8	483±36	0.31±0.02	0.35±0.02	0.15±0.01	1.21±0.08	0.79±0.05	
BghiP	0.92±0.06	25.2±1.8	0.43±0.03	28.4±1.9	0.35±0.02	30.3±2.1	0.22±0.02	11.7±0.9	7.10±0.50	207±14	12.7±0.9	232±15	7.50±0.51	160±10	0.21±0.01	0.18±0.01	0.91±0.06	0.59±0.04	0.81±0.06	
SUM [μg/kg]	1054±60	1119±121	1700±125	1783±111	1398±95.6	1633±9	675±16.3	724±51	2072±153	5073±334	228±165	3590±257	5494±380	6156±451	1061±73.0	196±13	207±14	360±25	473±33	

Values are mean of three repetitions. <LOD – bellow limit of detection. NAP - naphthalene; ACY - acenaphthylene; ACE - acenaphthene; FLO - fluorene; PHE - phenanthrene; ANT - anthracene; FLA - fluoranthene; PYR - pyrene; BaA - benz(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(g,h,i)perylene.

Table S5. The content of individual C_{free} PAHs [ng/L] in b-BCs and n-BCs.

PAHs	n-BC-WL-450	b-BC-WL-450	n-BC-WL-550	b-BC-WL-550	n-BC-WL-650	b-BC-WL-650	n-BC-WL-750	b-BC-WL-750	nS-BC-SSL1-550	b-BC-SSL1-550	n-BC-SSL2-500	b-BC-SSL2-500	n-BC-SSL3-500	b-BC-SSL3-500	n-BC-SSL3CO2-500	b-BC-SSL3CO2-500	n-BC-RH-550	b-BC-RH-550	n-BC-OSR-550	b-BC-OSR-550
NAP	64.7±6.2	59.5±6.3	72.2±7.4	63.02±4.51	67.7±4.9	60.1±5.5	63.5±5.3	64.5±3.8	69.6±6.8	74.2±6.3	66.9±6.6	80.2±8.5	88.2±8.9	77.6±6.1	78.1±8.1	67.5±6.9	22.8±2.6	30.4±2.8	22.9±1.7	60±5.0
ACY	0.19±0.01	0.13±0.01	0.23±0.01	0.19±0.01	0.11±0.01	0.17±0.01	0.21±0.02	0.26±0.01	0.18±0.02	0.12±0.02	0.07±0.01	0.28±0.03	0.19±0.03	0.14±0.03	0.12±0.03	0.23±0.02	0.06±0.006	0.11±0.01	0.05±0.004	0.18±0.02
ACE	1.25±0.09	1.18±0.08	1.47±0.10	3.45±0.29	2.38±0.22	1.12±0.09	6.08±0.41	2.21±0.20	5.9±0.36	1.73±0.15	1.42±0.13	3.31±0.26	1.29±0.11	1.43±0.12	0.44±0.04	2.41±0.21	0.28±0.02	0.48±0.04	0.41±0.04	1.41±0.09
FLO	2.13±0.16	11.1±0.9	3.35±0.28	12.32±0.80	2.19±0.14	13.4±1.2	1.67±0.12	2.16±0.17	2.03±0.17	13.5±0.9	14.8±1.3	2.33±0.19	1.94±0.16	14.3±1.08	2.64±0.19	14.02±0.83	1.98±0.16	5.36±0.37	1.80±0.15	1.9±0.10
PHE	20.3±1.1	18.5±1.6	19.7±2.1	19.96±2.14	19.7±1.7	18.9±1.4	17.6±1.5	20.7±2.1	20.8±2.3	20.3±1.2	20.8±1.7	21.2±2.4	19.3±1.7	21.7±2.2	4.76±0.52	21.8±1.9	3.34±0.31	6.55±0.58	3.32±0.22	19.2±1.6
ANT	0.05±0.01	0.06±0.01	0.04±0.01	0.1±0.01	0.22±0.02	0.19±0.02	0.03±0.002	0.26±0.03	0.21±0.02	0.2±0.01	0.17±0.02	0.14±0.01	0.02±0.002	0.24±0.01	0.15±0.04	0.15±0.01	0.24±0.01	0.09±0.01	0.32±0.03	0.04±0.004
FLA	<LOD	0.05±0.01	0.1±0.01	0.07±0.01	0.1±0.01	0.08±0.01	0.09±0.01	0.11±0.01	0.07±0.01	0.08±0.01	0.08±0.02	0.1±0.01	0.08±0.01	0.13±0.04	0.08±0.02	0.17±0.02	0.1±0.01	0.05±0.004	0.04±0.004	0.09±0.009
PYR	0.01±0.00	0.09±0.01	0.16±0.01	0.11±0.01	0.18±0.01	0.11±0.01	0.15±0.01	0.15±0.01	0.11±0.02	0.16±0.01	0.13±0.01	0.14±0.02	0.14±0.02	0.27±0.02	0.17±0.04	0.22±0.03	0.21±0.02	0.09±0.009	0.06±0.006	0.15±0.01
BaA	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
CHR	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
BbF	0.07±0.01	0.07±0.01	0.08±0.01	0.03±0.003	0.01±0.002	0.01±0.003	0.01±0.001	<LOD	<LOD	<LOD	<LOD	0.02±0.002	0.04±0.002	<LOD	<LOD	0.02±0.001	<LOD	<LOD	0.09±0.008	<LOD
BkF	<LOD	<LOD	0.01±0.00	<LOD	0.01±0.003	0.01±0.009	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.01±0.001
BaP	<LOD	0.05±0.01	0.08±0.01	0.01±0.005	0.11±0.01	0.01±0.007	0.01±0.001	0.01±0.001	0.34±0.02	0.01±0.001	0.42±0.03	0.16±0.03	0.56±0.03	0.03±0.002	0.12±0.01	0.01±0.001	<LOD	0.05±0.003	0.01±0.001	0.01±0.001
IcdP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
DahA	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.01±0.001	0.01±0.001	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
BghiP	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
SUM	88.7±6.3	90.7±6.7	97.4±7.0	99.3±6.45	92.7±7.3	94.1±6.2	89.3±6.4	90.4±5.9	99.2±7.2	110±7.4	105±6.5	108±7.7	112±8.6	116±9.1	86.6±6.5	107±6.7	28.9±1.9	43.1±3.1	29.1±1.91	83.3±5.9

Values are mean of three repetitions. <LOD – below limit of detection. NAP - naphthalene; ACY - acenaphthylene; ACE - acenaphthylene; FLO - fluorene; PHE - phenanthrene; ANT - anthracene; FLA - fluoranthene; PYR - pyrene; BaA - benz(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(g,h,i)perylene.

Table S6. The log K_d values for individual PAHs in b-BCs and n-BCs.

PAHs	n-BC-WL-450	b-BC-WL-450	n-BC-WL-550	n-BC-WL-650	b-BC-WL-650	n-BC-WL-750	b-BC-WL-750	n-BC-SSL1-550	b-BC-SSL1-550	n-BC-SSL2-500	b-BC-SSL2-500	n-BC-SSL3-500	b-BC-SSL3-500	n-BC-SSL3CO2-500	b-BC-SSL3CO2-500	n-BC-RH-550	b-BC-RH-550	n-BC-OSR-550	b-BC-OSR-550
NAP	3.81	3.51	4.08	4.27	4.19	3.99	3.76	3.74	3.51	4.17	3.90	4.41	4.23	4.12	3.93	3.79	3.61	4.08	3.64
ACY	4.21	4.09	4.88	5.27	4.32	4.56	5.23	4.82	6.35	5.22	5.26	4.90	6.40	3.57	3.43	4.48	4.43	5.04	4.71
ACE	3.93	3.95	4.68	3.66	4.95	3.21	4.22	3.88	5.40	4.41	4.10	4.77	4.53	3.70	3.01	3.75	3.93	4.33	3.87
FLO	4.16	3.07	5.21	3.98	3.88	3.62	4.70	5.12	4.46	3.69	4.28	5.91	4.11	3.12	3.34	3.77	2.98	3.84	4.00
PHE	4.03	3.81	3.76	3.20	3.17	3.14	2.99	4.15	4.44	3.92	3.03	4.21	3.84	1.95	3.25	3.50	3.94	4.01	3.80
ANT	5.74	4.47	5.27	5.38	5.39	4.76	4.81	4.50	5.54	4.85	5.66	6.00	6.00	3.38	4.26	5.00	4.05	4.20	5.43
FLA	7.06	5.78	5.10	4.42	5.61	4.67	5.14	6.62	6.14	6.47	6.44	5.46	5.93	4.17	5.26	3.71	4.84	5.22	5.18
PYR	6.97	5.77	4.61	3.96	5.50	4.17	5.22	6.23	6.02	6.15	6.20	6.08	6.09	3.98	5.11	3.85	3.70	4.96	4.96
BaA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CHR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BbF	5.78	6.56	5.69	4.48	6.63	4.71	-	-	-	-	6.65	5.48	-	-	6.20	-	-	4.13	3.36
BkF	-	-	5.15	4.14	7.01	-	-	-	-	-	-	-	-	-	-	-	-	-	4.35
BaP	-	6.44	4.25	4.11	6.98	5.11	6.02	5.65	7.74	5.94	6.63	5.57	7.25	5.42	6.01	-	5.23	5.15	5.04
IcdP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DahA	-	-	-	-	-	-	-	5.83	7.21	-	-	-	-	-	-	-	-	-	-
BghiP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Where red indicates an increase in affinity, green signifies a decrease in affinity, and gray represents no change compared to bulk counterparts.

Table S7. The content of metals [mg kg⁻¹] in b-BCs and n-BCs.

metals [mg kg ⁻¹]	n-BC-WL-450	b-BC-WL-450	n-BC-WL-550	b-BC-WL-550	n-BC-WL-650	b-BC-WL-650	n-BC-WL-750	b-BC-WL-750	n-BC-SSL1-550	b-BC-SSL1-550	n-BC-SSL2-500	b-BC-SSL2-500	n-BC-SSL3-500	b-BC-SSL3-500	n-BC-SSL3CO2-500	b-BC-SSL3CO2-500	n-BC-RH-550	b-BC-RH-550	n-BC-OSR-550	b-BC-OSR-550
Cd	0.49± 0.03	0.49± 0.04	0.13± 0.01	0.25± 0.03	0.11± 0.01	0.24± 0.02	0.39± 0.03	0.50± 0.04	0.79± 0.05	1.21± 0.09	1.11± 0.12	1.48± 0.18	0.49± 0.042	1.20± 0.14	0.78± 0.06	1.49± 0.17	0.11± 0.01	0.24± 0.03	0.14± 0.02	0.25± 0.02
Mn	120± 9.0	144± 14	95.3± 9.6	189± 21	92.5± 5.6	269± 24	91.5± 7.8	136± 12	897± 94	1104± 118	211± 19.1	226± 14	417± 33	492± 54	485± 49	501 ± 53	370± 40	452± 49	68.2± 7.1	69.4± 7.3
Cr	2.46± 0.23	3.58± 0.38	2.46± 0.18	5.09± 0.39	4.97± 0.28	11.7± 0.98	1.88± 0.17	4.76± 0.42	301± 31	440± 41	67.4± 7.2	74.3± 4.3	85.5± 10.3	101± 8.2	91.2± 10	109± 9.6	8.45± 1.01	55.9± 6.12	4.98± 0.57	6.87± 0.75
Ni	2.62± 0.18	4.81± 0.35	2.09± 0.20	4.71± 0.25	11.4± 1.1	49.9± 3.1	3.09± 0.23	3.38± 0.32	57± 5.4	75.1± 6.9	61.7± 6.2	63.9± 7.8	55.9± 5.4	69± 4.9	7.79± 0.29	67.2± 6.4	2.20± 0.18	28.8± 2.3	1.87± 0.16	4.91± 0.33
Cu	12.2± 1.1	12.4± 1.1	7.01± 0.67	13.2± 1.2	7.04± 0.63	13.9± 1.1	8.81± 0.77	14.8± 0.89	262± 29	355± 31	228± 27	242± 17	238± 134	259± 28	271± 20	281± 30	7.35± 0.78	16.7± 1.2	9.09± 1.00	10.1 0.89
Zn	119± 12	133± 15	93.4± 7.9	155± 8.8	114± 8.6	240± 19.	105± 9.3	388± 22	896± 801	1177± 113	1774± 181	1819± 206	1560± 152	1733± 183	1757± 182	1852± 138	37.8± 4.2	71.8± 4.6	41.3± 4.1	74.8± 7.9
Fe	84.1± 8.5	88.2± 6.4	103± 11	116± 12	118± 9.5	172± 11	116± 6.5	181± 1	547± 54	561± 63	1975± 219	2013± 225	1692± 187	2122± 230	1941± 214	2156± 195	300± 24	924± 95	913± 78	1093± 88.0
Co	0.12± 0.01	0.25± 0.02	0.15± 0.02	0.25± 0.03	0.24± 0.02	0.24± 0.02	0.48± 0.04	0.75± 0.06	13.7± 1.6	20.5± 2.1	3.82± 0.22	5.04± 0.39	6.43± 0.44	6.73± 0.62	6.34± 0.62	7.38± 0.78	1.22± 0.14	2.56± 0.29	0.25± 0.02	0.49± 0.06
Pb	0.27± 0.02	0.49± 0.05	0.66± 0.05	1.24± 0.12	0.73± 0.07	1.46± 0.16	0.67± 0.06	1.38± 0.12	20.6± 2.1	23.3± 2.0	38.6± 2.3	41.7± 3.7	19.7± 1.7	21.4± 1.8	22.7± 2.7	24.4± 2.7	13.6± 2.3	79.1± 8.1	11.7± 1.1	47.9± 5.7

Table S8. The content of available fraction of metals [mg kg⁻¹] in b-BCs and n-BCs dispersed into 15 mL of Milli-Q water or 15 mL 0.01 mol L⁻¹ CaCl₂ solution.

metals [mg kg ⁻¹]	n-BC-WL-450	b-BC-WL-450	n-BC-WL-550	b-BC-WL-550	n-BC-WL-650	b-BC-WL-650	n-BC-WL-750	b-BC-WL-750	n-BC-SSL1-550	b-BC-SSL1-550	n-BC-SSL2-500	b-BC-SSL2-500	n-BC-SSL3-500	b-BC-SSL3-500	n-BC-SSL3CO2-500	b-BC-SSL3CO2-500	n-BC-RH-550	b-BC-RH-550	n-BC-OSR-550	b-BC-OSR-550
Milli-Q water																				
Cd	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Mn	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Cr	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Ni	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Cu	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Zn	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Fe	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Co	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Pb	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
0.01 mol L ⁻¹ CaCl ₂																				
Cd	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Mn	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Cr	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Ni	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Cu	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Zn	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Fe	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Co	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Pb	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

Where <LOD means below the limit of detection (LOD).

Table S9. Relationship between physiochemical properties of b-BCs and n-BCs and PAHs content (statistically significant differences (P<0.05) are marked in red).

	C ^{total} Σ16 PAHs	C ^{free} Σ16 PAHs	S _{BET} [m ² /g]	S _{micro} [m ² /g]	S _{ext} [m ² /g]	V _p [cm ³ /g]	V _{micro} [cm ³ /g]	Pore width [nm]	pH	EC [μS/cm]	C [%]	H [%]	N [%]	O [%]	O/C [molar ratio]	H/C [molar ratio]	(O+N)/C [molar ratio]	Ash [%]
C ^{total} Σ16 PAHs	1.000	0.654	-0.188	-0.211	-0.187	0.042	0.105	0.285	-0.193	-0.271	-0.478	-0.127	0.617	-0.001	0.384	0.430	0.514	0.429
C ^{free} Σ16 PAHs	0.654	1.000	-0.012	0.012	-0.144	0.094	0.169	0.061	-0.320	-0.478	-0.073	0.053	0.505	-0.365	-0.065	0.268	0.061	0.123

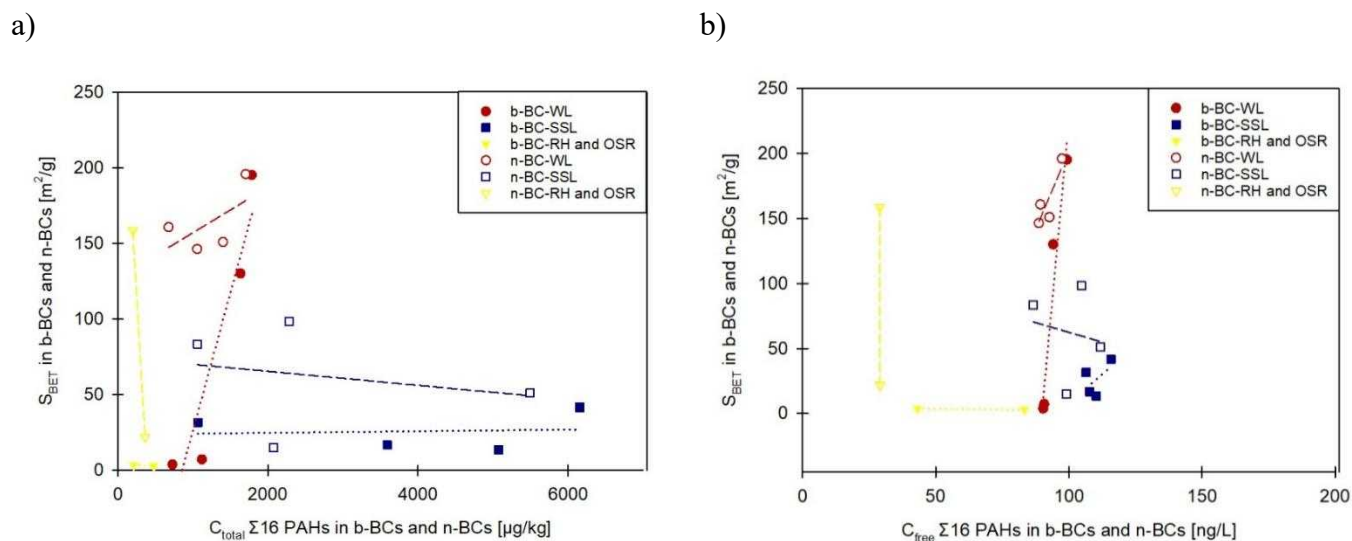


Figure S2. The relationship between S_{BET} and C_{total} $\Sigma 16$ PAHs (a) and C_{free} $\Sigma 16$ PAHs (b) in b-BCs and n-BCs.

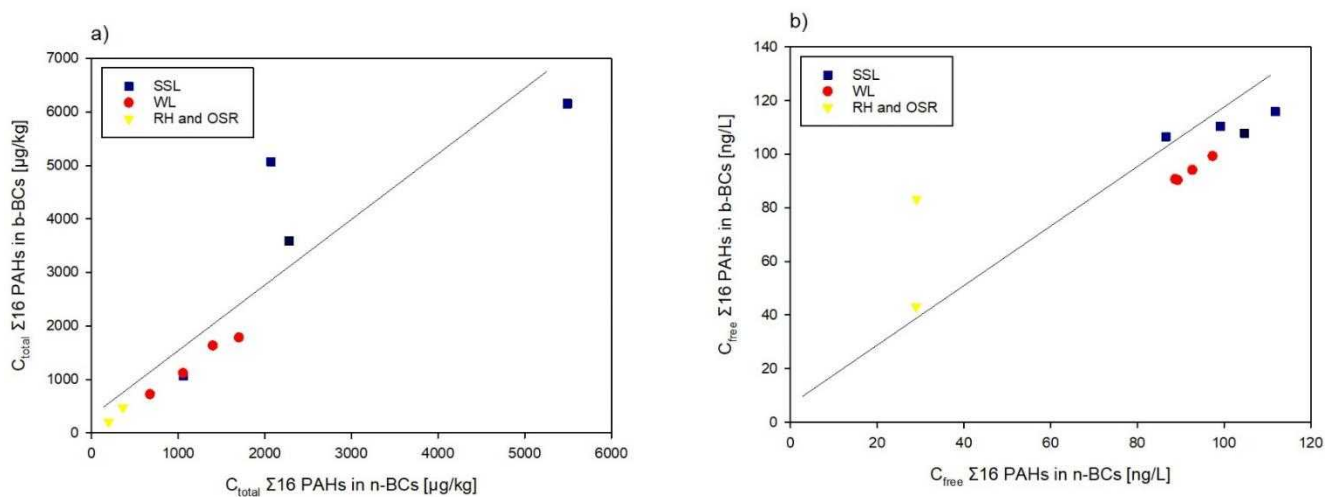


Figure S3. The linear relationship between C_{total} $\Sigma 16$ PAHs (a) and C_{free} $\Sigma 16$ PAHs (b) in n-BCs and b-BCs.

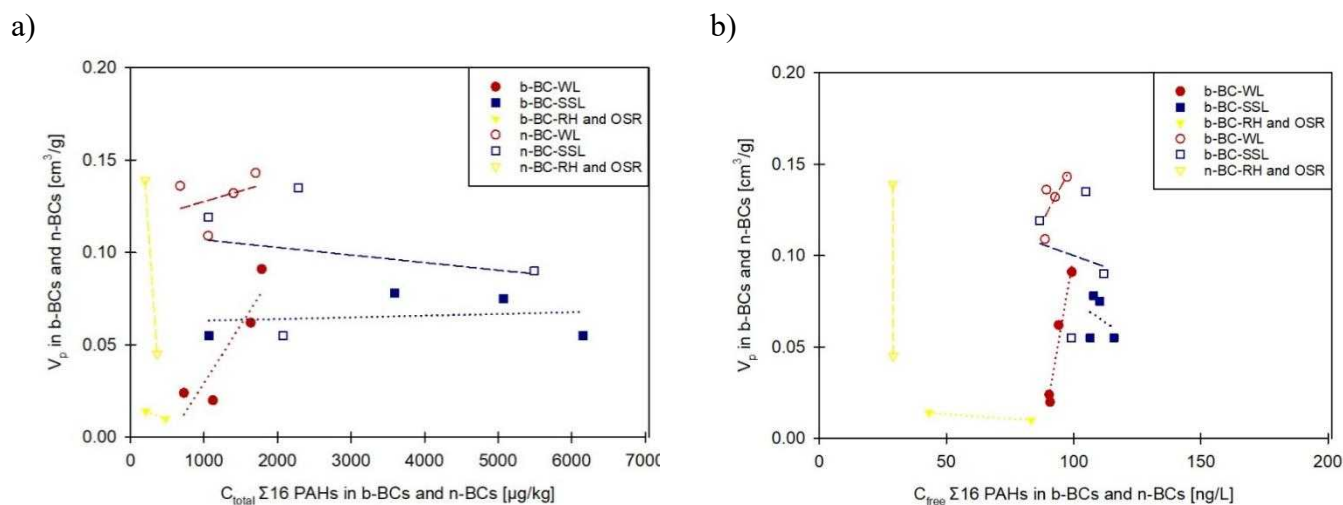


Figure S4. The linear relationship between V_p and $C_{\text{total}} \Sigma 16 \text{ PAHs}$ (a) and $C_{\text{free}} \Sigma 16 \text{ PAHs}$ (b) in b-BCs and n-BCs.

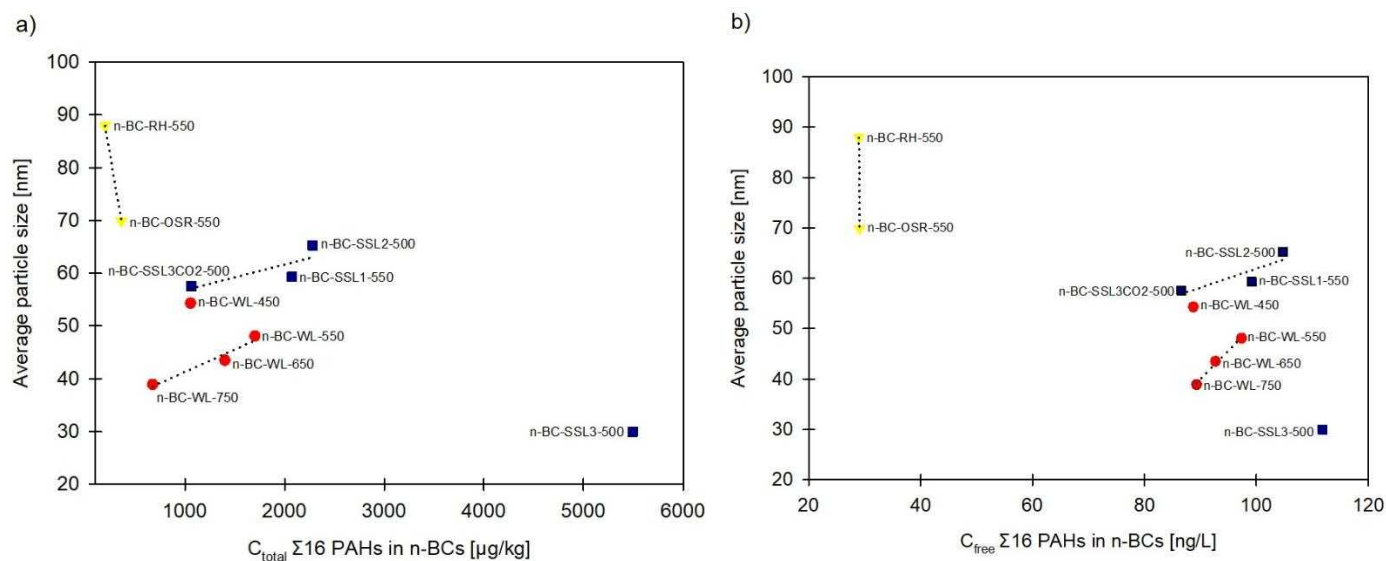
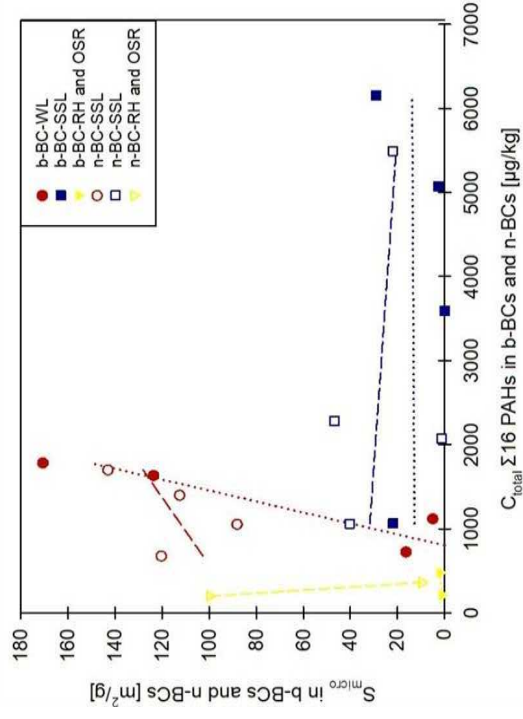


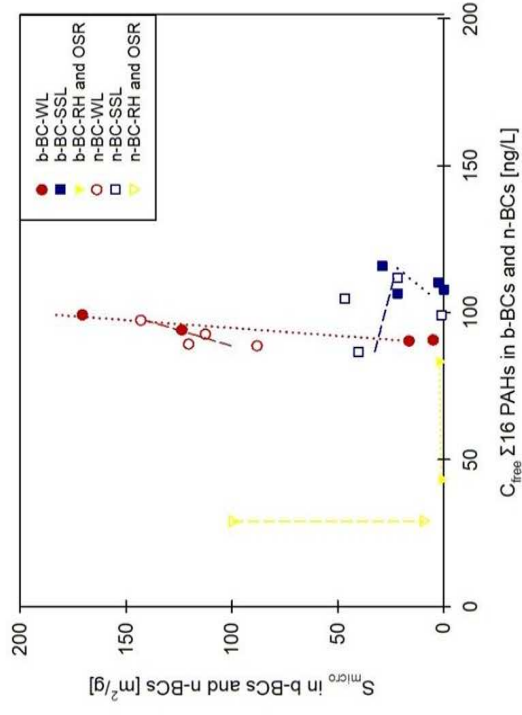
Figure S5. The relationship between average particle size and $C_{\text{total}} \Sigma 16 \text{ PAHs}$ (a) and $C_{\text{free}} \Sigma 16 \text{ PAHs}$ (b) in n-BCs.

Other releanships

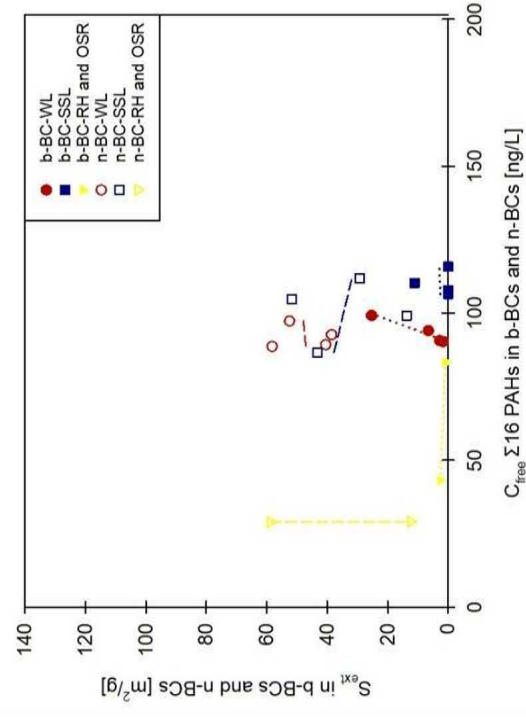
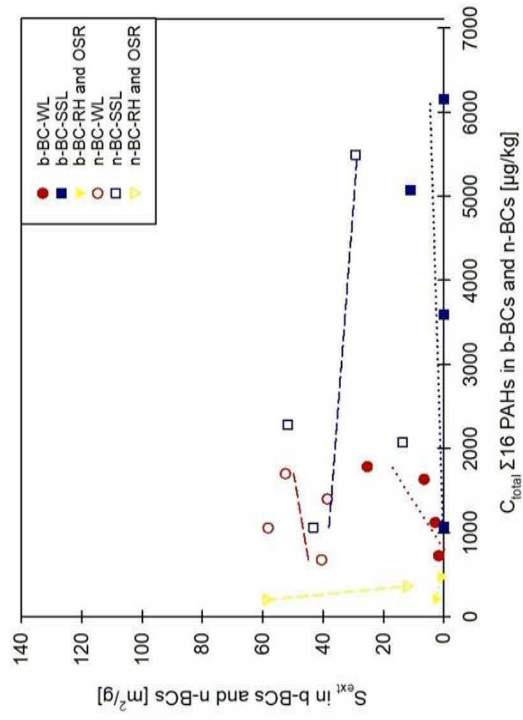
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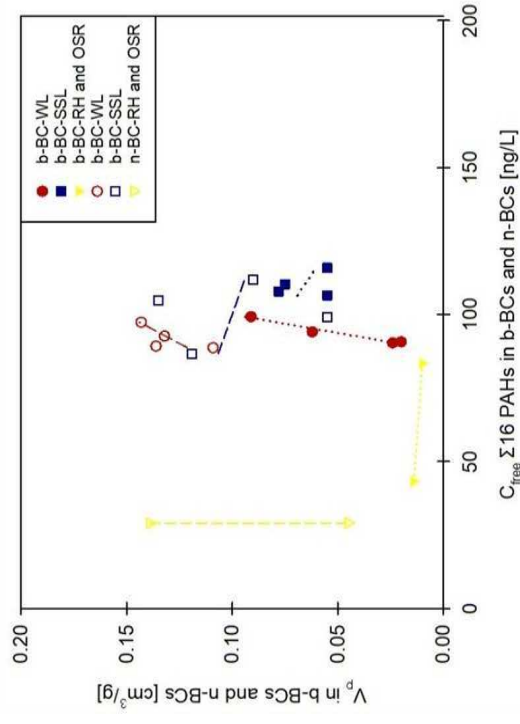
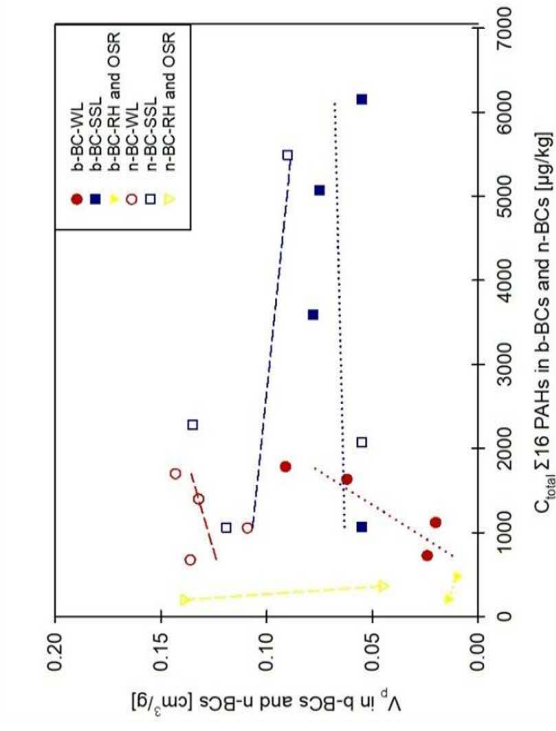
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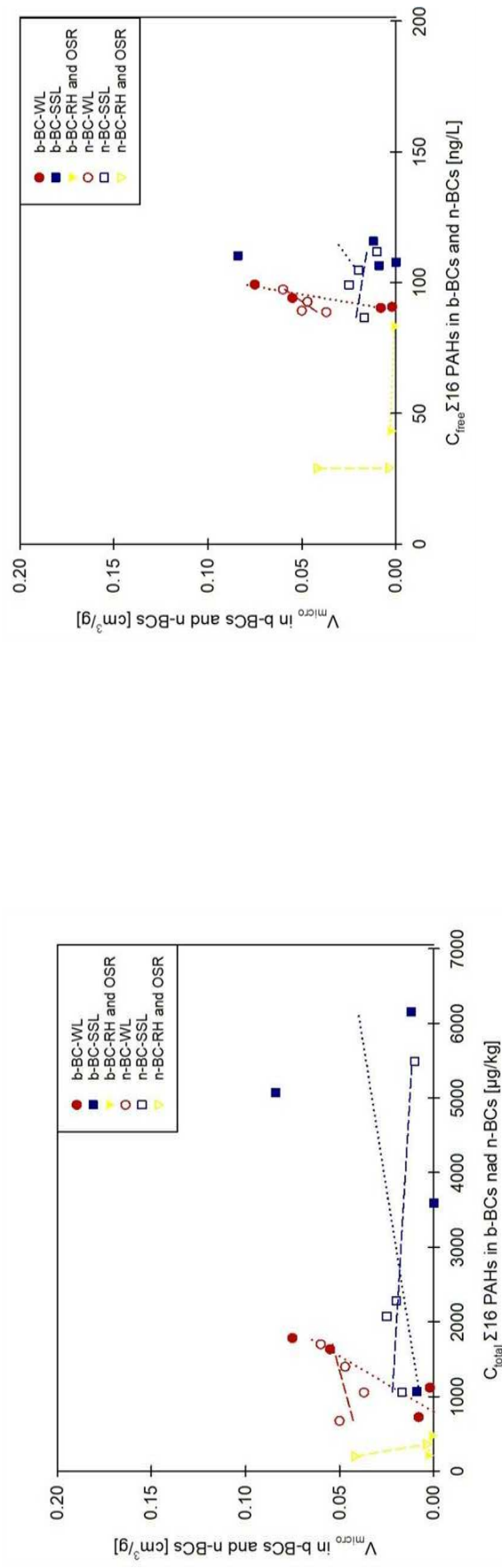
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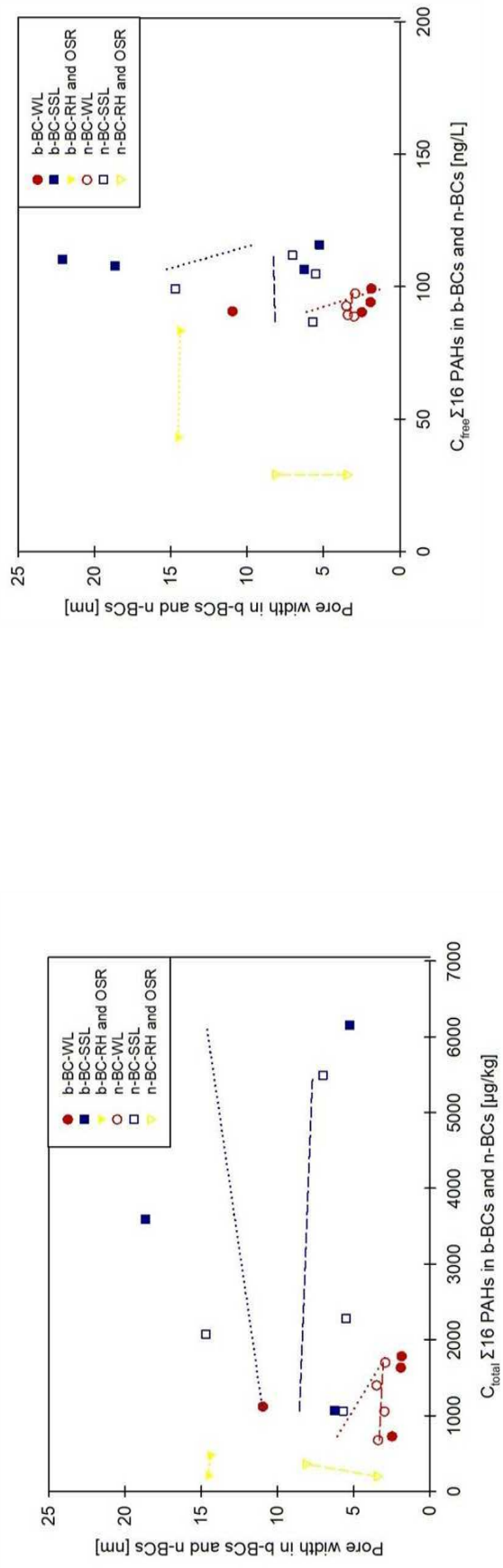
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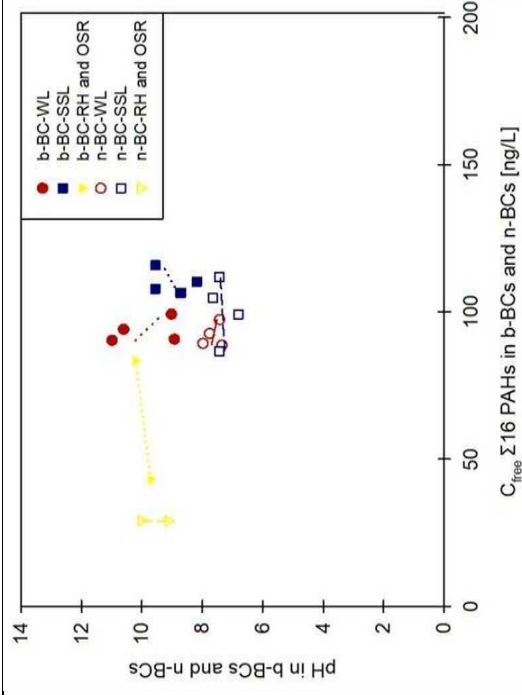
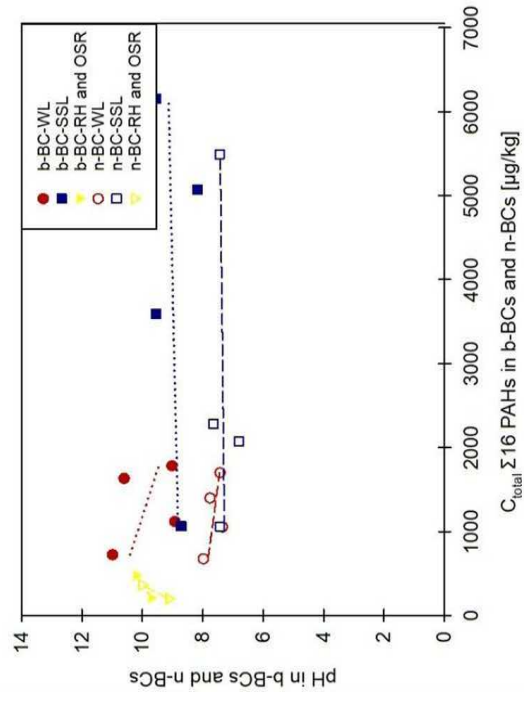


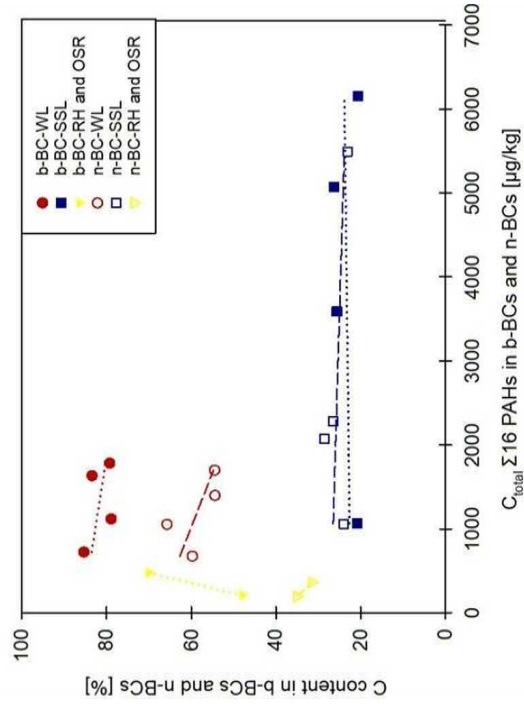
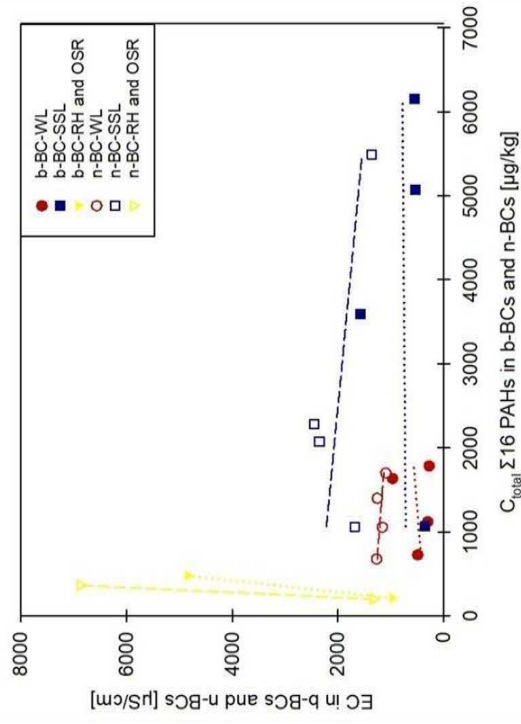
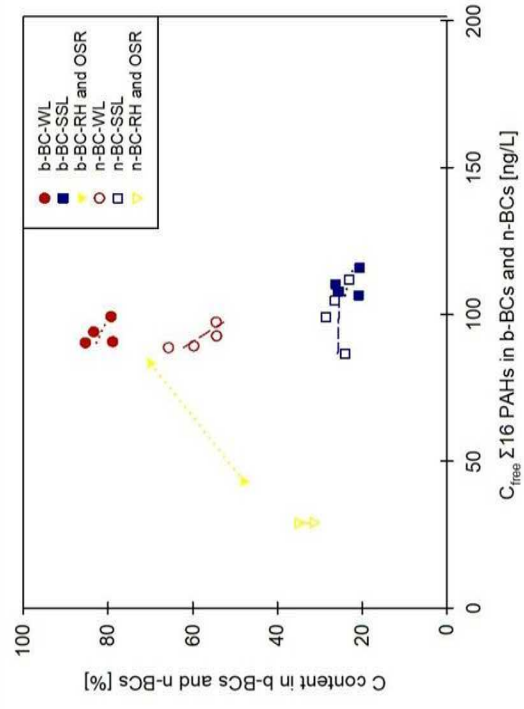
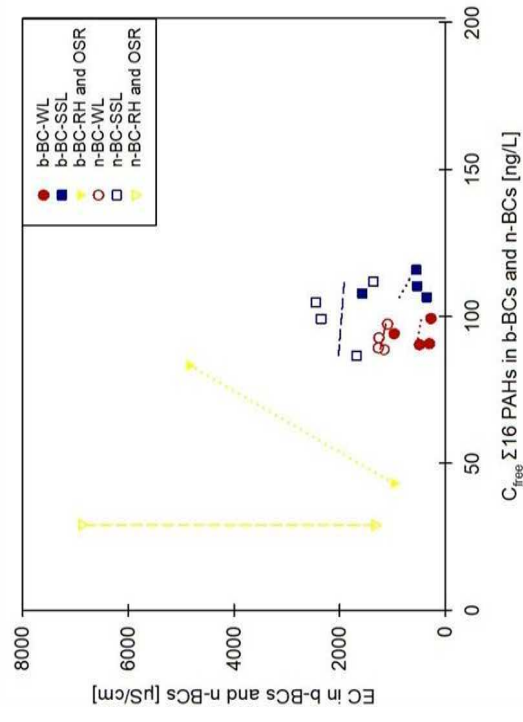
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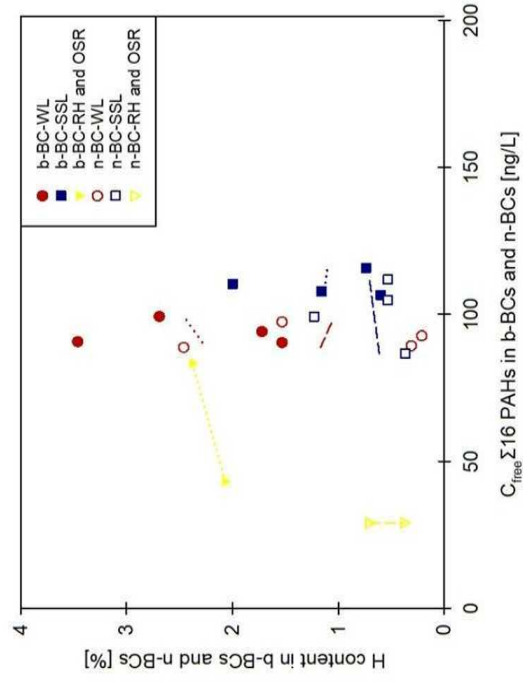
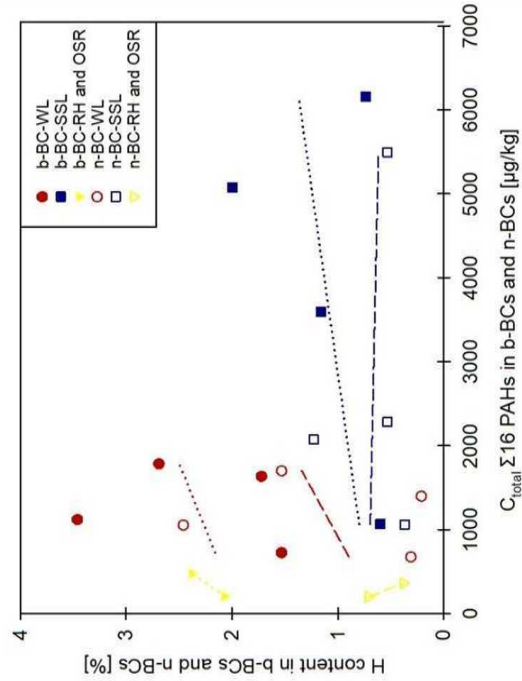
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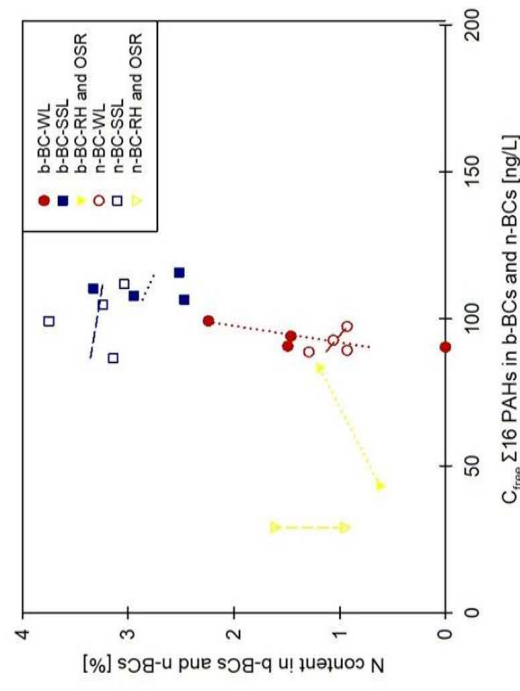
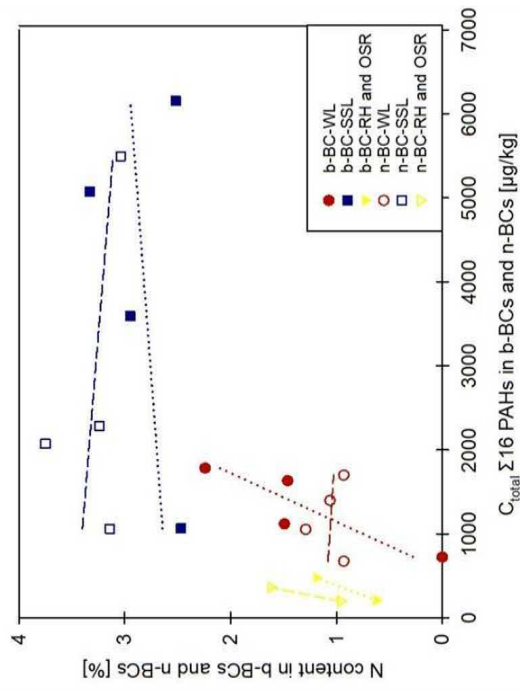




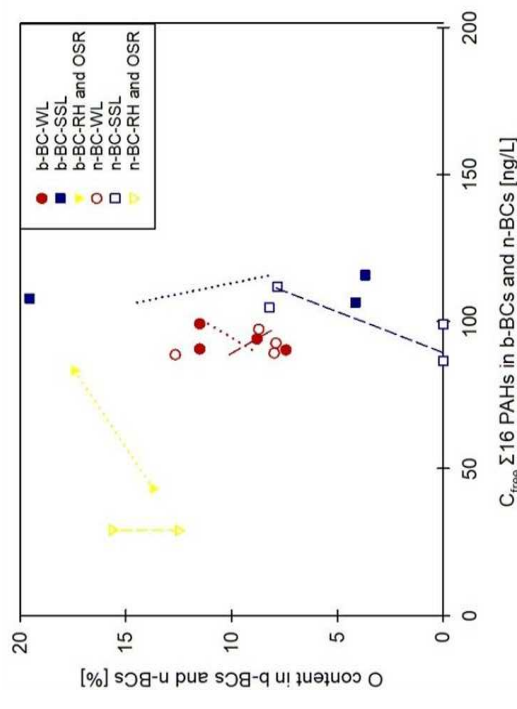
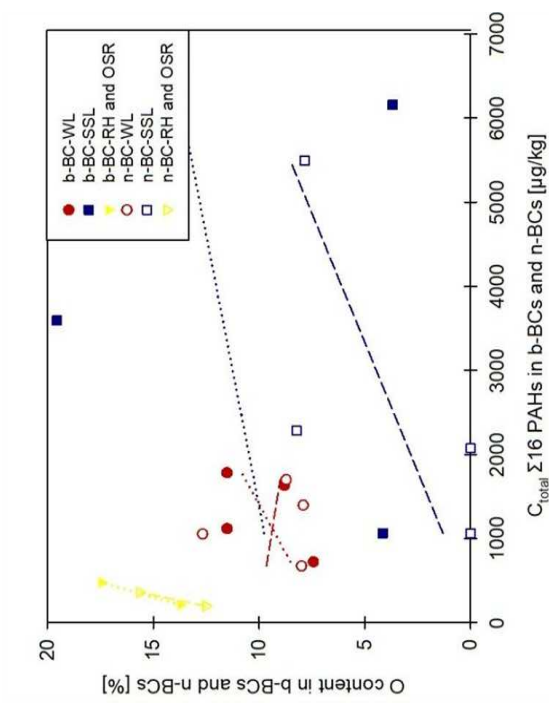
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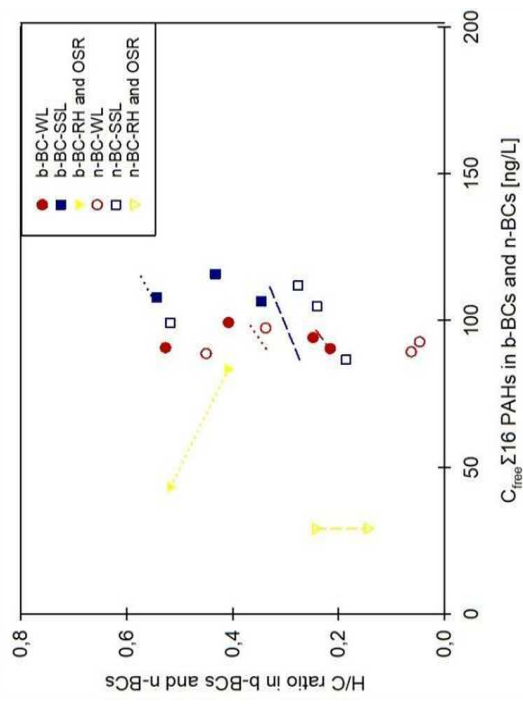
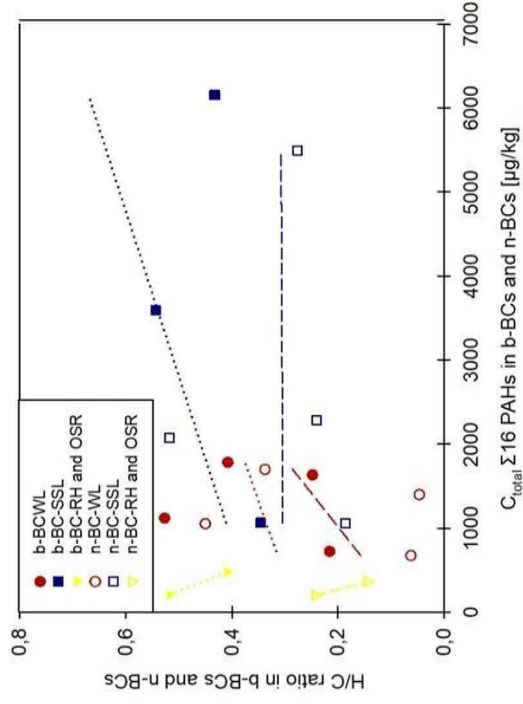
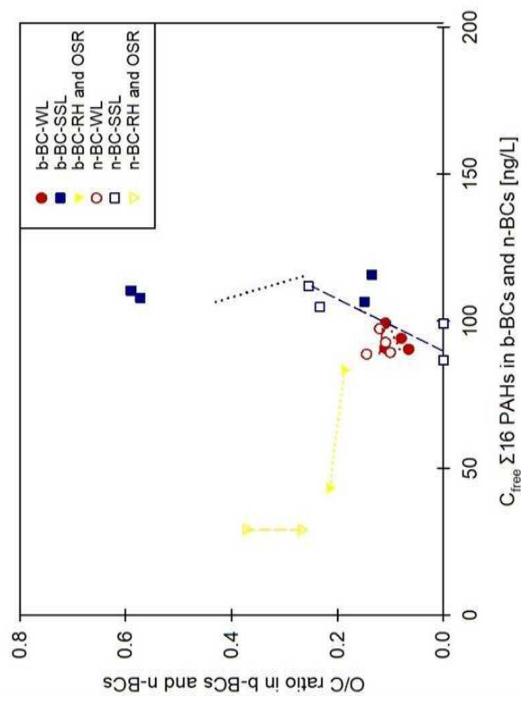
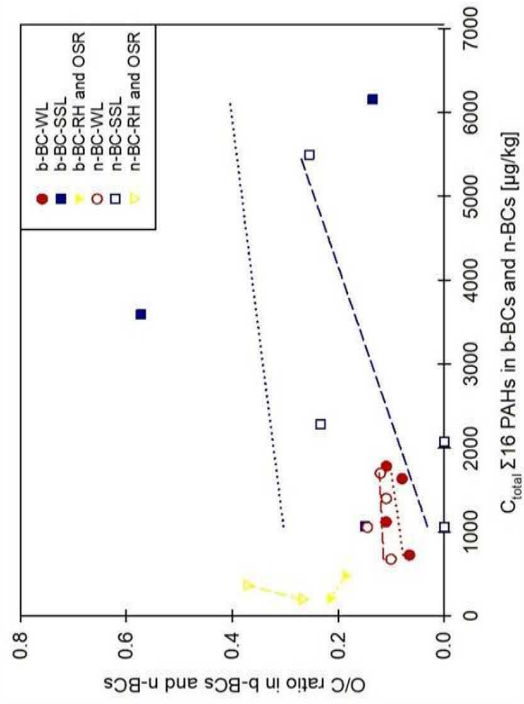
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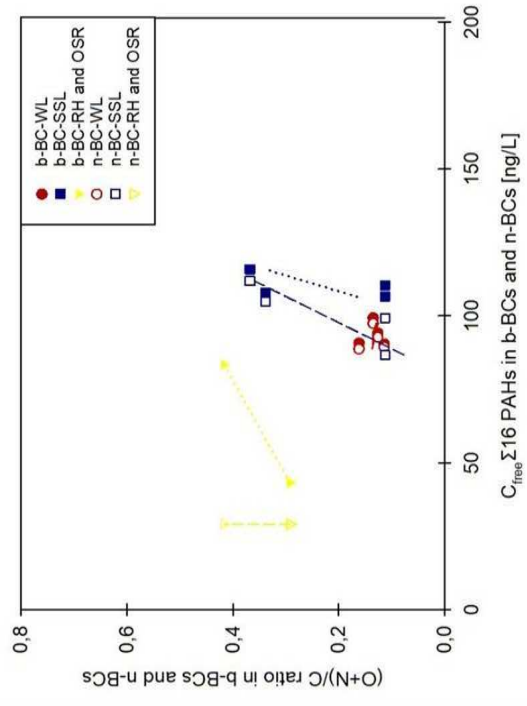
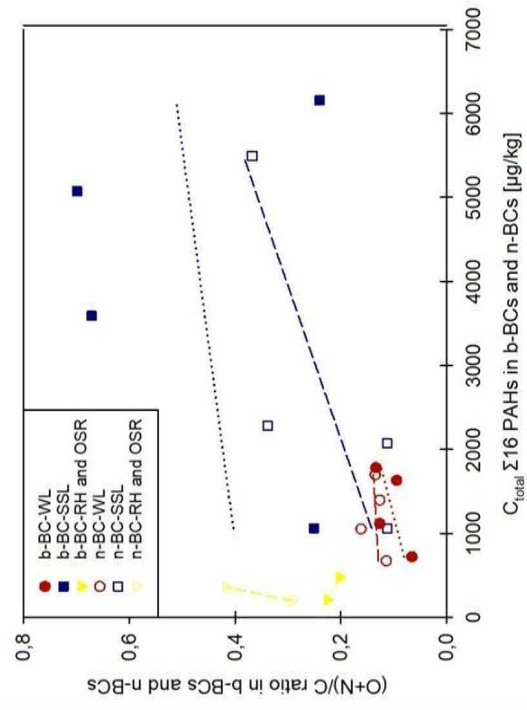
O/C



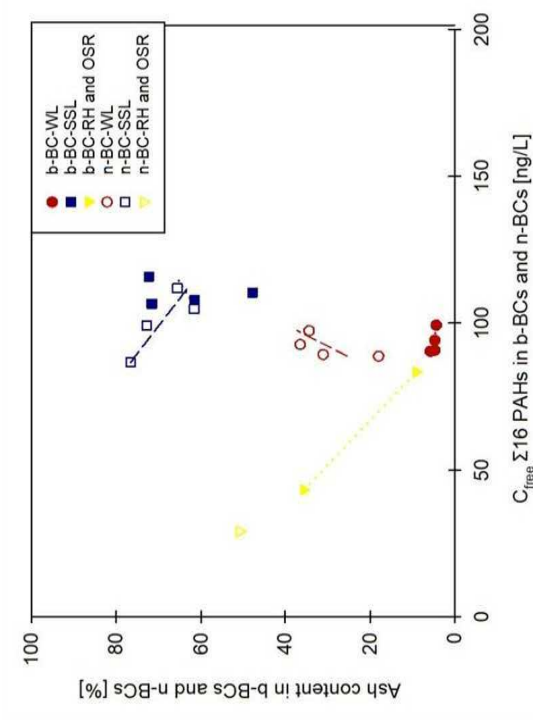
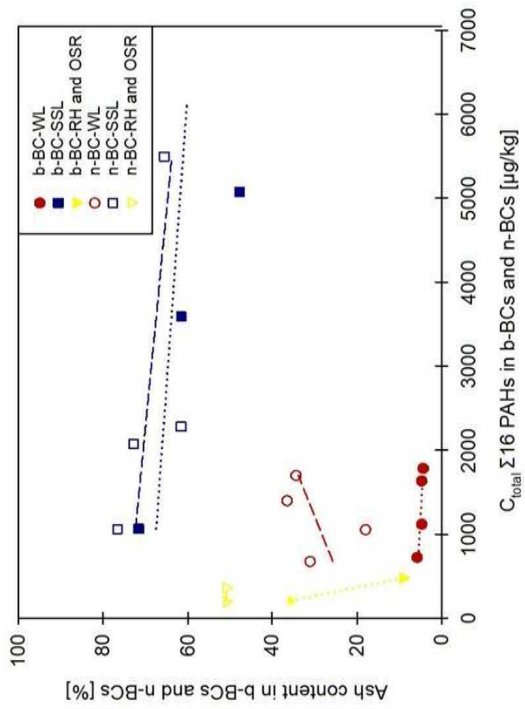
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Publikacja D3

M. Racziewicz, A. Bogusz, B. Pan, B. Xing, P. Oleszczuk

"Contrasting environmental impacts of nano–biochar and conventional biochar on various organisms"

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Contrasting environmental impacts of nano-biochar and conventional biochar on various organisms

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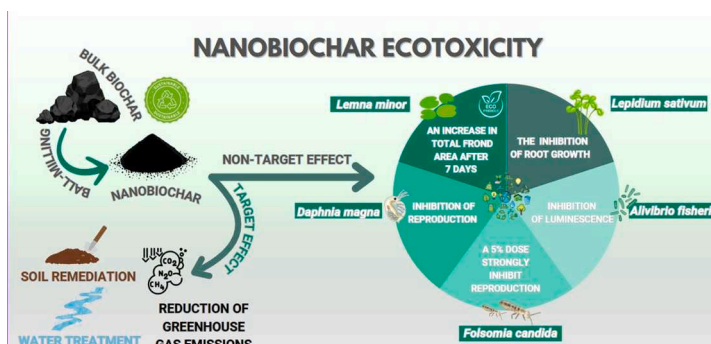
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HIGHLIGHTS

- Reducing biochar size boosts toxicity (18 %–2886 %) due to enhanced surface activity.
- Effects differed by species, biochar concentration, exposure type.
- Nano-particles of n-BC penetrate organisms more easily, which may increase toxicity.
- Toxic or stimulatory effects correlate significantly with contaminants in biochar.
- Findings underscore need to consider size, production methods in BC use.

GRAPHICAL ABSTRACT



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ABSTRACT

The environmental hazards of nanobiochar (n-BC) require attention due to limited knowledge. This study is the first to explore the effects of biochar size reduction across various organisms, including bacteria (*Allivibrio fischeri*), plants (*Lemna minor*, *Lepidium sativum*), and invertebrates (*Daphnia magna*, *Folsomia candida*). Bulk biochar (b-BC) and n-BC were applied in both liquid and solid-phase tests to assess their ecotoxicity. The resulting leachates were tested at concentrations of 2, 10, and 100 mg/L on organisms such as *Daphnia magna* and *Lemna minor*. In the solid-phase tests, b-BC and n-BC were added to the OECD soil at concentrations of 1 % and 5 % to evaluate toxicity in *Folsomia candida* and at concentrations of 1 % to evaluate toxicity in *Lepidium sativum*. We found n-BC to be significantly more toxic (by 18 % to 2886 %) to *A. fischeri* than b-BC, with toxicity increasing over time. Low doses (1 %) of both b-BC and n-BC did not cause mortality or inhibit reproduction in *F. candida*, though b-BC enhanced reproduction (by 30 % to 56 %) compared to n-BC. At a 5 % dose, both b-BC and n-BC inhibited reproduction *F. candida*, with n-BC being 0.5 to 1.8 times more toxic. Neither b-BC nor n-BC immobilized *D. magna*, but both inhibited reproduction (by 28 % to 35 %). The nanoscale dimensions of n-BC facilitated bioaccumulation in *D. magna*, leading to adhesion on the organism's body. The n-BC had a greater impact on plants, both b-BC and n-BC were non-toxic to *L. minor*, but all n-BC inhibited root growth in *L. sativum*.

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These findings highlight the importance of considering biochar size, feedstock, and pyrolysis conditions when evaluating environmental risks, ensuring safe use in sustainable agriculture.

1. Introduction

As global environmental challenges continue to grow, nanotechnology emerges as an essential tool for developing innovative solutions to pollution in water and soil. Among these advancements, carbonaceous-derived materials stand out as a promising material due to its remarkable ability to adsorb both organic and inorganic contaminants. Nano-biochar (n-BC) represents an innovative form of well-known carbonaceous material called biochar (BC) obtained through pyrolysis. The n-BC, defined as BC with particle sizes ranging from 1 to 100 nm (Rashid et al., 2023) is currently at the forefront of research. The current approaches to n-BC production and utilization include environmentally friendly synthesis methods and highlighting energy-efficient techniques according to the directions of green chemistry in nanotechnology (Lyu et al., 2018). Among various synthesis n-BCs methods, such as sonication (Oleszczuk et al., 2016) or conventional grinding followed by sieving the aqueous suspension of n-BC (Li et al., 2017), recently ball milling stands out as the most commonly employed technique (Naghdi et al., 2017).

The increased interest in n-BC can be attributed to n-BC's unique properties determined by particle size like higher specific surface area (Huang et al., 2020), reduced hydrodynamic radius, more negative zeta potential (Song et al., 2019), increased presence of oxygen (O)-containing functional groups, and the occurrence of carbon defects compared to pristine (regular) BC (Weber and Quicker, 2018). These distinctive properties potentially affect n-BC adsorption capacity to trace metals (TMs) and organic contaminants (e.g., polycyclic aromatic hydrocarbons - PAHs) (Ramanayaka et al., 2020), which can be used for wastewater treatment and immobilization of contaminants. However, by changing the mentioned properties during ball milling, the strength of interaction between hydrophobic organic contaminants and BC may decrease, making previously inaccessible PAHs, more available and mobile (Wang et al., 2017). Releasing these toxic substances into the environment and inducing harmful effects on the ecosystem. At the same time, more functional groups created during ball milling may increase the affinity (Liu et al., 2022) of n-BC to TMs and simultaneously decrease the TM's mobility. Moreover, n-BC exhibits a higher critical coagulation concentration (CCC) than other carbonaceous nanomaterials (e.g. carbon nanotubes or graphene oxides) (Song et al., 2019), which induces its greater aggregation or accumulation and colloidal stability (Sun et al., 2018). This better aggregation and stability of n-BC than other carbon materials may influence the toxicological character of nanoparticles. Aggregated forms of nanoparticles can have distinct biological effects compared to individual nanoparticles (Sani et al., 2023). For example, the reduced bioavailability of aggregated nanoparticles may influence their interactions with biological systems (Zhang, 2014).

Considering that bulk biochar (b-BC) and n-BC are primarily used in agriculture and soil remediation (Pathak et al., 2024; Pradhan et al., 2024; Sarma et al., 2024; Vadakkan et al., 2024), it is crucial to assess their impact on plants and different groups of organisms. The physico-chemical characterization and the contaminant content are insufficient for evaluating the environmental risks associated with n-BC (Ndoung et al., 2021; Osman et al., 2024). This is due to the complex interactions between n-BC and various environmental matrices, as well as the dynamic nature of contaminant release. Furthermore, the potential for bioaccumulation in ecosystems underscores the need for comprehensive ecotoxicological studies and long-term monitoring to fully understand their impacts. While chemical analyses still remain essential and give an overall look at possible behavior and indirect and potential risk of n-BC, ecotoxicological tests can complement information about the direct risk and offer valuable insights into the direct effects on living organisms,

such as their growth, behavior, reproduction, and overall physiological health over the long term (Domene et al., 2015; Oleszczuk et al., 2013). In this context, the biological assays should not be seen as a replacement for chemical analyses but rather as a supplementary tool, which extends our knowledge about potential threats and additionally allowing the explanation of interactions between various contaminants, providing crucial evidence in the assessment process.

Numerous studies have examined the effects of b-BC on organisms and plants (Godlewska et al., 2021; Kim et al., 2020; Marcińczyk et al., 2024; Wei et al., 2024). These studies have revealed that b-BC can have varying impacts on microorganisms, plants, and soil invertebrates (Kim et al., 2020; Marcińczyk et al., 2024; Wei et al., 2024) and the effect very often depends on the soil types. Currently, studies specifically focused on the ecotoxicity of n-BCs are extremely limited, highlighting the novelty and significance of our research. There is a lack of information regarding the influence of n-BC depending on feedstocks and pyrolysis conditions on different ecosystems. It is very crucial to explain the effect of the initial parameters of b-BC in the context of the toxicological properties of n-BC. A critical knowledge gap exists regarding whether size-related changes in n-BC properties influence potential toxicity. Addressing this gap is necessary for predicting n-BC's environmental impacts based on pristine BC properties and for the eventual widespread use of n-BC.

The aim of this study was to assess the impact of n-BC on various groups of organisms like bacteria (*Alivibrio fischerii*), plants (*Lemna minor*, *Lepidium sativum*), and invertebrates (*Daphnia magna*, *Folsomia candida*), specifically examining their effects on viability, reproduction, and growth. Evaluating organisms from various trophic groups can provide comprehensive information on the potential risks of introducing n-BC into the environment. Understanding interactions among organisms at different trophic levels is crucial for predicting n-BC's environmental impacts. Analyzing these interactions may reveal changes in ecosystem dynamics and potential cascading effects.

2. Materials and methods

2.1. Bulk and nano-biochars

Detailed information about production conditions and physico-chemical properties of bulk- (b-BC) and nano-biochar (n-BC) was presented in our previous work (Racziewicz et al., 2024b). The b-BCs used in this experiment were obtained from willow (WL), rice husk (RH), oilseed rape (OSR), and various sewage sludges 1–3 (SSL 1–3) collected from three wastewater treatment plants. The b-BCs were produced at 550 °C under a nitrogen atmosphere. The WL-derived b-BC were produced at additional temperatures of 450 °C, 650 °C, and 750 °C to examine the temperature-dependent effects on b-BC properties (Tomczyk et al., 2020), which may influence n-BC properties and ecotoxicity. The SSL-derived b-BC was also produced in a carbon dioxide atmosphere, which may alter b-BC properties and toxicity (Kończak et al., 2020). Afterward, the resulting b-BCs were frozen (−80 °C) and ground using a ball milling machine (PM100, Retsch Corporation, German) to achieve BC particle sizes smaller than 100 nm according to the method described previously (Racziewicz et al., 2024b). The contaminants content (PAHs and TMs,) and their bioavailability are presented in our previous work (Racziewicz et al., 2024a).

2.2. Ecotoxicological tests

The effect of the b-BC and n-BC on tested organisms was evaluated in liquid- and solid-phase tests. The “liquid-phase tests” investigate the

impact of leachates obtained from BC on organisms (mainly water organisms) and can be called indirect test because the part of chemical (leached from solid) is evaluated, whereas solid-phase tests examine the direct effect of the whole material on the tested organism. Five ecotoxicological tests were employed to assess and compare the ecotoxicity of b-BC and n-BC. The liquid phase (leachate) was prepared according to the EN 12457-2 protocol (2003). The b-BC and n-BC were mixed with deionized water at a proportion of 1:10 (solid:liquid, w/v) and were subsequently shaken on a horizontal shaker for 24 h (Model 358 A, ELPINSC+, Poland). The resulting extract was filtered through a 0.45 µm syringe filter.

2.3. Liquid phase test

The evaluation of the leachate toxicity to *Alivibrio fischeri* was performed based on Microtox® tests using a Microtox M500 analyzer (Microtox M500, Modern Water, UK) according to the test protocol (SDI, 1992). Before the test, the pH was adjusted to the range of 6–8 using 0.1 M HCl/NaOH to ensure test accuracy. Luminescence inhibition was measured after 5 and 15 min of exposure of *A. fischeri* to the leachates. The Microtox Omni software was employed for the analysis of the results.

The toxicity assessment of b-BC and n-BC to *Daphnia magna* was conducted following the standard procedures according to OECD 202 (OECD, 2004) and OECD 211 guidelines (OECD, 1998). The immobilization and reproduction of *D. magna* are the endpoints of this test. To assess the impact of b-BC and n-BC on immobilization, three concentrations (2, 10, and 100 mg/L) of each b-BC or n-BC were prepared by adding specific amounts in deionized water. In exposure containers, five young daphnids were placed in 100 mL of the test solution, with three replicates for each concentration of b-BC or n-BC. The containers without BCs were used as a control. Test conditions, including light and temperature, were consistent with the culture conditions, and the exposure medium was not aerated. Following the immobilization test, the impact of b-BC and n-BC on reproduction was further assessed for selected b-BC/n-BC (where the effect of the adhesion induced by n-BC was observed under a microscope). Two concentrations of b-BC or n-BC (2 and 10 mg/L) were selected. A young female *D. magna* was exposed to each concentration for 21 days, and at the end of the test, the total number of living offspring produced by the exposed *D. magna* was assessed.

In order to assess the toxicity of b-BC and n-BC solutions to aquatic plants, the duckweed *Lemna minor* was used according to the procedure proposed by OECD 221 guidelines (OECD, 2006). The primary objective of the test was to quantify substance-related effects on vegetative growth over a test period, based on assessments of selected measurement variables, including frond number and total frond area. Three concentrations of b-BC or n-BC (2, 10, and 100 mg/L) were applied. For each concentration, 3 colonies with 3–4 visible fronds each were placed into measurement vessels with a total volume of 100 mL. The cultures were incubated in a temperature-controlled environment (at 24 ± 2 °C) under continuous illumination with fluorescent lamps). The frond counting was manually performed at the beginning of the test (0 days) and after 2, 5, and 7 days using the ImageJ 1.54 software (ImageJ 1.54, Java, USA). The average specific growth rate (μ_i) was calculated for each replicate after 7 days, to assess the health status of plants following equation:

$$\mu_i = \frac{\ln(N_j) - \ln(N_i)}{t}$$

Where μ_i -average specific growth rate from time i to j, N_i - measurement variable in the test or control vessel at the time i, N_j -measurement variable in the test or control vessel at the time j, t-time period from i to j.

2.4. Solid phase test

An ecotoxicological test with Collembola (*Folsomia candida*) was performed following the standard procedure based on OECD 232 guidelines (OECD, 2016). The test examines the mortality and reproduction of *F. candida*. A detailed description of the test is presented in our previous publications (Kottowski et al., 2017; Oleszczuk et al., 2019; Tomczyk et al., 2021). Briefly, ten adult individuals (aged 10–12 days) were placed in a petri dish with standard soil (OECD) mixed with 1 % and 5 % of b-BC or n-BC. The standard soil (OECD) was used as a control. After 28 days, the individuals were separated from the soil using the flotation method and counted. The calculations were manually performed using the ImageJ 1.54 software (ImageJ 1.54, Java, USA).

Lepidium sativum was used as a test plant to assess the impact of b-BC/ n-BC on plant growth. The Phytotoxkit test was conducted according to the method presented in ISO (2016). The endpoints included seed germination inhibition and root growth inhibition after 72 h of exposure to the mixture of standard soil (OECD) with a 1 % addition of b-BC/n-BC at 25 ± 1 °C. The bioassays were conducted in three replicates. Analysis and length measurements were performed using Image Tool 1.28 software (Image Tool 1.28, CMEIAS, USA).

2.5. Statistical analysis

The data were subjected to statistical analysis using Statistica 13.3 software. Mean values were obtained from a triple set of data. Differences between ecotoxicological endpoints in individual tests and various b-BC/n-BC were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test at a significance level of 0.05. Pearson's correlation coefficient test was conducted to examine the relationships between physicochemical properties, pollutant contents (PAHs and TMs), and test results in Statistica 13.3. Significance was established at $p \geq 0.05$.

3. Results and discussion

3.1. Toxicity to *alivibrio fischeri*

Fig. 1 illustrates the effect of b-BC and n-BC on inhibition of luminescence of *Alivibrio fischeri*. Among all b-BC, the most pronounced toxicity towards *A. fischeri* was observed for b-BC-RH-550, exhibiting inhibition levels from 59 % to 62 % depending on exposure time. Regarding the feedstock, the highest toxicity towards *A. fischeri* was observed for agricultural residues-derived b-BCs (RH and OSR) (Fig. 1). The highest toxicity observed for this group of BCs may be attributed to the highest concentration of Pb in these b-BCs (Fig. S1) than in other investigated b-BCs. The b-BCs obtained from other feedstocks (SSL and WL) demonstrated a weak toxic effect (<20 %), which may be considered non-harmful according to guidelines (Doe et al., 2005), with the lowest toxicity observed for those derived from SSL1–3. In our previous work (Marcinińczyk et al., 2023) we also observed a similar trend where b-BC derived from SSL exhibited the lowest toxicity towards *A. fischeri* than BCs produced from biomass. Gondek et al. (2016) studied leachates from BCs produced from mixed sewage sludge with wheat straw, bark, or sawdust at temperatures of 300 °C or 600 °C and did not observe the harmful effect towards *A. fischeri*. The lower toxicity of SSL-derived b-BC compared to those from plant feedstocks (WL, RH and OSR) can be attributed to the content of essential nutrients (Racziewicz et al., 2024b). SSL-derived b-BC often contain higher levels of beneficial nutrients, such as nitrogen, phosphorus, and potassium, because sewage sludge is rich in these elements (Yuan et al., 2016). These nutrients can support the growth of microorganisms, potentially counteracting the negative effects of contaminants present in BC (Goldan et al., 2022).

The temperature of pyrolysis and used carrier gas also determined the toxic effect of b-BC on *A. fischeri* (Fig. 1). Regarding the temperature (WL-derived b-BC in the range of 450–750 °C), the luminescence

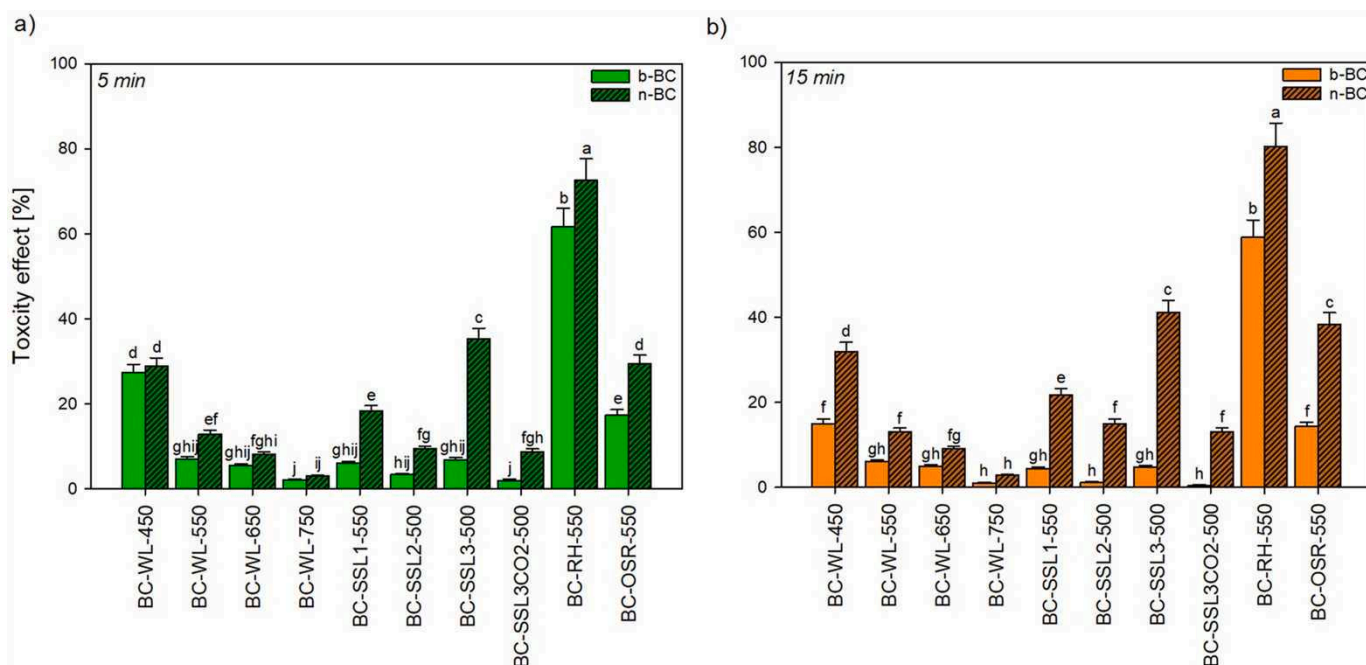


Fig. 1. *Alivibrio fischeri* luminescence inhibition (%) in Microtox® test. Error bars represent the standard deviation of the mean ($n = 3$). Biochars produced from willow (WL), rice husk (RH), oilseed rape (OSR), and various sewage sludges 1–3 (SSL 1–3). Different letters indicate significant differences among the treatments (Tukey test, $p \leq 0.05$).

inhibition decreased with increasing pyrolysis temperature (both after 5 and 15 min of exposure) which resulted in a 13-fold reduction in luminescence inhibition for b-BC-WL-750 compared to b-BC-WL-450 (Fig. 1). A similar trend was also noted previously by Lyu et al. (2016) for pine tree wood sawdust-derived BCs obtained at temperatures between 250 and 700 °C. BC obtained at the lowest temperatures (250–300 °C) exhibited greater toxicity to bacteria than BC produced at 700 °C. Regarding the carrier gas used during pyrolysis, it was observed that changing N₂ to CO₂ reduced the luminescence inhibition by 3.5 and 11 times after 5 and 15 min of exposure, respectively. A similar relationship was previously observed by Kończak et al. (2020) who also reported the luminescence inhibition reduction for SSL-derived BCs after changing CO₂ to N₂ atmosphere.

Moreover, a noteworthy observation was the toxicity exhibited a discernible decline with extended exposure of *A. fischeri* to the BC's leachate. The adverse effects decreased over time, emphasizing the dynamic nature of BC's toxicity under exposure conditions. These results may suggest the potential degradation or disintegration of initially toxic constituents occurred in BC's leachates. Chemical or biochemical processes may instigate a diminution in the concentration of deleterious compounds, consequently translating into attenuated toxicity (Bala et al., 2022). This can also indicate that bacteria might adapt to the presence of toxins, potentially activating defense mechanisms that reduce their sensitivity to the toxins over time, resulting in a smaller decrease in luminescence after 15 min compared to 5 min. Additionally, some toxins might be metabolized or broken down by the bacteria used in the test, leading to a reduction in their concentration and toxicity over time.

The reduction of BC's size to the nanoscale (n-BC) significantly increased the n-BC's leachate toxicity to *A. fischeri*, with the Tukey test results indicating that n-BC derived from specific feedstocks and pyrolysis conditions exhibits higher toxicity than b-BC, highlighting the critical role of production parameters—such as feedstock type, pyrolysis temperature, and carrier gas—in shaping the ecotoxicological properties of BC (Fig. 1). Comparable to b-BC, n-BC-RH-550 consistently demonstrated the highest toxicity (Fig. 1), with luminescence inhibition ranging from 73 % to 80 % depending on exposure duration. The n-BC-

SSL3–500 demonstrated the next highest level of toxicity, showing luminescence inhibition rates of 35 % and 41 % after 5 and 15 min, respectively. In the comparison to feedstocks, it was observed that n-BC-OSR-500 and n-BC-WL-550 exhibited lower toxicity, with inhibition rates ranging from 13 % to 29 % after 5 min (Fig. 1a) and from 13 % and 38 % after 15 min (Fig. 1b). Notably, the highest increase (from 179 % to 410 % and from 381 % to 288 %, respectively after 5 and 15 min) in luminescence inhibition after size reduction was observed for SSL1–3-derived n-BC. However, despite this, a strong toxic effect (>20 %) was observed only for n-BC-SSL1–550 and n-BC-SSL3–500.

For WL-derived n-BC, within a temperature range of 450–750 °C, toxicity increased from 5 % to 83 % after 5 min and from 81 % to 159 % after 15 min, when compared to b-BC. For BCs produced in the CO₂ atmosphere, size reduction led to a 30-fold increase in toxicity after 15 min of exposure (n-BC-SSL3CO2–500 vs. b-BC-SSL3CO2–500) (Fig. 1b), which was not as pronounced for BCs produced in an N₂ atmosphere from the same feedstock (8-fold increase). Nonetheless, the luminescence inhibition for n-BC produced at CO₂ was still significantly lower than for n-BC produced at N₂ (n-BC-SSL3CO2–500 vs n-BC-SSL3–500) (Fig. 1).

It is noteworthy that n-BC showed corresponding trends in changes concerning the applied pyrolysis conditions as observed for b-BC. These trends suggest that the reduction of the b-BC size, surface area of n-BC (Racziewicz et al., 2024b) may also affect surface activity, which subsequently may impact the observed toxic effect. Consequently, the increase of surface activity may impact the reactivity and gradual leaching of toxic substances from the n-BC with higher intensity than it occurs for b-BC. Furthermore, n-BC demonstrated a lower pH than b-BC (Table S2), potentially enhancing the leaching efficiency of TMs during the leachates preparation, which subsequently resulted in toxicity towards *A. fischeri*. Directly before the test, the pH of the leachates is adjusted to neutral. However, during the extraction process (leachates preparation), the pH may influence the efficiency of TMs release from BCs. Consequently, leaching of TMs can be more effective from BCs with lower pH (such as n-BCs) compared to those with higher pH (like b-BCs), as acidic conditions can increase the solubility of metals in water and facilitate their leaching (Yakubu et al., 2018).

3.2. Toxicity to *Lemna minor*

For all tested concentrations of b-BC and n-BC, the average specific growth rates (μ_i) were very similar to those for the control, indicating that adding b-BC or n-BC did not affect the rate of biomass growth or the size of *L. minor* throughout the entire test (Fig. 2), which is confirmed by Tukey's test. There was also no relationship between the applied concentration and μ_i for b-BC and for lower doses (2 and 10 mg/L) of n-BC. However, an exception was observed for certain SSL-derived (e.g. n-BC-SSL1-550, n-BC-SSL2-500, n-BC-SSL3-500, and n-BC-SSL3CO2-500) and agriculture residues-derived n-BC (n-BC-RH-550 and n-BC-OSR-550), where higher μ_i were observed at the highest concentration of BC (100 mg/L). These values were from 20 % to 44 % higher compared to the control (Fig. 2), suggesting a stimulating effect of those BCs on plant growth. It may be attributed to their higher nutrient content, including essential elements, which are more readily available in n-BC.

For all concentrations of b-BC tested, no significant changes ($p > 0.05$) in the total frond surface area of *L. minor* were observed compared to the control at any evaluation points during exposure (Fig. S2). This clearly indicates that b-BC does not have a toxic effect on *L. minor*. A different trend was observed in relation to n-BC. For the selected concentrations of n-BC (specifically at a concentration of 100 mg/L), a statistically significant ($p \leq 0.05$) increase of the total frond area was observed after 7 days of *L. minor* exposure to SSL-derived n-BC (n-BC-SSL1-550, n-BC-SSL2-500, n-BC-SSL3-500, and n-BC-SSL3CO2-500) and agriculture residues-derived n-BC (n-BC-RH-550 and n-BC-OSR-550) (Fig. S2) compared to the control. This observation supports previous observations related to μ_i for these materials (Fig. 2). For the remaining concentrations (2 and 10 mg/L), no statistically significant ($p > 0.05$) differences between control and solutions with n-BC were observed throughout the whole test.

The differences between b-BCs derived from different feedstocks were relatively small in terms of *L. minor*'s frond area across all tested concentrations. However, with respect to n-BC, these differences became more pronounced after 7 days, and the increase in *L. minor*'s frond area intensified depending on the used feedstock and the

increasing tested concentrations of n-BC (Fig. S2). This indicates that when BC is in nano size, certain substances are leached out that determine the potential positive effect on *L. minor*'s frond area. This observation highlights that the feedstock material and its particle size can significantly influence the efficacy of BC in promoting frond area growth.

To date, no studies have examined the effects of BC on the growth of *L. minor*. The most relevant research on BC studies was conducted by Jagodzinski et al. (2018), who evaluated the impact of wood ash on the growth rate of *L. minor*. BC, in addition to organic carbon, also consists of inorganic minerals, which are referred to as ash content. Ash content is a crucial parameter because it indicates the potential of BC to supply essential nutrients to plants. The authors also demonstrated a significant increase in *L. minor* growth rate in ultraoligotrophic media supplemented with ash at a dose of up to 5 g/L.

In our research, we also observed the statistically significant relationships (Fig. 3) between the ash content ($p \leq 0.001$) and additionally nitrogen content ($p \leq 0.012$) in n-BC and μ_i . However, these correlations were only observed for the highest concentration of n-BC. At lower concentrations, the amount of ash was likely insufficient to stimulate plant growth. For b-BC, the availability of nutrients may have been reduced due to the larger particle size, which made access to them more difficult. SSL-derived n-BC (n-BC-SSL1-550, n-BC-SSL2-500, n-BC-SSL3-500, and n-BC-SSL3CO2-500), along with agriculture residues-derived n-BC (n-BC-RH-550 and n-BC-OSR-550) exhibit a high proportion of both ash and nitrogen. This likely contributes to the growth-promoting effects observed at higher concentrations of n-BC due to an enhanced nutrient supply. Given the critical role nitrogen plays in plant growth, it is reasonable to expect that a higher dose of n-BC will improve nutrient leaching, leading to more pronounced growth stimulation. Additionally, the ash present in n-BC may contain essential nutrients such as potassium, phosphorus, and calcium (Hossain et al., 2020), which are beneficial for plant growth. Consequently, a higher ash content in n-BC may result in a greater availability of nutrients for *L. minor* (Bisane et al., 2023).

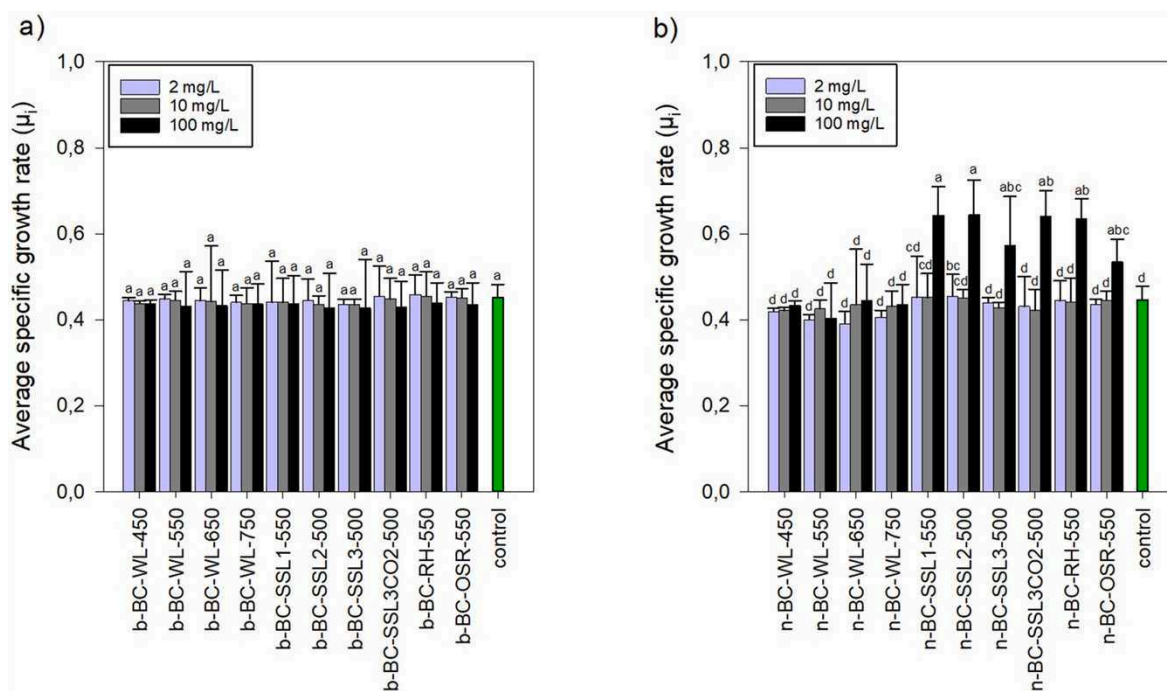


Fig. 2. *Lemna minor* average growth rates (μ_i) after 7 days calculated for the number of fronds for b-BC (a) and n-BC (b). Biochars produced from willow (WL), rice husk (RH), oilseed rape (OSR), and various sewage sludges 1–3 (SSL 1–3). Error bars represent the standard deviation of the mean ($n = 3$). Different letters indicate significant differences among the treatments (Tukey test, $p \leq 0.05$).

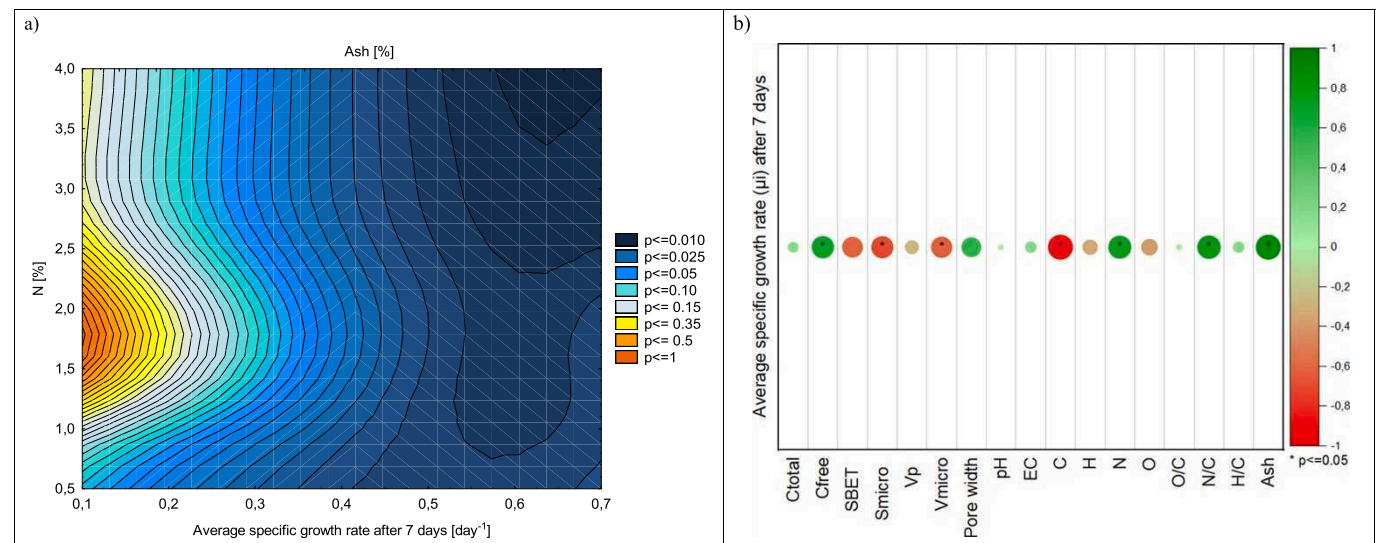


Fig. 3. Three-way interaction plot of *Lemna minor* average growth rates after 7 days in a 100 mg/L n-BC solution, highlighting parameters influencing growth, with optimal performance represented by blue regions (a) and correlation plot presenting the values of Pearson correlation factor between physicochemical properties of n-BC and *Lemna minor* average growth rates after 7 days in a 100 mg/L n-BC solution (b).

3.3. Toxicity to *Daphnia magna*

The *Daphnia magna* immobilization test revealed that none of the tested concentrations caused statistically significant immobilization of these organisms for b-BCs and n-BCs (Table S3). However, detailed microscopic observations of the organisms revealed a significant adherence of BC's particles to the bodies of *D. magna* (Fig. 4). This adherence may pose long-term risks, potentially leading to chronic toxicity. The adherence effect was not observed in the system with b-BC. Following this observation, n-BC and b-BC representing different feedstocks were selected for a reproduction test to assess the long-term effects of the particles. Both n-BC and b-BC affected the reproduction of *D. magna* (Table 1), but a higher reduction compared to control was observed for n-BC than b-BC, especially in the system with a higher concentration of particles. The harmful effect was observed for n-BC for two applied concentrations, while for b-BC the effect was only noted for the highest concentration of particles. Reproduction inhibition clearly depended on the feedstock used for BC production. Except for b-BC-WL-

750 and n-BC-WL-750, higher reproduction inhibition was observed for n-BC than b-BC (Table 1). Regarding the age of first reproduction, it was generally noted that the values were very close to those of the control group. However, for n-BC-OSR-550 and b-BC-WL-750, the age of first reproduction was significantly longer (>11 days) compared to the control (9 days). This delay may be explained by the potential presence of inhibitory compounds in the specific feedstocks or by the larger impact of these particular BC on the organisms' overall energy budget, possibly due to the leaching of specific TMs or organic compounds that interfere with reproductive timing.

3.4. Toxicity to *Lepidium sativum*

No notable impact or trend was detected regarding the inhibition of *Lepidium sativum* seed germination (Table S6). Other studies report that under stress conditions, the addition of n-BC can increase the germination rate of plants. Xin et al. (2024) observed maximum increases in germination rates of 24.8 % and 20.8 % for BC's nanoparticles and BC,

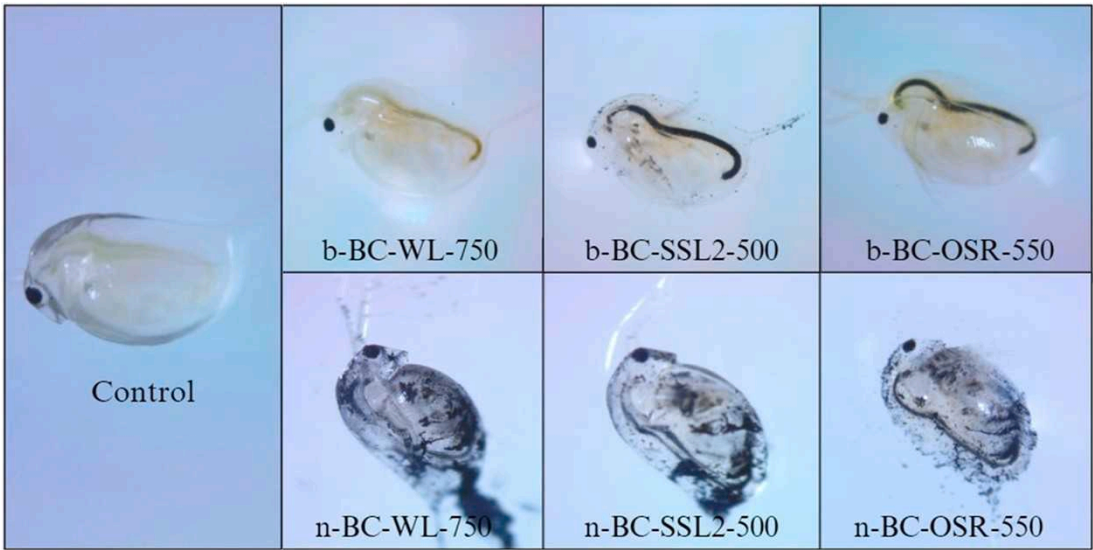


Fig. 4. Photographs of *Daphnids* in the control and suspension of selected b-BC and n-BC. Biochars produced from willow (WL), oilseed rape (OSR), and sewage sludges 2 (SSL2).

Table 1*Daphnia magna* reproduction test results for selected b-BC and n-BC.

Sample	Concentration [mg/L]	Cumulative offspring per introduced parent (mean)	Reduction compared to control [%]	Age of First Reproduction [day]
Control	0	96.3 ± 9.3ab	–	9.0
BC-WL-750	Bulk	98.2 ± 9.4ab	0	9.7
		69.2 ± 7.7 cd	27.1	11.3
	Nano	76.2 ± 5.1 cd	20.9	8.7
		70.2 ± 7.8 cd	28.1	9.1
BC-SSL2-500	Bulk	102.2 ± 8.1a	0	8.7
		92.4 ± 10.6abc	4.0	9.3
	Nano	82.0 ± 6.6abcd	14.8	10.5
		76.6 ± 9.7abcd	20.5	9.7
BC-OSR-500	Bulk	90.6 ± 11.3abc	4.7	8.9
		77.8 ± 10.2abcd	19.2	9.3
	Nano	91.8 ± 9.3abc	5.9	9.5
		62.6 ± 6.4d	35.0	11.7

Biochars produced from willow (WL), oilseed rape (OSR), and sewage sludge 2 (SSL2).

respectively, compared to the control group. In contrast, Zhang et al. (2020) did not observe any effect of BC's nanoparticles at different concentrations (0.5, 5, and 50 mg/L) on the seed germination rates of rice and tomato.

Nevertheless, a statistically significant effect ($p \leq 0.05$) of BCs was observed on root growth inhibition (Fig. 5). When comparing different feedstocks, only the SSL-derived b-BC (b-BC-SSL1-550, b-BC-SSL2-500, and b-BC-SSL3-500) among the tested b-BC exhibited toxicity towards *L. sativum*, causing root growth inhibition ranging from 18 % to 23 %. In contrast, the agricultural residues-derived b-BC (b-BC-RH-550 and b-BC-OSR-550) and WL-derived b-BC (b-BC-WL-550) produced at the same temperature were found to be non-toxic and even stimulated the root growth by 6 % to 15 %. SSL-derived b-BC produced at lower temperatures (500–550 °C) tends to have higher concentrations of volatile organic compounds (VOCs), which can disrupt root development (Goldan et al., 2022). Additionally, SSL-derived b-BC has lower carbon content and higher levels of ash (Raczkiewicz et al., 2024b). Higher ash content is associated with an increased presence of inorganic contaminants, such as heavy metals, which can be toxic to plants (Goyal et al.,

2020) and in BC can also cause fluctuations in soil pH and electrical conductivity (EC), creating an unfavorable environment for plant growth (Marra et al., 2018). Increasing the pyrolysis temperature also enhanced the positive effect of b-BC on *L. sativum*, which correlated with a reduction in the content of the contaminants in these BC (Fig. 7b) (Table S4). Statistical analysis revealed a strong negative correlation between the C_{total} PAHs and the root growth of *L. sativum*. It was previously suggested that PAHs contained in BCs could be responsible for their negative impact on plants (Godlewska et al., 2022; Jajoo, 2017; Stefaniuk and Oleszczuk, 2016). Additionally, a positive correlation was observed between the carbon content (C) and root growth, while a negative correlation was found between the nitrogen content (N) in b-BC and root growth. These correlations could be attributed to the differing physicochemical properties of the BC associated with its chemical composition. Higher C content may enhance soil structure, water retention, and nutrient availability, thereby promoting plant growth (Murtaza et al., 2024). On the other hand, the N content in BC may influence microbial activity in the soil, which could also affect plant growth (Liu et al., 2018). Insufficient N levels may lead to adverse effects such as inhibited plant growth, explaining the observed negative correlation. Furthermore, replacing the carrier gas from N_2 to CO_2 showed no significant impact ($p > 0.05$) on the inhibition of *L. sativum* root growth. These findings align with similar trends observed in previous studies regarding the influence of pyrolysis conditions on BC's toxicity (Kończak et al., 2020; Sun et al., 2023).

The reduction of BC's size to the nanoscale resulted in a shift from a stimulatory effect to toxicity (BC-WL, BC-RH, BC-OSR) or a significant increase in the inhibition of *L. sativum* root growth (Fig. 5). The Tukey test results in the Fig. 5 indicate that n-BC generally causes higher root growth inhibition in *L. sativum* compared to b-BC, as indicated by the higher percentage inhibition values for n-BC bars. Statistically significant differences ($p \leq 0.05$) in root growth effects are denoted by different letters. Zhang et al. (2020) indicated that BC's nanoparticles could pose a potential threat to biota, primarily due to phenolic compounds deposited on the larger BC's specific surface area, which have been shown to negatively impact plants. Therefore, the risk posed by n-BC to biota likely originates from these phenolic compounds on its increased surface. All n-BCs displayed a consistent trend of changes similar to those observed with b-BC concerning the feedstock used and pyrolysis conditions. Statistical analysis indicated that the physicochemical factors determining toxicity are the same in both b-BC and n-BC (Table S4). However, due to the reduction in size, harmful substances may be released more easily. These trends between b-BC and n-BC were also observed for *F. candida* (Fig. 6). Furthermore, n-BCs demonstrated significantly higher EC values compared to b-BCs (Table S2) (Raczkiewicz et al., 2024b). These elevated EC values may exceed the tolerance levels of plants to salt presence, especially as BC concentration increases. Numerous studies have reported that high EC value resulting

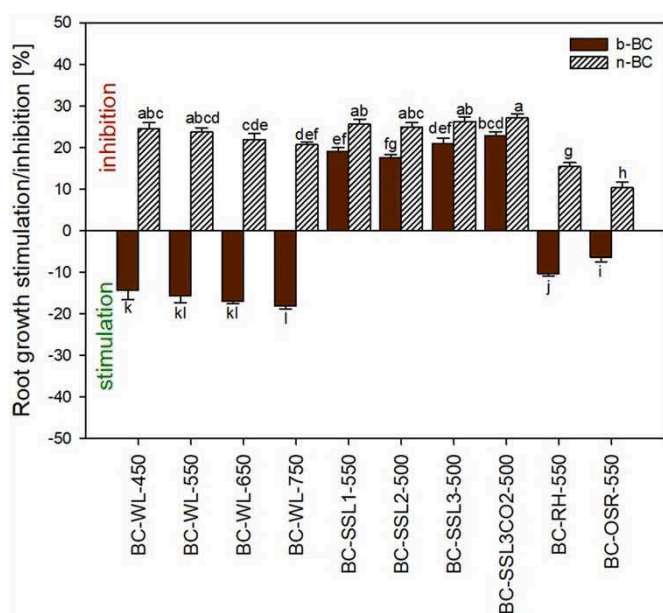


Fig. 5. Inhibition/stimulation of seed germination and root growth of *Lepidium sativum* in b-BC and n-BC-amended OECD soil at the dose of 1 %. Biochars produced from willow (WL), rice husk (RH), oilseed rape (OSR), and various sewage sludges 1–3 (SSL 1–3). Error bars represent the standard deviation of the mean ($n = 3$). Different letters indicate significant differences among the treatments (Tukey test, $p \leq 0.05$).

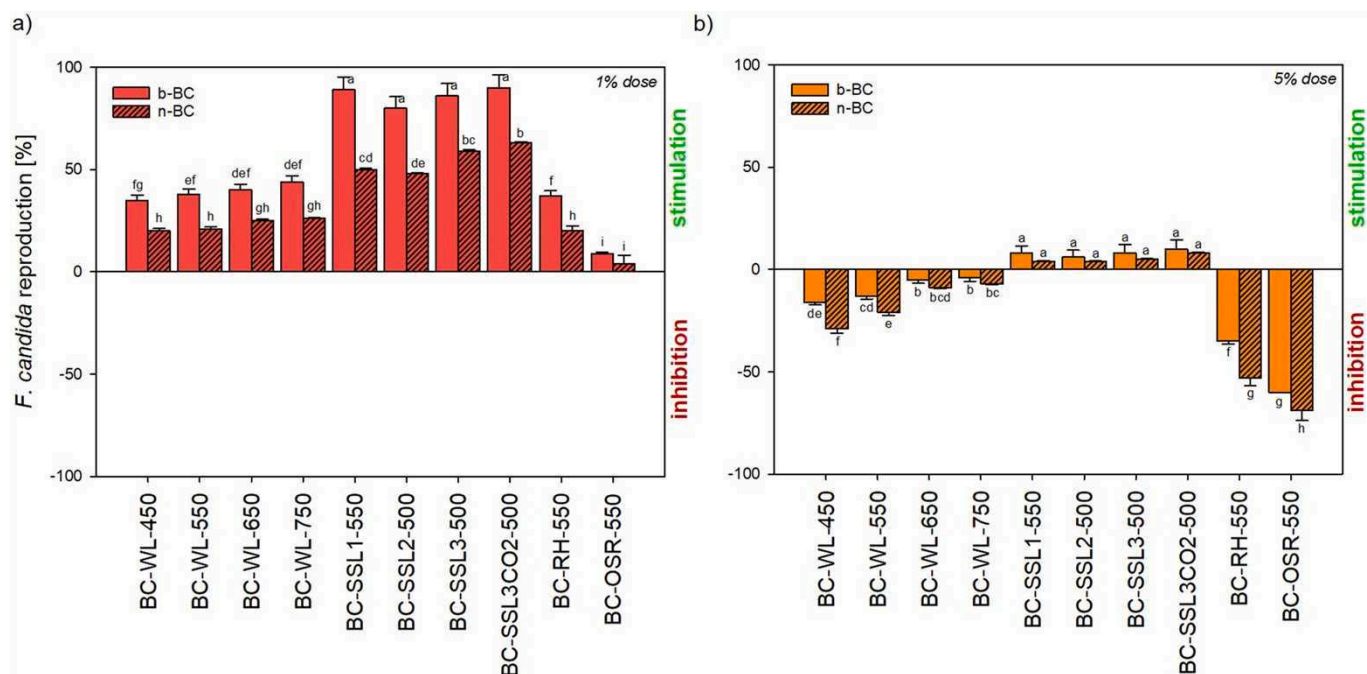


Fig. 6. *Folsomia candida* reproduction in b-BC/n-BC-amended soil. Error bars represent the standard deviation of the mean ($n = 3$). Biochars produced from willow (WL), rice husk (RH), oilseed rape (OSR), and various sewage sludges 1–3 (SSL 1–3). Different letters indicate significant differences among the treatments (Tukey test, $p \leq 0.05$).

from soil amendments with BC is a key factor contributing to the detrimental effects of BC on *L. sativum* (Gell et al., 2011; Muhammad and Hussain, 2010; Nhan et al., 2019).

3.5. Toxicity to *Folsomia candida*

Both b-BC and n-BC, when added to soil at two different doses did not affect the mortality of *F. candida* (Table S7), nevertheless, they did influence their reproduction (Fig. 6). At 1 % dose, b-BC stimulated the reproduction of *F. candida* in the range from 9 % to 90 %. Concerning the feedstock used, the most visible effect was observed for SSL-derived b-BCs, while the lowest values were noted for b-BC-OSR-550. An increase in pyrolysis temperature resulted in a gradual increase in reproduction with the highest temperature yielding a reproduction rate 25 % higher than that observed at the lowest temperature (Fig. 6). Changing the carrier gas from N_2 (b-BC-SSL3-500) to CO_2 (b-BC-SSL3CO2-500) did not affect reproduction of *F. candida* (Fig. 6).

The addition of 1 % of n-BC had a less favorable impact on the stimulation of *F. candida* reproduction to their respective bulk counterparts (Fig. 6a). The reproduction stimulation of n-BC was from 38 % to 45 % lower for WL-derived n-BCs, from 30 % to 44 % for SSL-derived n-BCs, and from 46 % to 56 % for agricultural residues-derived n-BC (n-BC-RH-550 and n-BC-OSR-550) compared to corresponding b-BC. The observed trend in response to the applied feedstock and pyrolysis parameters for n-BC exhibited a strong correlation with the trend noted for b-BC.

An increase of the b-BC dose to 5 % significantly diminished the stimulatory effect observed at lower doses (Fig. 6), resulting in the inhibition of *F. candida* reproduction for WL- (from 4 % to 16 %) and for agricultural residues-derived b-BC (from 35 % to 60 %) (Fig. 6b). Only SSL-derived b-BC did not negatively affect the reproduction of *F. candida*, showing no significant difference from the control. An increase pyrolysis temperature, which reduced toxicity and replacing N_2 with CO_2 , which showed no significant effect, exhibited a consistent trend as observed at lower b-BC dose.

At higher dose of n-BC, a similar trend was observed as for b-BC regarding to the applied feedstock and pyrolysis conditions (Fig. 6b).

The n-BCs exhibited a greater toxic effect on *F. candida* than corresponding b-BCs. In general, the reproduction inhibition was from 62 % to 81 % higher for WL-derived n-BC and from 15 % to 51 % for agricultural waste-derived n-BCs than b-BCs.

The observed toxic or stimulatory effects were significantly correlated ($p \leq 0.05$) with the levels of contaminants in the BC, including PAHs and TMs (Fig. 7). This observation aligns with findings from previous researchers (Eom et al., 2007; Sverdrup et al., 2002), who demonstrated the sensitivity of springtails (*Folsomia candida* and *Folsomia fimetaria*) to PAHs, particularly regarding reproduction juvenile. In the case of the 5 % dose of b-BC, another potential explanation for the detrimental impact on reproduction could be the pH levels. Prior studies (Luo et al., 2022; Styrisshave et al., 2010) have shown that the alkaline pH may negatively affect *F. candida*, especially in terms of reproduction. The optimal pH for the growth and reproduction of *F. candida* falls within the range of 5 to 6. The pH values for the tested b-BC ranged from 8.2 to 11. With the addition of 1 % b-BC, the pH impact was minimal and did not cause a toxic effect, but rather a stimulating one. This could be due to the relatively low concentration of b-BC, which might not significantly alter the overall pH or create conditions that are harmful to the organisms. Instead, the small amount of b-BC might provide essential nutrients or create a mildly favorable environment that enhances reproduction. In the case of n-BC, the pH after size reduction decreased to the range of 7.4 to 10. For n-BC, besides the ability to adsorb nutrients, it may be easier for the particles to penetrate organisms or accumulate, increasing the chance of introducing contaminants that could have a direct toxic effect. Additionally, nano-sized particles may more easily deposit in tissues/cells than macro-sized particles, which pass through the organism and are excreted or not even absorbed.

Nanoparticles, due to their small size, are more prone to accumulation, potentially causing long-term adverse effects on organisms (Rajput et al., 2020). Due to its larger specific surface area and size effect, n-BC exhibits greater adsorption properties than b-BC. When added to soil, n-BC can adsorb essential native nutrients from the soil solution, leading to their depletion. This process can induce the translocation of nutrients into deeper soil layers due to the enhanced migratory capabilities of n-BC, which are associated with their greater surface charge in soil

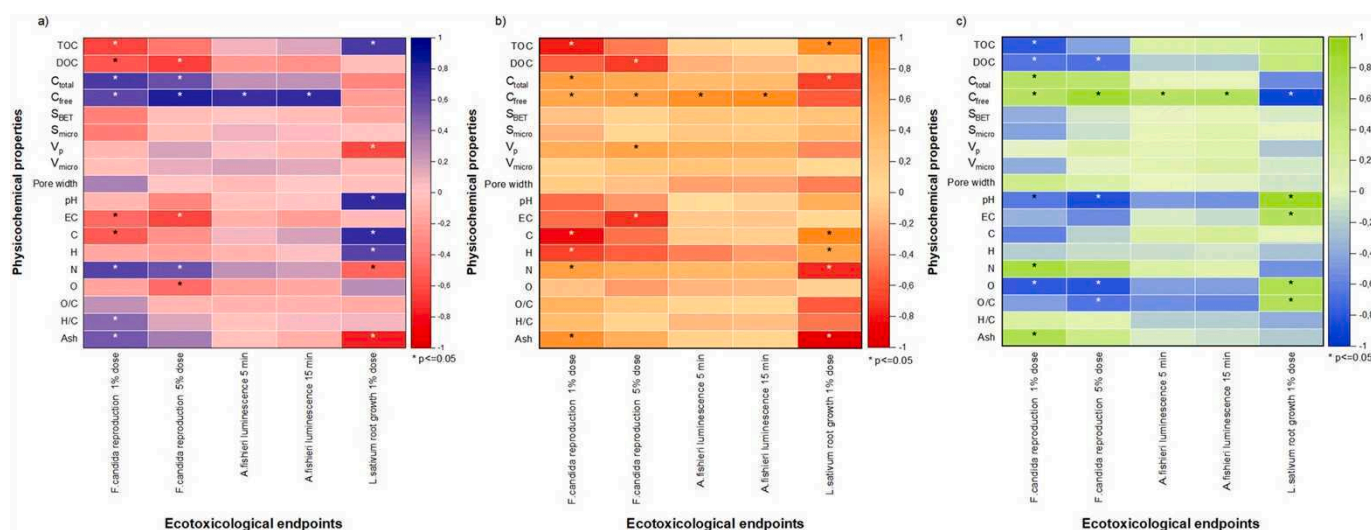


Fig. 7. Correlation plot presenting the values of Pearson correlation factor between physicochemical properties and ecotoxicological endpoints for b-BC and n-BC calculated together (a), calculated only for b-BC (b), and calculated only for n-BC (c). The significant correlations were indicated by $* p < 0.05$.

profiles, thereby diminishing nutrient retention within the surface horizons (Li et al., 2024). Consequently, the reduced physical availability of these elements in the surface layers for *F. candida* may exacerbate toxicological effects. Our study showed that low doses of n-BC (1 % dose) do not lead to significant soil nutrient depletion sufficient to inhibit reproduction or cause mortality in adult individuals, but it is evident that reproduction is reduced compared to b-BC (Fig. 6a). The observed detrimental effect at elevated doses of n-BC (5 % dose) may be attributable to the adsorption capacity of n-BC surpassing the natural replenishment rate of soil nutrients at this concentration. Furthermore, a higher dose of n-BC is associated with increased nutrient adsorption, which then migrates deeper into the soil profile, thereby reducing their availability in the surface horizons. The pronounced opposing effect of BC, attributable to the applied dose (stimulation vs. detrimental effect) may suggest the existence of a threshold concentration of n-BC/b-BC beyond which its presence in the soil becomes toxic to springtails. While lower doses of n-BC may still confer benefits without reaching this threshold, the cumulative effect of higher doses could prove detrimental. Bielská et al. (2018) observed that with increasing doses of BC, the toxicity towards *F. candida* also increased. In their study, reproduction rates in soil with the addition of 1 % wood and rice husk BC were comparable to those of control soil. However, when the BC dose was increased to 10 %, reproduction rates decreased by 38 % and 27 % for wood BC and rice husk BC, respectively, compared to control soil. Moreover, the elevated content of dissolved organic carbon (DOC) in n-BC may influence its ecotoxicity by enhancing the bioavailability and mobility of toxic substances (like PAHs or TMs) associated with DOC (Ma et al., 2021). This is confirmed by the statistical analysis of correlations between DOC and reproduction of *F. candida* (Fig. 7c). Despite the reduced organic-solvent extractable (C_{total}) and freely dissolved (C_{free}) PAHs content in n-BC than in b-BC, the increased specific surface area and DOC content in n-BC facilitate enhanced interactions between PAHs in the soil-n-BC system.

4. Conclusion

This study provides a systematic assessment of the ecotoxicological effects of nano-biochar (n-BC) across multiple biological systems, elucidating the environmental implications of its application. The findings highlight the pivotal role of feedstock properties, pyrolysis conditions, and particle size in influencing BC's interactions with organisms. The key outcomes are as follows: (1) n-BC exhibited significantly higher toxicity to *A. fischeri* compared to bulk biochar (b-BC),

likely due to enhanced surface activity, leaching of toxic substances, and lower pH, (2) while n-BC was non-toxic to *L. minor*, promoting frond growth potentially due to the release of bioavailable nutrients, it inhibited root growth in *L. sativum*, possibly as a result of elevated electrical conductivity (EC) surpassing the plant's salt tolerance; (3) higher concentrations of n-BC induced reproductive toxicity to *F. candida*, which may be attributed to increased dissolved organic carbon (DOC) and deeper soil migration of n-BC particles; (4) exposure to elevated doses of n-BC inhibited *D. magna*'s reproduction and resulted in notable particle adhesion to the organisms' surfaces, indicating potential bioaccumulation and long-term toxic effects.

Our research reveals the pivotal role of feedstock and particle size in determining the environmental risks of biochar. By methodically analyzing both bulk- and nano-sized biochar across various trophic levels, we unlock new perspectives on the ecological impacts of biochar usage. These insights can drive future innovations in the development and responsible application of biochar technologies within environmental management. Ultimately, our study underscores the imperative of integrating multiple factors, such as feedstock, pyrolysis conditions, and particle size, when evaluating the toxicological profiles of biochar. Further investigations should focus on long-term ecological monitoring, mechanistic studies of biochar-organism interactions, and the development of guidelines for the safe and sustainable deployment of biochar in environmental management.

CRedit authorship contribution statement

Monika Racziewicz: Writing – original draft, Visualization, Methodology, Investigation, Data curation, Conceptualization. **Aleksandra Bogusz:** Methodology, Investigation. **Bo Pan:** Writing – review & editing, Validation, Data curation. **Baoshan Xing:** Writing – review & editing, Formal analysis, Data curation. **Patryk Oleszczuk:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

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.scitotenv.2024.177629>.

Data availability

Data will be made available on request.

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Contrasting influences of nano-biochar and bulk biochar on various organisms: A comprehensive analysis of environmental impact

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Physicochemical analysis

The analysis of the content of total elemental carbon (C), hydrogen (H) and nitrogen (N) oxygen (O) was determined with an elemental analyzer (MicroCube, Elementar, Germany), where 2 mg of samples were placed in a sheet metal boat and heated in an incineration furnace at a temperature range of 25-1150°C. In the carbon mode, the pressure He was 0.12MPa and the flow rate was 200 ml / min; The O₂ pressure was 0.2MPa and the flow rate was 11 ml/min. The temperature of the reflux furnace was 850°C. In the oxygen mode, the pressure He was 0.12MPa and the flow rate was 130 ml / min; in this mode, there is no need for a reduction furnace.

The specific surface area (S_{BET}) and the volume of biochar micropores were determined by the one-point Barrett-Brunauer-Emmett-Teller (BET) method using a BET analyzer (ASAP 2020, American Mike, USA). 100 mg of biochar powder was placed in a test tube at 120 °C overnight for degassing, and then the isothermal N₂ adsorption at 77K was measured. Pore volume was calculated by the Barrett-Joyner-Halend (BJH) method. The ash content of n- and b-BC samples was determined using the ASTM standard technique by burning 1 g of each sample at 760 °C for 6 h and weighing the residue after ashing (Abdel-Fattah et al., 2015). The ash content was calculated using the equation below:

$$\% Ash = \frac{\text{weight after ashing} - \text{weight of crucible}}{\text{dry weight of BC sample}} \cdot 100\% \quad (1)$$

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Table S1. Process parameters of obtaining bulk-biochar (b-BC).

Feedstock material	Biochar	Temperature [°C]	Heating time [min]	Heat rate [°C/min]	Gas flow [L/min]	Gas type
willow	b-BC-WL-450	450	180	10	1	N ₂
willow	b-BC-WL-550	550	180	10	1	N ₂
willow	b-BC-WL-650	650	180	10	1	N ₂
willow	b-BC-WL-750	750	180	10	1	N ₂
rice husk	b-BC-RH-550	550	15	98	10	N ₂
oilseed rape	b-BC-OSR-550	550	12	78	10	N ₂
sewage sludge SCOTLAND EDINBURGH 55°56' N, 3°14' W	b-BC-SSL1-550	550	13	86	10	N ₂
sewage sludge POLAND ZAMOŚĆ 50°42' N, 23°13' E	b-BC-SSL2-500	500	180	7	1	N ₂
sewage sludge POLAND LUBLIN 51°14' N, 22°35' E	b-BC-SSL3-500	500	180	10	1	N ₂
sewage sludge POLAND LUBLIN 51°14' N, 22°35' E	b-BC-SSL3-CO2-500	500	180	10	1	CO ₂

Where, b-BC= bulk biochar; WL=willow; SSL 1-3=different sewage sludges; RH=rice husk; OSR=oilseed rape; 450,500,550,650,750=temperature od pyrolysis; CO2= CO₂ as carrier gas.

Table S2. Physicochemical properties of bulk (b-BC) and nanobiochars (n-BC) produced at various temperatures and conditions by ball milling.

Sample	S _{BET} [m ² /g]	S _{micro} [m ² /g]	S _{ext} [m ² /g]	V _p [cm ³ /g]	V _{micro} [cm ³ /g]	Pore width [nm]	pH	EC [μS/cm]	C [%]	H [%]	N [%]	O [%]	O/C [molar ratio]	N/C [molar ratio]	H/C [molar ratio]	(O+N)/C [molar ratio]	Ash [%]
b-BC-WL-450	7.2	5.0	2.8	0.020	0.002	10.95	8.92	299	78.87	3.46	1.49	11.50	0.1094	0.0162	0.5264	0.1256	4.68
n-BC-WL-450	146.3	88.1	58.2	0.109	0.037	2.99	7.35	1157	65.66	2.46	1.29	12.66	0.1446	0.0168	0.4496	0.1614	17.93
b-BC-WL-550	195.2	170.5	25.3	0.091	0.075	1.85	9.02	270	79.23	2.69	2.24	11.51	0.1089	0.0242	0.4074	0.1331	4.34
n-BC-WL-550	195.8	142.9	52.4	0.143	0.060	2.92	7.43	1089	54.46	1.53	0.93	8.71	0.1199	0.0146	0.3371	0.1346	34.37
b-BC-WL-650	130.1	123.6	6.5	0.062	0.055	1.91	10.6	966	83.35	1.72	1.46	8.79	0.0791	0.0150	0.2476	0.0941	4.68
n-BC-WL-650	150.9	112.5	38.5	0.132	0.047	3.49	7.76	1253	54.36	0.21	1.06	7.90	0.1090	0.0167	0.0464	0.1257	36.47
b-BC-WL-750	3.8	16.4	1.6	0.024	0.008	2.48	10.98	486	85.30	1.53	0	7.42	0.0653	-	0.2152	0.0653	5.75
n-BC-WL-750	160.7	120.3	40.5	0.136	0.050	3.39	7.98	1265	59.73	0.31	0.93	7.98	0.1002	0.0133	0.0623	0.1136	31.05
b-BC-RH-550	3.7	1.1	2.6	0.014	0.003	14.52	9.71	970									
n-BC-RH-550	158.6	99.8	58.8	0.139	0.042	3.49	9.13	1328	47.97	2.07	0.62	13.71	0.2143	0.0111	0.5178	0.2254	35.63
b-BC-OSR-550	2.9	2.1	0.9	0.010	0.001	14.40	10.20	4840									
n-BC-OSR-550	21.9	9.7	12.3	0.045	0.004	8.15	10.01	6880	69.95	2.38	1.19	17.43	0.1869	0.0146	0.4083	0.2015	9.05
b-BC-SSL1-550	13.6	2.6	11.1	0.055	0.084	22.11	8.18	535	31.52	0.38	1.62	15.64	0.3722	0.0441	0.1447	0.4163	50.836
n-BC-SSL1-550	15.0	1.2	13.8	0.075	0.025	14.69	6.82	2350	26.22	2.00	3.33	20.63	0.5901	0.1089	0.9153	0.6990	47.82
b-BC-SSL2-500	16.7	-	-	0.078	-	18.65	9.56	1573	28.51	1.23	3.75	-	-	0.1127	0.5177	0.1127	72.78
n-BC-SSL2-500	98.3	46.7	51.7	0.135	0.020	5.50	7.66	2450	25.61	1.16	2.95	19.55	0.5725	0.0987	0.5435	0.6713	61.53
b-BC-SSL3-500	41.6	29.1	-	0.055	0.012	5.27	9.55	552	26.41	0.53	3.24	8.22	0.2333	0.1052	0.2408	0.3385	61.60
n-BC-SSL3-500	51.2	22.0	29.2	0.090	0.010	7.03	7.45	1358	20.53	0.74	2.52	3.69	0.1348	0.1052	0.4325	0.2400	72.31
b-BC-SSL3-CO2-500	31.5	21.9	-	0.055	0.009	6.26	8.72	352	23.06	0.53	3.04	7.83	0.2548	0.1130	0.2758	0.3677	65.54
n-BC-SSL3-CO2-500	83.5	40.3	43.2	0.119	0.017	5.70	7.44	1685	20.82	0.60	2.47	4.15	0.1495	0.1017	0.3458	0.2512	71.60
									23.93	0.37	3.14	-	-	0.1125	0.1855	0.1125	76.63

Where, b-BC= bulk biochar; n-BC= nanobiochar; WL=willow; SSL 1-3=different sewage sludges; RH=rice husk; OSR=oilseed rape; 450,500,550,650,750=temperature of pyrolysis; CO2= CO₂ as carrier gas; S_{BET}= specific surface area of biochar; S_{micro}= specific surface area of micropores; S_{ext}= external surface area of biochars; V_p= pore volume; V_{micro} = micropore volume; pH= in H₂O; EC= electrical conductivity; C, H, N, O [%] = total content; O [%]= total content calculated by difference; O/C, N/C, H/C, (O + N)/C = molar ratios; Ash [%]= total content; The values are mean of three repetitions.

Table S3. *Daphnia magna* immobilization test results for various concentrations (2, 10 and 100 mg/L) of b-BC or n-BC.

Sample	Concentration [mg/L]	The number of floating <i>D.magna</i> (cumulative counts from replicates)			Immobilization [%]
		t=0	t=24 h	t=48 h	
Control	K1	15	15	15	0
	K2	15	15	15	0
	K3	15	15	15	0
	K4	15	15	15	0
b-BC-WL-450	100	15	15	15	0
	10	15	15	15	0
	2	15	15	15	0
n-BC-WL-450	100	15	15	15	0
	10	15	15	15	0
	2	15	15	15	0
b-BC-WL-550	100	15	15	15	0
	10	15	14	14	6.67
	2	15	15	15	0
n-BC-WL-550	100	15	14	14	6.67
	10	15	15	15	0
	2	15	15	15	0
b-BC-WL-650	100	15	15	15	0
	10	15	15	15	0
	2	15	15	15	0
n-BC-WL-650	100	15	14	14	6.67
	10	15	15	15	0
	2	15	15	15	0
b-BC-WL-750	100	15	14	14	6.67
	10	15	15	15	0
	2	15	15	15	0
n-BC-WL-750	100	15	15	15	0
	10	15	14	14	6.67
	2	15	15	15	0
b-BC-SSL1-550	100	15	15	15	0
	10	15	15	15	0
	2	15	15	15	0
n-BC-SSL1-550	100	15	15	15	0
	10	15	15	15	0
	2	15	15	15	0
b-BC-SSL2-500	100	15	15	15	0
	10	15	15	15	0

	2	15	15	15	15	0
n-BC-SSL2-500	100	15	15	15	15	0
	10	15	15	15	15	0
	2	15	15	15	15	0
b-BC-SSL3-500	100	15	15	15	15	0
	10	15	15	15	15	0
	2	15	15	15	15	0
n-BC-SSL3-500	100	15	15	15	15	0
	10	15	15	15	15	0
	2	15	15	15	15	0
b-BC-SSL3CO2-500	100	15	15	15	15	0
	10	15	15	15	15	0
	2	15	15	15	15	0
n-BC-SSL3CO2-500	100	15	15	15	15	0
	10	15	15	15	15	0
	2	15	15	15	15	0
b-BC-RH-550	100	15	15	15	15	0
	10	15	15	15	15	0
	2	15	15	15	15	0
n-BC-RH-550	100	15	15	15	15	0
	10	15	15	15	15	0
	2	15	15	15	15	0
b-BC-OSR-550	100	15	15	15	15	0
	10	15	15	15	15	0
	2	15	15	15	15	0
n-BC-OSR-550	100	15	15	15	15	0
	10	15	15	15	15	0
	2	15	15	15	15	0

Table S4. Pearson correlation between physicochemical properties and contaminations content in b-BC and n-BC and ecotoxicological test endpoints. The significant correlations (* $p \leq 0.05$.) were indicated by red color.

	TOC	DOC	C _{total}	C _{free}	S _{BET}	S _{micro}	V _p	V _{micro}	Pore width	pH	EC	C	H	N	O	O/C	H/C	Ash
<i>F.candida</i> reproduction 1% dose b-BC	-0.81	-0.56	0.72	0.64	-0.14	-0.21	0.58	0.15	0.20	-0.52	-0.51	-0.85	-0.70	0.73	-0.14	0.51	0.38	0.87

<i>F.candida</i> reproduction 1% dose n-BC	-0.81	-0.67	0.63	0.64	-0.39	-0.45	-0.01	-0.39	0.38	-0.66	-0.36	-0.61	-0.20	0.84	-0.81	-0.47	0.20	0.74
<i>F.candida</i> reproduction 5% dose b-BC	-0.46	-0.71	0.61	0.71	0.13	0.09	0.67	0.28	-0.14	-0.33	-0.75	-0.48	-0.56	0.56	-0.32	0.27	0.13	0.55
<i>F.candida</i> reproduction 5% dose n-BC	-0.46	-0.70	0.57	0.90	-0.09	-0.11	0.21	-0.01	0.19	-0.86	-0.56	-0.19	-0.12	0.61	-0.82	-0.67	0.11	0.39
<i>A.fishieri</i> luminescence inhibition 5 min b-BC	-0.05	-0.22	0.41	0.89	0.29	0.28	0.60	0.32	-0.28	0.00	-0.10	-0.10	-0.42	0.45	-0.18	0.10	-0.17	0.15
<i>A.fishieri</i> luminescence inhibition 5 min n-BC	0.19	-0.21	0.06	0.64	0.01	0.08	0.09	0.11	0.13	0.15	0.16	0.18	0.20	0.22	0.23	0.25	-0.22	-0.04
<i>A.fishieri</i> luminescence inhibition 15 min b-BC	-0.02	-0.15	0.40	0.91	0.24	0.23	0.54	0.26	-0.26	-0.05	-0.11	-0.06	-0.32	0.45	-0.18	0.08	-0.14	0.11
<i>A.fishieri</i> luminescence inhibition 15 min n-BC	0.23	-0.23	0.05	0.66	0.09	0.16	0.23	-0.23	0.05	0.66	0.09	0.16	0.23	-0.23	0.05	0.66	-0.20	-0.10
<i>L.sativum</i> root growth inhibition 1% dose b-BC	0.94	0.24	-0.69	-0.58	0.31	0.41	-0.40	0.04	-0.43	0.55	0.07	0.96	0.67	-0.78	-0.04	-0.60	-0.46	-0.94
<i>L.sativum</i> root growth inhibition 1% dose n-BC	0.43	0.46	-0.56	-0.91	-0.04	0.02	-0.25	-0.25	-0.25	-0.25	-0.25	-0.25	-0.25	-0.25	-0.25	-0.25	-0.39	-0.23

TOC= total organic carbon [mg]; DOC=dissolved organic carbon [mg/L], C_{total}= organic-solvent extractable sum of 16 PAHs [µg/kg]; C_{free}= freely dissolved sum of 16 PAHs [ng/L]; S_{BET}= specific surface area of biochar [m²/g] ; S_{micro}= specific surface area of micropores [m²/g] ; V_p= pore volume [cm³/g] ; V_{micro} = micropore volume [cm³/g] ; pore width in [nm]; pH= in H₂O; EC= electrical conductivity [µS/cm]; C, H, N, O = [%] total content; O/C, H/C = molar ratios; Ash = [%] total content;

Table S5. Pearson correlation between each heavy metal content in b-BC and n-BC and ecotoxicological test endpoints. The significant correlations (* $p \leq 0.05$.) were indicated by red color.

	Pb	Zn	Cd	Ni	Cu	Fe	Cr	Co	Mn
<i>F.candida</i> reproduction 1% dose b-BC	-0.78	-0.37	-0.36	-0.39	-0.46	-0.66	-0.38	-0.42	-0.34
<i>F.candida</i> reproduction 1% dose n-BC	-0.70	-0.39	-0.26	-0.42	-0.46	-0.59	-0.38	-0.43	-0.44
<i>F.candida</i> reproduction 5% dose b-BC	-0.82	0.12	0.12	0.05	0.01	-0.30	-0.11	-0.09	-0.05
<i>F.candida</i> reproduction 5% dose n-BC	-0.70	-0.39	-0.26	-0.42	-0.46	-0.59	-0.38	-0.43	-0.44
<i>A.fishieri</i> luminescence inhibition 5 min b-BC	-0.80	0.29	0.26	-0.02	0.09	0.05	-0.26	-0.20	-0.36
<i>A.fishieri</i> luminescence inhibition 5 min n-BC	0.10	0.48	0.47	0.40	0.39	0.40	0.08	0.14	-0.10
<i>A.fishieri</i> luminescence inhibition 15 min b-BC	-0.83	0.31	0.30	0.03	0.17	-0.00	-0.10	-0.05	-0.23
<i>A.fishieri</i> luminescence inhibition 15 min n-BC	0.17	0.56	0.54	0.47	0.47	0.46	0.13	0.20	-0.04
<i>L.sativum</i> root growth inhibition 1% dose b-BC	0.67	0.71	0.70	0.70	0.76	0.85	0.53	0.61	0.55
<i>L.sativum</i> root growth inhibition 1% dose n-BC	0.22	-0.10	-0.29	-0.14	-0.10	0.24	-0.15	-0.12	-0.02
<i>L.minor</i> average growth rates (μ_i) after 7 days b-BC	0.64	0.07	0.19	0.11	0.24	0.25	0.37	0.38	0.42
<i>L.minor</i> average growth rates (μ_i) after 7 days n-BC	-0.87	-0.89	-0.76	-0.94	-0.92	-0.86	-0.67	-0.75	-0.59

Pb, Zn, Cd, Ni, Cu, Fe, Cr, Co, Mn= total content [mg/kg]

Table S6. *Lepidum sativum* germination test results for b-BC or n-BC after 72H.

Sample	Replicates	The number of germinated seeds
Control	K1	10
	K2	10
	K3	10
b-BC-WL-450	I	10
	II	10
	III	10
n-BC-WL-450	I	10
	II	10
	III	10
b-BC-WL-550	I	10
	II	9
	III	10
n-BC-WL-550	I	10
	II	10
	III	10
b-BC-WL-650	I	10
	II	10
	III	10
n-BC-WL-650	I	9
	II	10
	III	10
b-BC-WL-750	I	10
	II	10
	III	9
n-BC-WL-750	I	10
	II	10
	III	10
b-BC-SSL1-550	I	9
	II	10
	III	10
n-BC-SSL1-550	I	10
	II	9
	III	10
b-BC-SSL2-500	I	9
	II	10
	III	10
n-BC-SSL2-500	I	10
	II	10
	III	10

b-BC-SSL3-500	I	10
	II	10
	III	10
n-BC-SSL3-500	I	10
	II	9
	III	10
b-BC-SSL3CO2-500	I	9
	II	10
	III	10
n-BC-SSL3CO2-500	I	10
	II	10
	III	10
b-BC-RH-550	I	10
	II	10
	III	10
n-BC-RH-550	I	10
	II	9
	III	10
b-BC-OSR-550	I	10
	II	10
	III	10
n-BC-OSR-550	I	10
	II	10
	III	10

Table S7. *Folsomia candida* mortality test results for b-BC or n-BC after 28 days.

Sample	Replicates	The number of adult <i>F.candida</i> (counts from each replicates)		
		t=0 (1% dose)	t=28 days (1% dose)	t=28 days (5% dose)
Control	K1	10	10	10
	K2	10	10	10
	K3	10	10	10
b-BC-WL-450	I	10	10	10
	II	10	9	10
	III	10	10	10
n-BC-WL-450	I	10	10	10
	II	10	10	10
	III	10	10	10
b-BC-WL-550	I	10	10	10
	II	10	10	9
	III	10	10	10
n-BC-WL-550	I	10	10	10
	II	10	10	10
	III	10	10	10
b-BC-WL-650	I	10	10	10
	II	10	10	10
	III	10	10	10
n-BC-WL-650	I	10	10	10
	II	10	10	10
	III	10	10	10
b-BC-WL-750	I	10	10	10
	II	10	10	9
	III	10	9	10
n-BC-WL-750	I	10	10	10
	II	10	10	10
	III	10	10	10
b-BC-SSL1-550	I	10	10	10
	II	10	10	10
	III	10	10	10
n-BC-SSL1-550	I	10	9	9
	II	10	10	10
	III	10	10	10
b-BC-SSL2-500	I	10	10	10
	II	10	10	10
	III	10	10	10
n-BC-SSL2-500	I	10	10	10
	II	10	10	10

	III	10	10	10	10	10
b-BC-SSL3-500	I	10	10	10	10	10
	II	10	10	10	10	10
	III	10	10	10	10	10
n-BC-SSL3-500	I	10	10	10	10	10
	II	10	10	10	10	10
	III	10	10	10	10	10
b-BC-SSL3CO2-500	I	10	10	10	10	10
	II	10	10	10	10	10
	III	10	10	10	10	10
n-BC-SSL3CO2-500	I	10	10	10	10	10
	II	10	10	10	10	10
	III	10	10	10	10	10
b-BC-RH-550	I	10	10	10	10	10
	II	10	10	10	10	10
	III	10	10	10	10	10
n-BC-RH-550	I	10	10	10	10	10
	II	10	10	10	10	10
	III	10	10	10	10	10
b-BC-OSR-550	I	10	10	10	10	10
	II	10	10	10	10	10
	III	10	10	10	10	10
n-BC-OSR-550	I	10	10	10	10	10
	II	10	10	10	10	10
	III	10	10	10	10	10

Table S8. Pearson correlation between each C_{total} PAHs content in b-BC and n-BC and ecotoxicological test endpoints. The significant correlations (* p ≤ 0.05.) were indicated by red color.

C _{total} PAHs	NAP	ACY	ACE	FLO	PHE	ANT	FLA	PYR	BaA	CHR	BbF	BkF	BaP	IcdP	DahA	BghiP
<i>F.candida</i> reproduction 1% dose b-BC	0.43	0.61	0.38	0.53	0.33	0.54	0.60	0.68	0.57	0.67	0.50	0.61	0.68	0.84	0.67	0.89
<i>F.candida</i> reproduction 1% dose n-BC	0.59	0.05	0.35	0.42	0.44	-0.26	0.61	0.60	0.56	0.54	-0.19	0.43	0.60	0.58	0.56	0.57
<i>F.candida</i> reproduction 5% dose b-BC	0.51	0.46	0.35	0.49	0.18	0.44	0.50	0.56	0.46	0.50	0.46	0.54	0.54	0.73	0.53	0.71
<i>F.candida</i> reproduction 5% dose n-BC	0.60	0.35	0.39	0.32	0.41	-0.09	0.53	0.52	0.50	0.39	-0.05	0.40	0.52	0.49	0.48	0.50
<i>V.fishieri</i> luminescence inhibition 5 min b-BC	0.50	0.25	0.27	0.39	0.11	0.29	0.38	0.36	0.29	0.29	0.21	0.28	0.33	0.51	0.36	0.46
<i>V.fishieri</i> luminescence inhibition 5 min n-BC	0.24	0.37	0.11	-0.14	-0.02	-0.17	0.05	0.05	0.07	-0.18	0.02	0.11	0.13	0.09	0.04	0.12
<i>V.fishieri</i> luminescence inhibition 15 min b-BC	0.46	0.24	0.24	0.35	0.11	0.26	0.37	0.36	0.27	0.27	0.26	0.32	0.33	0.48	0.34	0.45
<i>V.fishieri</i> luminescence inhibition 15 min n-BC	0.23	0.40	0.12	-0.13	-0.02	-0.13	0.03	0.02	0.05	-0.19	0.06	0.07	0.08	0.06	0.00	0.08
<i>L.sativum</i> root growth inhibition 1% dose b- BC	-0.35	-0.61	-0.27	-0.40	-0.35	-0.54	-0.61	-0.69	-0.58	-0.66	-0.45	-0.59	-0.66	-0.82	-0.69	-0.89
<i>L.sativum</i> root growth inhibition 1% dose n-BC	-0.54	-0.25	-0.45	-0.36	-0.53	0.02	-0.48	-0.50	-0.49	-0.40	-0.32	-0.43	-0.46	-0.39	-0.47	-0.42
<i>L.minor</i> average growth rates (μ_i) after 7 days b-BC	-0.83	-0.34	0.12	-0.05	0.18	-0.60	-0.59	-0.66	-0.60	-0.28	-0.42	-0.56	-0.37	-0.82	-0.71	-0.57
<i>L.minor</i> average growth rates (μ _i) after 7 days n- BC	-0.01	-0.35	-0.05	0.02	0.15	-0.30	0.51	0.49	0.49	0.24	-0.52	0.40	0.52	0.54	0.45	0.53

NAP - naphthalene; ACY - acenaphthylene; ACE - acenaphthene; FLO - fluorene; PHE - phenanthrene; ANT - anthracene; FLA - fluoranthene; PYR - pyrene; BaA - benz(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(g,h,i)perylene

Table S9. Pearson correlation between each C_{free} PAHs content in b-BC and n-BC and ecotoxicological test endpoints. The significant correlations (* $p \leq 0.05$.) were indicated by red color.

C_{free} PAHs	NAP	ACY	ACE	FLO	PHE	ANT	FLA	PYR	BaA	CHR	BbF	BkF	BaP	IcdP	DahA	BghiP
<i>F.candida</i> reproduction 1% dose b-BC	0.62	0.14	0.31	0.45	0.41	0.58	0.58	0.66	-	-	-0.22	-0.51	0.26	-	0.42	-
<i>F.candida</i> reproduction 1% dose n-BC	0.72	0.15	0.12	0.26	0.22	-0.29	0.27	0.27	-	-	-0.57	-0.26	0.75	-	0.29	-
<i>F.candida</i> reproduction 5% dose b-BC	0.64	0.26	0.40	0.53	0.52	0.73	0.46	0.47	-	-	-0.07	-0.43	0.17	-	0.28	-
<i>F.candida</i> reproduction 5% dose n-BC	0.90	0.42	0.42	0.29	0.59	-0.49	0.31	0.25	-	-	-0.54	0.06	0.64	-	0.27	-
<i>V.fishieri</i> luminescence inhibition 5 min b-BC	0.88	0.60	0.64	0.24	0.95	0.55	0.60	0.49	-	-	-0.04	0.09	-0.07	-	0.15	-
<i>V.fishieri</i> luminescence inhibition 5 min n-BC	0.60	0.33	0.45	0.26	0.48	-0.26	0.05	-0.10	-	-	-0.04	0.31	0.11	-	0.07	-
<i>V.fishieri</i> luminescence inhibition 15 min b-BC	0.89	0.57	0.61	0.25	0.98	0.48	0.55	0.44	-	-	0.12	0.06	-0.04	-	0.13	-
<i>V.fishieri</i> luminescence inhibition 15 min n-BC	0.62	0.40	0.49	0.21	0.53	-0.31	0.08	-0.06	-	-	-0.04	0.36	0.08	-	0.08	-
<i>L.sativum</i> root growth inhibition 1% dose b- BC	-0.61	-0.08	-0.22	-0.28	-0.38	-0.28	-0.63	-0.75	-	-	0.20	0.36	-0.29	-	-0.38	-
<i>L.sativum</i> root growth inhibition 1% dose n- BC	-0.93	-0.55	-0.22	-0.23	-0.63	0.63	-0.09	-0.07	-	-	0.33	-0.05	-0.55	-	-0.23	-
<i>L.minor</i> average growth rates (μ_i) after 7 days b- BC	-0.74	-0.44	-0.55	-0.30	-0.67	-0.29	-0.59	-0.62	-	-	-0.01	0.01	-0.29	-	0.25	-
<i>L.minor</i> average growth rates (μ_i) after 7 days n- BC	-0.11	-0.54	-0.16	0.34	-0.38	0.44	0.17	0.23	-	-	-0.60	-0.54	0.48	-	0.36	-

NAP - naphthalene; ACY - acenaphthylene; ACE - acenaphthene; FLO - fluorene; PHE - phenanthrene; ANT - anthracene; FLA - fluoranthene; PYR - pyrene; BaA - benz(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(g,h,i)perylene

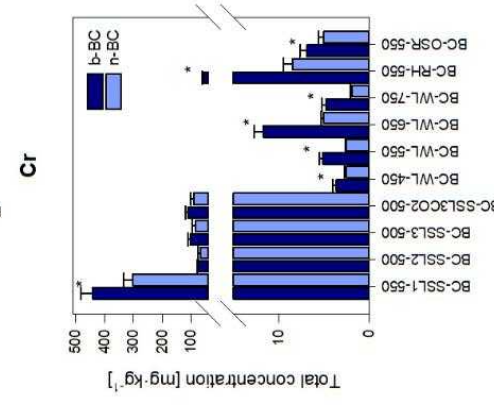
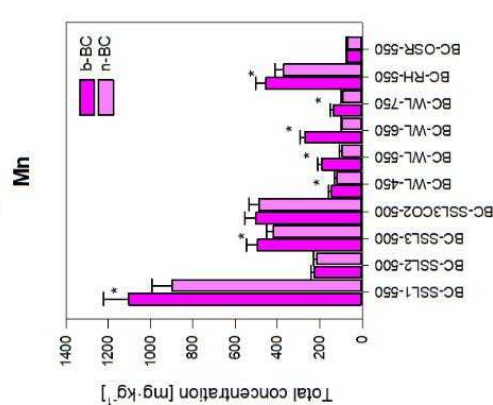
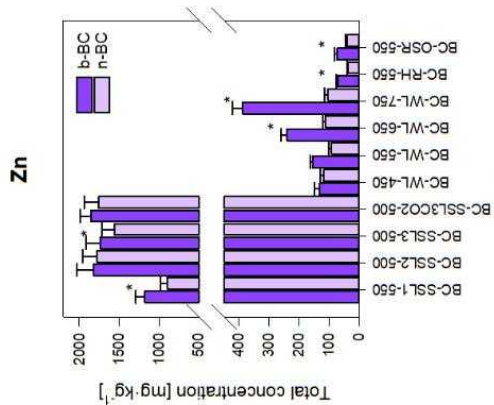
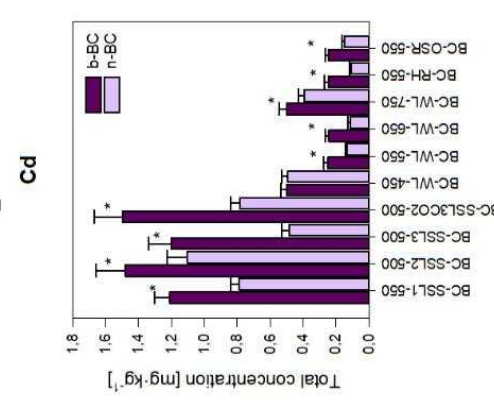
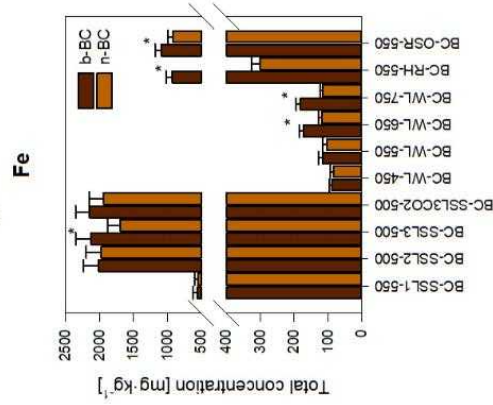
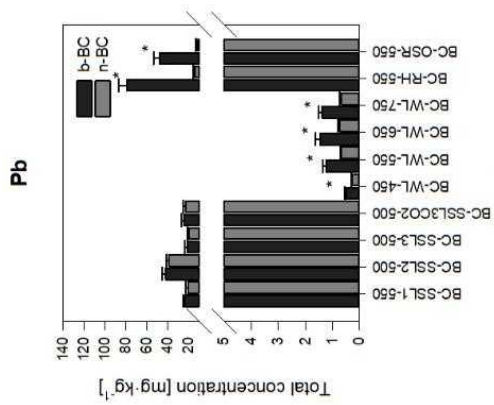
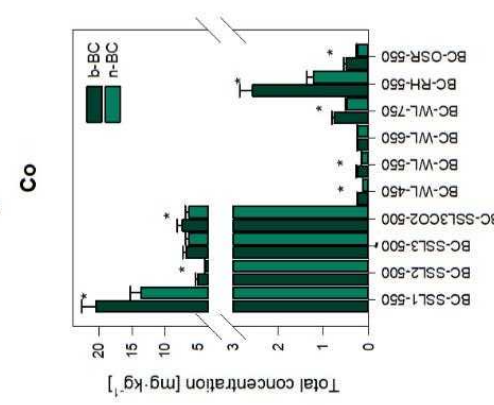
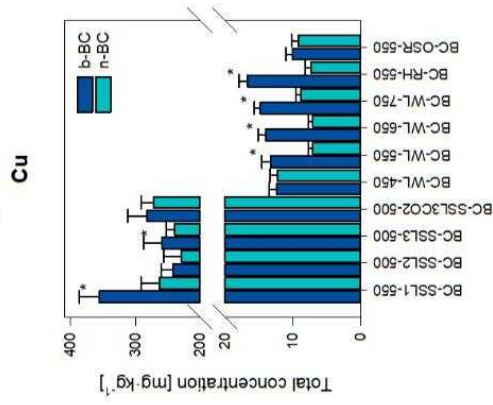
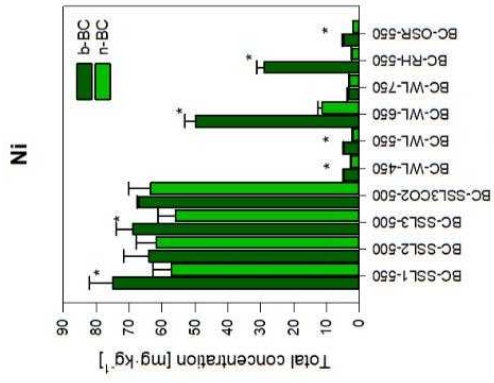


Figure S1. Total trace metals (TMs) [$\text{mg} \cdot \text{kg}^{-1}$] content in bulk- and nanobiochar, where * means statistically significant change ($p \leq 0.05$). Error bars represent the standard deviation of the mean ($n = 5$).

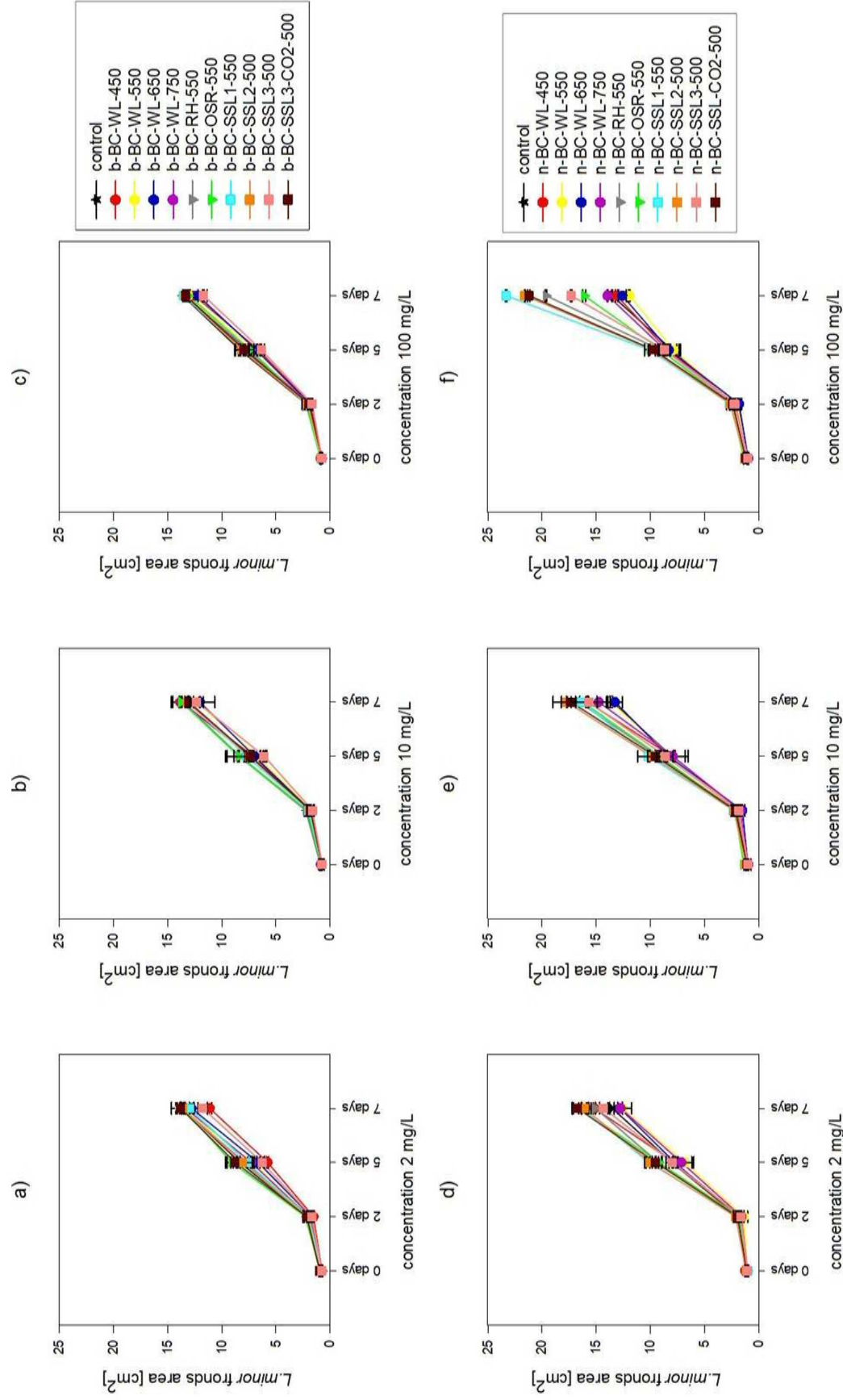


Figure S2. Growth curves of total frond area for *Lemna minor* exposed to 2, 10 and 100 mg/L of b-BC (a-c) and n-BC (d-f).

Publikacja D4

M. Raczkiewicz, P. Oleszczuk

“Enhanced heavy metal removal using nano–engineered biochars synthesized under variable pyrolysis conditions and feedstocks”

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Research article

Enhanced heavy metal removal using nano-engineered biochars synthesized under variable pyrolysis conditions and feedstocks

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ABSTRACT

This study investigates the adsorption efficiency of nano-biochars (n-BC) produced via ball-milling technique from various feedstocks (willow-WL, sewage sludge-SSL, rice husk-RH, oilseed rape-OSR) under different pyrolysis conditions. The most notable differences between n-BC and bulk biochars (b-BC) were observed in specific surface area (S_{BET}), total pore volume (V_p), and ash content—all of which increased in n-BCs, potentially enhancing their adsorption capacity. Adsorption kinetics, isotherms, and the impact of feedstock, pyrolysis temperature, atmosphere, and particle size on PTEs removal were analyzed. The adsorption performance toward Cd(II), Zn(II), and Pb(II) ions was evaluated separately in individual experiments to avoid competitive effects. The results demonstrated that n-BC significantly enhances the adsorption of Cd(II), Zn(II), and Pb(II) ions, with Pb(II) showing the highest adsorption capacity ranged from 276.4 to 431.9 mg/g. Adsorption followed a pseudo-second-order model, indicating chemisorption as the dominant mechanism. Reducing b-BC to the nanoscale increased adsorption capacity by up to 332 %, with specific feedstocks and pyrolysis conditions optimizing performance. This study provides valuable insights into BC's potential for sustainable PTEs remediation and highlights the critical role of nanostructuring and process optimization in enhancing adsorption efficiency. Moreover, this study offers a unique and systematic comparison of n-BCs derived from a wide range of feedstocks, spanning from lignocellulosic materials to more complex, nutrient- and ash-rich sources such as SSL. This comprehensive approach enables an in-depth exploration of how the intrinsic physicochemical properties of different feedstocks influence the PTEs adsorption performance of n-BCs. In addition, the study highlights the critical role of N_2 as a pyrolysis atmosphere in significantly enhancing Zn(II) adsorption. This distinctive observation emphasizes the importance of tailoring pyrolysis conditions to maximize the functional performance of biochar (BC) materials.

By providing a detailed understanding of the interplay between feedstock characteristics and pyrolysis parameters, this work makes a substantial contribution to the development of next-generation, sustainable technologies for the removal of PTEs.

1. Introduction

Potentially toxic elements (PTEs) represent a critical environmental contaminant class, characterized by persistence, non-biodegradability, and toxicity at trace concentrations. This group encompasses both acute toxicants (Cd, Pb, Hg, As) and essential micronutrients (Fe, Mn, Cu, Zn, Mo) that exhibit toxicity at elevated levels. Their environmental mobility and bioaccumulation potential make PTEs particularly hazardous to aquatic ecosystems. Given their increasing environmental burden from anthropogenic activities, adsorption-based remediation has emerged as an optimal mitigation strategy, offering superior efficiency

and cost-effectiveness compared to alternative treatment methods.

Biochar (BC), a carbon-rich material produced from the pyrolysis of biomass, has gained considerable attention as an alternative adsorbent for various contaminants (He et al., 2022; Cheng et al., 2021; Krasucka et al., 2021; Bogusz et al., 2015). However, recent studies indicated that nanoscale biochar (n-BC) outperforms bulk biochars (b-BCs) in adsorption due to their surface area, more active sites, greater negative zeta potential and diverse functional groups, which expand interaction with contaminants (Pathak et al., 2024; Raczkiewicz et al., 2024a; Sani et al., 2023; Ramanayaka et al., 2020). Moreover, n-BC may contain fewer native contaminants, like polycyclic hydrocarbons (PAHs) or PTEs, than

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b-BC (Raczkiewicz et al., 2024a). While recent research has largely concentrated on n-BCs capacity for removing organic pollutants (Aziz et al., 2023; Huang et al., 2020; Li et al., 2020; Xiang et al., 2020a, 2020b; Q. Zhang et al., 2019; Lyu et al., 2018b), its potential for addressing a PTEs warrants deep and further exploration.

The b-BC as an adsorbent for PTEs has been extensively studied and has shown promising results through a process known as adsorption (Bayar et al., 2024). Research on b-BC has demonstrated that, depending on the feedstock used and the pyrolysis conditions applied, the resulting b-BCs vary in their physicochemical properties (Kwak et al., 2019; Monga et al., 2022). In the context of the sorption of PTEs, the presence of functional groups on the BC's surface play a key role (Tag et al., 2016). This is also closely related to the appropriate selection of feedstock, pyrolysis conditions, and any applied modification (Liu et al., 2022; Wang et al., 2020). For example, Islam et al. (2021) reported that b-BC derived from rice straw and pulp mill sludge exhibited high adsorption capacities for Ni(II), Cu(II), Cd(II), and Pb(II), achieving maximum adsorption capacities ranging from 18.5 to 256.4 mg/g depending on the metal ion. Park et al. (2016) examined the adsorption behavior of PTEs using sesame straw-derived b-BC and compared its performance in single-metal and multi-metal systems. Their results showed that sesame straw-derived b-BC exhibited strong adsorption efficiency toward PTEs. In single-metal systems, the adsorption capacity followed the order: Pb(II) (102 mg/g) > Cd(II) (86 mg/g) > Cr(VI) (65 mg/g) > Cu(II) (55 mg/g) > Zn(II) (34 mg/g), while in multi-metal systems, the order was: Pb(II) (88 mg/g) > Cu(II) (40 mg/g) > Cr(VI) (21 mg/g) > Zn(II) (7 mg/g) > Cd(II). Notably, the adsorption capacity in the presence of multiple metals was significantly higher than in individual metal systems.

Although b-BC has been widely studied and shown effective in removing PTEs from aqueous solutions, research on the adsorption capacities of PTEs by n-BC, particularly regarding the influence of feedstock type and production conditions, remains limited (Li et al., 2020; Cao et al., 2019; Nath et al., 2019; Lyu et al., 2018a). Existing research demonstrates that ball-milling enhances n-BCs affinity for PTEs. For instance, Xiao et al. (2020) found that ball-milled n-BC produced at 600 °C achieved adsorption capacities of 165.77 mg/g for Cd(II), 287.58 mg/g for Cu(II), and 558.88 mg/g for Pb(II) at 298 K and pH 5.0. These values represent significant improvements over non-milled BC-increases of 93.91 %, 75.56 %, and 64.61 % respectively. In a similar study, Cao et al. (2019) observed that ball-milling only slightly improved the Pb(II) adsorption capacity of wheat straw-derived n-BC from 119.55 to 134.68 mg/g. While there have been a few studies on n-BC, we still lack comprehensive research that accounts for both the diverse production conditions and feedstock used in its production. A comprehensive evaluation of how factors like feedstock type and n-BC production conditions impact adsorption properties for various PTEs is missing. Different feedstocks (including agricultural residues, woody waste, and sewage sludge) have distinct chemical compositions and structures affecting the resulting b-BC and n-BCs properties. To optimize n-BC production and use in remediation, it is essential to understand how feedstock variation impacts PTEs adsorption.

This study explores and contrasts the adsorptive capabilities of n-BC, synthesized from a variety of feedstocks and produced under varying pyrolysis conditions, focusing on their efficacy in removing Cd(II), Zn(II), and Pb(II) from aqueous solutions. The primary focus is on evaluating their efficiency and mechanisms in PTEs adsorption. This study aims to elucidate the intricate relationships between feedstock composition, pyrolysis parameters, and the resultant physicochemical properties of n-BCs that contribute to their enhanced pollutant removal performance.

2. Materials and methods

2.1. Adsorbent materials

The adsorption properties of twenty BCs were examined, including ten n-BC, and their corresponding ten bulk counterparts (b-BC). The production processes for these b-BC and n-BC, including the feedstock preparation, are detailed elsewhere (Raczkiewicz et al., 2024b; Stefaniuk and Oleszczuk, 2015). In brief, to investigate the influence of feedstock type, pyrolysis was conducted at 550 °C under N₂ atmosphere using four distinct feedstocks: willow (WL), sewage sludge (SSL1-3), rice husk (RH), and oilseed rape (OSR). The feedstock selection encompassed lignocellulosic biomass (WL, RH), oleaginous material (OSR), and organic waste (SSL1-3). This selection allows for a comprehensive evaluation of how feedstock composition and origin influence the adsorption properties of the resulting BC. While feedstock properties such as WL, RH, and OSR are usually similar, SSL can vary significantly depending on its source, which determines the subsequent properties of BC. Therefore, it was decided to examine SSLs from different wastewater treatment plants (SSL 1–3). Moreover, the influence of pyrolysis temperature (450–750 °C for WL) and carrier gas (N₂ or CO₂ for SSL3) on selected feedstocks was systematically evaluated. Detailed methodology for the production and physicochemical characterization for both b-BC and n-BC are extensively documented in the Supporting Information (SI).

2.2. Chemicals

The initial stock solutions of Cd(II), Zn(II), and Pb(II) ions, each with a concentration of 2000 mg/L, were prepared by dissolving salts (Cd(NO₃)₂·4H₂O, Zn(NO₃)₂·6H₂O, and Pb(NO₃)₂) (POCH, Gliwice, Poland) in MilliQ water. These stock solutions were subsequently used to prepare solutions for kinetic and adsorption studies. PTEs quantification was conducted via a six-point calibration curve established for each PTEs, spanning concentration from 0.01 to 5 mg/L. Calibration curve parameters were prepared through dilution of a 1000 mg/L of each PTEs stock solution (Centipur, Merck, Germany) with MilliQ water, followed by acidification with 1 % HNO₃ (v/v). Calibration curve parameters are detailed in Table S2 of the Supporting Information (SI).

2.3. Adsorption kinetics experiment

The effect of contact time on Cd(II), Zn(II), or Pb(II) adsorption was separately evaluated via batch equilibrium studies. Initial concentrations were 250 mg/L for Cd(II) and Zn(II), and 500 mg/L for Pb(II). Adsorption capacities of b-BC and n-BC were measured at 0.25, 0.5, 1, 6, 24, and 48 h intervals. Each setup contained 15 ± 0.5 mg of b-BC/n-BC in a glass flask containing 25 mL PTEs ion solution at pH 5.0 ± 0.1 using 0.01 mol/L HNO₃ or NaOH to prevent the precipitation of metal hydroxides (Da Conceição et al., 2021). The flasks were agitated at 190 rpm at 24 ± 2 °C. Then, 6 mL aliquots were filtered through 0.45 µm PTFE hydrophilic syringe filters (AlfaChem, Poland) and analyzed via ICP-OES. Additionally, the pH of the aliquots after adsorption was measured to exclude the influence of pH changes on the adsorption process and was found to be 5.2 ± 0.3. Simultaneously, blank samples without b-BC/n-BC were monitored over the same time intervals as the experimental setups, and no changes in PTEs concentrations compared to the initial values were detected. Comprehensive details on the ICP-OES methodology are provided in the Supporting Information (SI).

2.4. Adsorption isotherm experiments

The correlation between the adsorbed amounts of Cd(II), Zn(II), or Pb(II) ions and the equilibrium concentration of the solution was separately examined through adsorption isotherms using the batch equilibrium method. Initial PTEs concentrations in the solutions were set at 50,

75, 100, 150, 250, and 500 mg/L for Cd(II) and Zn(II), and 250, 500, 750, 1000, 1500 and 2000 mg/L for Pb(II). To conduct the experiment, 15 ± 0.5 mg of b-BC/n-BC was placed in a glass flask containing 25 mL of the PTEs solution at the specified concentration. The pH of the solution was adjusted to 5.0 ± 0.1 using 0.01 mol/L HNO_3 or NaOH. The flasks were shaken on a horizontal shaker at a constant speed of 190 rpm for 24 h at a temperature of 24 ± 2 °C to reach equilibrium. After shaking, 6 mL aliquots were taken and filtered using a 0.45 μm PTFE hydrophilic syringe filter (AlfaChem, Poland). Simultaneously, blank samples without b-BC/n-BC were monitored over the same time intervals as the experimental setups, and no significant changes in PTEs concentrations compared to the initial values were detected. The PTEs concentrations in each sample were then measured using the ICP-OES technique as previously described. Additionally, the pH of the aliquots after adsorption was measured to exclude the influence of pH changes on the adsorption process and was found to be 5.2 ± 0.3 .

2.5. Surface characteristics: elemental and functional group analysis

Transmission electron microscopy (TEM) analysis of selected b-BC and n-BC samples with adsorbed Cd(II), Zn(II), or Pb(II) ions was conducted using a Titan G2 60–300 kV microscope (FEI Company) equipped with a field emission gun (FEG), monochromator, a system of three condenser lenses, an HAADF detector, and an energy dispersive X-ray spectroscopy (EDS) spectrometer. To visualize the spatial distribution of specific selected elements (C, Si, O, Cd, Zn, Pb), EDS spectra were collected point by point in STEM mode, producing images where distinct colors and intensities correspond to the elements' concentration. Phase identification was conducted using the FFT method with the Gatan Digital Micrograph software package. The functional groups of selected b-BC and n-BC samples before and after PTEs adsorption were

characterized using FTIR-ATR spectroscopy ($400\text{--}4000\text{ cm}^{-1}$, 1 cm^{-1} resolution; Nicolet 8700A, Thermo Scientific, USA) and X-ray photoelectron spectroscopy (XPS) (UHV Prevac analytical system, Poland) to identify the dominant adsorption mechanisms.

2.6. Data modeling

Adsorption kinetic models, including the pseudo-first-order (PFO) and pseudo-second-order (PSO) (Table S3) and intraparticle diffusion model (IPD) models (Table S4), as well as adsorption isotherm models, such as the Langmuir and Freundlich models, were applied to analyze the adsorption data (Table S5). Detailed information regarding the model fittings and their corresponding statistical evaluations can be found in the Supporting Information (SI). All fitting procedures and statistical analyses were conducted using OriginPro 2024 (OriginLab Corporation, USA), ensuring precise data modeling and interpretation of the adsorption mechanisms.

3. Results and discussion

3.1. Adsorption kinetics

Fig. 1 illustrates the adsorption kinetics of Cd(II), Zn(II), or Pb(II) ions on b-BC and n-BC. All the tested PTEs ions exhibited relatively slow adsorption kinetics on both b-BCs and n-BCs compared to other carbon adsorbents (Wang et al., 2024; Xiao et al., 2020; Xiang et al., 2018; Bogusz et al., 2015). Adsorption on both b-BCs and n-BCs for all ions studied proceeded in three distinct stages: an initial rapid phase within the first 60 min, a subsequent slower phase from 60 min to 48 h as equilibrium was reached, and finally a plateau (Fig. 1). The initial rapid step is attributed to the surface adsorption of PTEs ions on the BC's outer

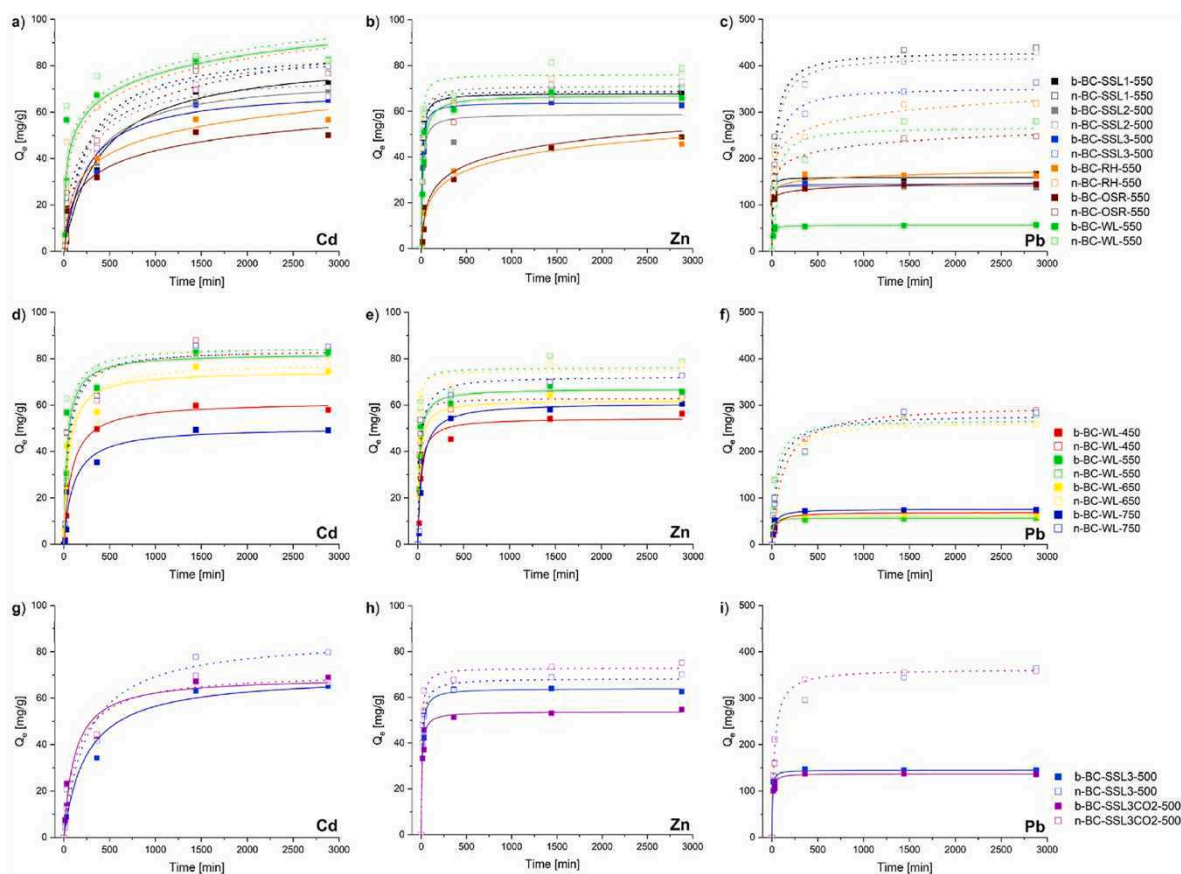


Fig. 1. Adsorption kinetics fit with pseudo-second order of Cd(II), Zn(II) and Pb(II) ions onto b-BC and n-BC. Positions a–c represent a comparison of different feedstocks, while d–f illustrate comparison of different pyrolysis temperatures and g–i illustrate comparison of different carrier gases.

surface. The subsequent slower step is associated with the redistribution of adsorbed ions from the external to the internal surfaces and pore filling of the BCs (Sun et al., 2021).

The time required to reach equilibrium was consistent amongst all evaluated b-BCs, irrespective of the feedstock or pyrolysis parameters applied, yet differed amongst the various PTEs ions. For Cd(II) and Zn(II) adsorption equilibrium was reached at 24 h, and for Pb(II) at 6 h, with adsorbed amounts ranging from 31.7 to 51.8 mg/L for Cd(II), 27.0–43.7 mg/L for Zn(II), and 33.4–94.8 mg/L for Pb(II), depending on the type of b-BC. The faster equilibrium for Pb(II) ions can be attributed to their larger ionic radius compared to other studied PTEs (Table S6). This results in greater polarizability and thus stronger electrostatic interactions with the negatively charged BCs surface, leading to more rapid adsorption (Bogusz et al., 2015). Additionally, Pb(II) exhibits a lower affinity for the solvent and is less reactive with hydroxides compared to other studied PTEs, as indicated by the solubility (K_{sp} values) of metal hydroxides (Table S6). When combined with its lowest hydration energy relative to Zn(II) and Cd(II) (Table S6), this suggests that water molecules in the hydration shell of Pb(II) are less tightly bound. This reduced hydration, along with its lower solubility, facilitates the desolvation process, allowing Pb(II) ions to interact more readily with the BCs surface, leading to stronger and faster adsorption dynamics. BC, possessing a negative surface charge, effectively adsorb cationic PTEs. Specifically, Pb(II), as a larger cation, exhibits a heightened tendency to rapidly bind to these sites (Kumkum and Kumar, 2024; Wang et al., 2018). The adsorption trend for n-BCs with Cd(II) and Zn(II) paralleled that of b-BC, although Pb(II) equilibrium occurred at 24 h. The equilibrium adsorption time for Pb(II) ions differs notably between n-BC (24 h) and b-BC (6 h), reflecting their distinct porous structures and adsorption mechanisms. N-BC exhibits both a larger specific surface area and a higher concentration of micropores and mesopores than b-BC. These features provide more adsorption sites but require Pb(II) ions to spend more time diffusing through the intricate microporous network (Tan et al., 2015). Additionally, n-BC's higher zeta potential (Table 1) creates stronger electrostatic attractions with Pb(II) ions, contributing to longer equilibration periods as these bonds stabilize. In contrast, b-BC achieves faster equilibrium due to its more straightforward porous structure and reduced surface area, with adsorption occurring on the external surface and in easily accessible mesopores. While n-BC demonstrates superior adsorption capacity, its complex pore structure and stronger electrostatic interactions affect the overall kinetics of the process. We propose the ionic radius of the PTEs ions primarily influenced the PTEs adsorption rate on n-BC particles. The ionic radius sequence of the PTEs studied, $Zn(II) < Cd(II) < Pb(II)$ (Table S6), suggests Pb(II) adsorption on n-BCs was slower than on b-BCs (Chen et al., 2024; Guzel et al., 2008; Hung et al., 2023). The adsorbed amounts ranged from 43.4 to 53.3 mg/L for Cd(II), 39.6–49.6 mg/L for Zn(II), and 151.8–256.7 mg/L for Pb(II), depending on the type of n-BC.

Tables S3 and S4 summarizes the kinetic parameters obtained from the adsorption systems examined. The adsorption kinetics of Cd(II), Zn(II), or Pb(II) ions onto b-BCs and n-BCs were best characterized by the pseudo-second-order (PSO) kinetic model, as it provided a significantly better fit to the experimental data compared to the pseudo-first-order (PFO) model. The results show slight differences between the experimental and calculated equilibrium adsorption capacities (Table S3). These findings indicate that the adsorption of investigated ions by b-BC and n-BC is chemically controlled and occurs through chemical interactions, such as electron exchange or sharing (Fawzy et al., 2022). The strong fit to PSO model indicates chemisorption, rather than physical adsorption, is the main mechanism for ions removal from the solution. The fitting to the PSO kinetic model also suggests that the adsorption of PTEs occurred primarily through the chemisorption of the ions onto the surface of the BCs (Chen et al., 2018; Vijayaraghavan and Yun, 2008). Furthermore, the strong fit to the PSO model also indicates that the initial adsorption rate is high, followed by a slowdown as equilibrium is approached. This is consistent with the experimental data

showing an initially rapid, followed by a slower, adsorption phase (Fig. 1). While the PSO models provided valuable insights into the adsorption process, they were insufficient in determining the underlying diffusion mechanisms. Therefore, the intraparticle diffusion (IPD) model was employed as a complementary approach to address this limitation. The adsorption kinetics for b-BC and n-BC were non-linear, indicating that intraparticle diffusion was not the sole rate-controlling process. As shown in Fig. 2, the slope of the linear portion of each curve (steps I–IV) provides the rate constants, while the intercept C, obtained by extrapolating the first step of the curves to the time axis, reflects additional diffusion effects. As presented in Table S4, negative C values (step II) suggested that the boundary layer in some b-BC and n-BC systems impeded intraparticle diffusion, highlighting the complexity of the adsorption process. Intraparticle diffusion was not the only rate-limiting step. The four slopes observed on each curve indicated that at least four diffusion stages occurred during the adsorption process. Adsorption on the external surface or diffusion through macropores occurred during steps I and II until the external surface reached saturation. Subsequently, ions of PTEs entered less accessible pores during steps III and IV, leading to increased diffusion resistance and a reduction in diffusion rate. The slope of the linear sections represented the diffusion rate, with larger k values corresponding to faster diffusion. The trend $(k_1 + k_2) > k_3 > k_4$ suggested that the available free path for diffusion decreased as pore dimensions diminished, resulting in a slower diffusion process during steps I and II. Step III, controlled primarily by intraparticle diffusion, corresponded to a gradual adsorption stage associated with surface reactions. Finally, step IV represented the equilibrium stage, where PTEs ions moved slowly from larger pores into micropores, leading to a reduced adsorption rate. This final stage was constrained by the availability of adsorption sites, indicating a balance between diffusion and site accessibility.

The k_2 values for the PSO adsorption systems with b-BCs demonstrated that the adsorption process occurred in the following order (from fastest to slowest): $Pb(II) > Zn(II) > Cd(II)$. Conversely, for the n-BCs systems, the order was different $Zn(II) > Cd(II) > Pb(II)$. This indicates that the adsorption rate for Cd(II) increased significantly compared to other PTEs with the reduction of particle size to the nanoscale for n-BC-RH-550, n-BC-SSL1-550, and n-BC-SSL2-500, showing respective increases in the k_2 constant by factors of 2.5-, 1.6-, and 1.2-, respectively. For Zn(II), a significant increase in the adsorption rate was observed for n-BCs, except for those derived from SSL, with a 1.5- to 7.6-fold increase in the k_2 constant. Notably, for Pb(II), the reduction of particle size to the nanoscale led to a significant decrease in k_2 values, with reduction ranging from 74 % to 98 % across all tested n-BCs. This effect is attributable to the relatively large ionic radius of Pb(II) ions compared to other PTEs studied (Table S6). The larger size of Pb(II) ions may hinder their diffusion into the internal pores of the nano-adsorbent, thus affecting the overall kinetics of the adsorption process (Long et al., 2023; Panayotova and Velikov, 2002).

The kinetic parameters depended on the feedstock and pyrolysis condition for specific PTEs (Fig. 1a–c). The fastest adsorption for Cd(II) based on the type of feedstock used to produce b-BC was observed with WL-derived b-BCs (b-BC-WL-550), while the slowest for SSL-derived b-BCs (b-BC-SSL1-550 < b-BC-SSL2-500 < b-BC-SSL3-500). This trend was also maintained when b-BC were reduced to nanoscale. Conversely, for Zn(II) ions, the fastest adsorption was observed on SSL-derived b-BCs (b-BC-SSL2-550 < b-BC-SSL1-500 < b-BC-SSL3-500). The slowest adsorption capacity was observed for agricultural residues-derived b-BCs (b-BC-RH-550 < b-BC-OSR-550). Interestingly, reducing the particle size to the nanoscale altered the adsorption rates for Zn(II) compared to the corresponding b-BCs from different feedstocks. For n-BCs, the fastest adsorption of Zn(II) ions was observed for WL-derived n-BC (n-BC-WL-550) and the slowest for agricultural-derived n-BCs (n-BC-RH-550 > n-BC-OSR-550). For Pb(II) ions, the fastest adsorption on b-BCs was observed for b-BC-SSL2-500, while the slowest was recorded for b-BC-RH-550 and b-BC-OSR-550. The remaining two SSL-derived b-BCs (b-

Table 1
Physicochemical properties of bulk (b-BC) and nano-biochars (n-BC).

Biochar	S _{BET} [m ² /g]	V _p [cm ³ /g]	Pore width [nm]	pH	EC [μS/ cm]	O/C [molar ratio]	H/C [molar ratio]	(O + N)/C [molar ratio]	Ash [%]	Zeta potential pH = 5.0 [mV]	Average particle size (by volume mean) [nm]
b-BC-WL-450	7.2 ± 0.144	0.02 ± 0.0003	10.95 ± 0.0987	8.92 ± 0.0723	299 ± 19.680	0.1094 ± 0.0011	0.5264 ± 0.0039	0.1256 ± 0.0014	4.68 ± 0.6639	−17.48 ± 0.2939	-*
n-BC-WL-450	146.3 ± 2.926	0.109 ± 0.0014	2.990 ± 0.0526	7.350 ± 0.2878	1157 ± 17.615	0.1446 ± 0.0024	0.4496 ± 0.0018	0.1614 ± 0.0011	17.93 ± 0.5243	−34.44 ± 0.4012	54.30 ± 1.671
b-BC-WL-550	195.2 ± 1.904	0.091 ± 0.002	1.850 ± 0.0326	9.020 ± 0.1045	270.0 ± 9.237	0.1089 ± 0.0018	0.4074 ± 0.0031	0.1331 ± 0.0015	4.340 ± 0.1615	−23.42 ± 0.7172	-*
n-BC-WL-550	195.8 ± 3.916	0.143 ± 0.002	2.920 ± 0.1460	7.430 ± 0.0861	1089 ± 7.168	0.1199 ± 0.0017	0.3371 ± 0.0029	0.1346 ± 0.0016	34.37 ± 1.568	−31.36 ± 0.5387	48.10 ± 1.5943
b-BC-WL-650	130.1 ± 1.602	0.062 ± 0.0007	1.910 ± 0.0955	10.60 ± 0.1229	966.0 ± 6.358	0.0791 ± 0.0013	0.2476 ± 0.002140	0.09410 ± 0.0075	4.680 ± 0.6470	−15.38 ± 0.9060	-*
n-BC-WL-650	150.9 ± 3.618	0.1320 ± 0.0017	3.490 ± 0.0237	7.760 ± 0.0724	1253 ± 11.21	0.1090 ± 0.0013	0.0464 ± 0.0031	0.1257 ± 0.0026	36.47 ± 1.928	−31.76 ± 0.6815	43.50 ± 2.537
b-BC-WL-750	3.800 ± 0.0760	0.024 ± 0.0003	2.480 ± 0.1240	10.98 ± 0.1148	486.0 ± 3.237	0.06530 ± 0.0011	0.2152 ± 0.0026	0.06530 ± 0.0021	5.750 ± 0.1568	−16.88 ± 0.2114	-*
n-BC-WL-750	160.7 ± 3.214	0.136 ± 0.0017	3.390 ± 0.1695	7.980 ± 0.0995	1265 ± 8.326	0.1002 ± 0.0012	0.06230 ± 0.0069	0.1136 ± 0.0276	31.05 ± 0.4404	−23.04 ± 0.7172	38.90 ± 1.441
b-BC-RH-550	3.700 ± 0.0240	0.014 ± 0.0002	14.52 ± 0.2514	9.710 ± 0.1125	970.0 ± 6.385	0.2143 ± 0.0025	0.5178 ± 0.0044	0.2254 ± 0.0258	35.63 ± 1.103	−27.56 ± 0.4387	-*
n-BC-RH-550	158.6 ± 2.172	0.1390 ± 0.0017	3.490 ± 0.1745	9.130 ± 0.1058	1328 ± 8.741	0.2683 ± 0.0012	0.2435 ± 0.0021	0.2919 ± 0.0150	50.82 ± 1.568	−37.31 ± 0.7860	87.90 ± 2.086
b-BC-OSR-550	2.900 ± 0.0480	0.010 ± 0.0001	14.40 ± 0.5139	10.20 ± 0.1488	4840 ± 21.86	0.1869 ± 0.0017	0.4083 ± 0.0033	0.2015 ± 0.0015	9.050 ± 0.6470	−34.60 ± 0.7347	-*
n-BC-OSR-550	21.90 ± 0.4980	0.045 ± 0.0006	8.150 ± 0.4075	10.01 ± 0.1735	6880 ± 35.28	0.3722 ± 0.0016	0.1447 ± 0.0030	0.4163 ± 0.0032	50.84 ± 1.822	−37.37 ± 0.6817	69.90 ± 3.864
b-BC-SSL1-550	13.60 ± 0.9720	0.055 ± 0.0007	22.11 ± 0.2373	8.180 ± 0.07236	535.0 ± 4.521	0.5901 ± 0.0037	0.9153 ± 0.0025	0.6990 ± 0.0020	47.82 ± 1.673	−3.240 ± 0.2152	-*
n-BC-SSL1-550	15.00 ± 0.4960	0.075 ± 0.0009	14.69 ± 0.7345	6.820 ± 0.2454	2350 ± 11.03	– ±	0.5177 ± 0.0458	0.1127 ± 0.0156	72.78 ± 0.9622	−6.439 ± 0.6574	59.30 ± 3.732
b-BC-SSL2-500	16.70 ± 0.3340	0.078 ± 0.0004	18.65 ± 0.9325	9.560 ± 0.1108	1573 ± 14.08	0.5725 ± 0.0026	0.5435 ± 0.05826	0.6713 ± 0.0053	61.53 ± 2.104	−4.120 ± 0.1712	-*
n-BC-SSL2-500	98.30 ± 0.9660	0.135 ± 0.0007	5.50 ± 0.2750	7.660 ± 0.1754	2450 ± 16.13	0.2333 ± 0.0010	0.2408 ± 0.0379	0.3385 ± 0.0026	61.60 ± 1.878	−13.42 ± 0.4815	65.30 ± 2.806
b-BC-SSL3-500	41.61 ± 2.459	0.055 ± 0.0030	5.27 ± 0.303	9.55 ± 0.5318	552 ± 31.98	0.433 ± 0.02523	0.135 ± 0.0076	0.2400 ± 0.01243	72.31 ± 4.285	−6.50 ± 0.052	-*
n-BC-SSL3-500	51.23 ± 2.674	0.090 ± 0.0050	7.03 ± 0.3584	7.75 ± 0.3897	1358 ± 72.5	0.276 ± 0.0145	0.255 ± 0.01332	0.3677 ± 0.0214	65.54 ± 3.392	−12.52 ± 0.560	30.00 ± 3.020
b-BC-SSL3-CO2-500	31.50 ± 0.6310	0.055 ± 0.0007	6.26 ± 0.1927	8.720 ± 0.2315	352.0 ± 8.612	0.1495 ± 0.0058	0.3458 ± 0.0145	0.2512 ± 0.0032	71.60 ± 2.085	−3.700 ± 0.9147	-*
n-BC-SSL3-CO2-500	83.50 ± 1.167	0.119 ± 0.0015	5.70 ± 0.2850	7.440 ± 0.1862	1685 ± 12.15	– ±	0.1855 ± 0.0184	0.1125 ± 0.0022	76.63 ± 1.161	−11.19 ± 0.2180	57.60 ± 2.511

Where, b-BC-bulk biochar; n-BC- nanobiochar; WL-willow; SSL 1-3-sewage sludges; RH-rice husk; OSR-oilseed rape; S_{BET}-specific surface area of biochars; V_p-total pore volume; pH = in H₂O; EC = electrical conductivity; O/C, H/C, (O + N)/C - molar ratios; Ash [%]- total content; Average particle size-measured in 1M NaCl and -* the particle size was not determined due to the limitations of the measurement method, which provides accurate results only for particles up to several hundred nm in size.

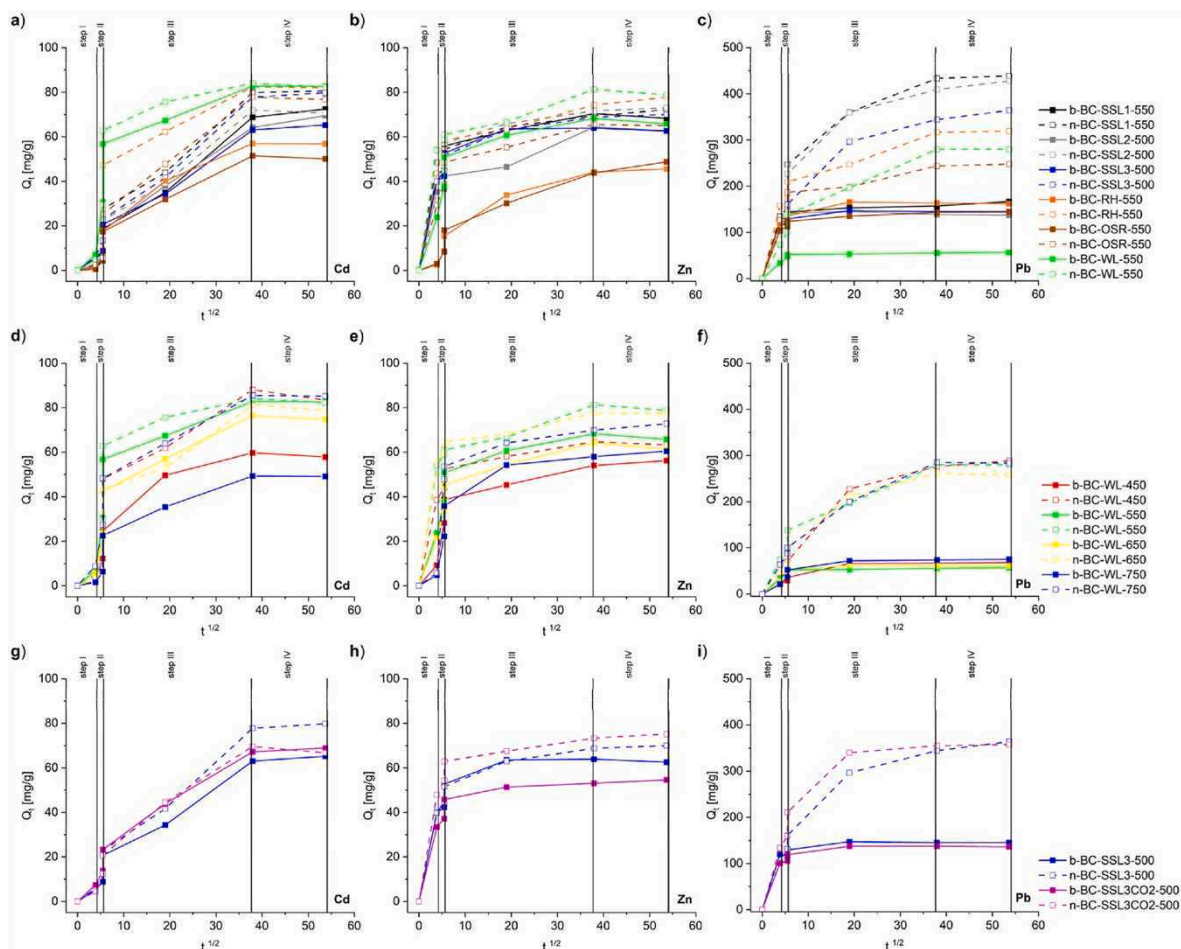


Fig. 2. Adsorption kinetics fit with intraparticle diffusion model (IPD) of Cd(II), Zn(II) and Pb(II) ions onto b-BC and n-BC. Positions a-c represent a comparison of different feedstocks, while d-f illustrate comparison of different pyrolysis temperatures and g-i illustrate comparison of different carrier gases.

BC-SSL1-550 and b-BC-SSL3-500), as well as b-BC-WL-550, exhibited similar adsorption kinetics. The trend for Pb(II) adsorption after changing the size was also reversed. The fastest adsorption was observed for agricultural-derived n-BC (n-BC-RH-550 < n-BC-OSR-550), while the slowest for all SSL-derived n-BCs.

Increasing the pyrolysis temperature from 450 to 650 °C enhanced the adsorption kinetics of Cd(II), Zn(II), and Pb(II) ions on both b-BC and n-BC (Fig. 1d-f). However, a subsequent increase to 750 °C resulted in a decrease in adsorption rate for both b-BC and n-BC. The k_2 values indicate that b-BC and n-BC produced at 550 °C exhibited the highest PTEs ion adsorption rates compared to other temperatures. These findings concur previous studies, which have reported that elevated pyrolysis temperatures can decelerate adsorption kinetics (Hu et al., 2022; Shakya et al., 2022; Liu et al., 2018).

Regarding the pyrolysis atmosphere, no statistically significant ($p > 0.05$) changes in the adsorption rates of Zn(II) and Pb(II) ions were observed for either b-BC or n-BC produced in N₂ or CO₂ atmosphere (Fig. 1g-i). However, the use of CO₂ during pyrolysis resulted in approximately 2.1-fold faster adsorption of Cd(II) on b-BC and n-BC (BC-SSL3-500 vs. BC-SSL3CO2-500). This observed increase in the adsorption rate for Cd(II) ions on BC produced at CO₂ atmosphere could be attributed to a higher number of oxygen-containing functional groups, such as carboxyl and hydroxyl groups (Fig. S2) (Feng et al., 2017; J. Zhang et al., 2019; Zhang et al., 2016). These groups can enhance the chemisorption of specific PTEs ions as previously noted by Kim et al. (2019). Cd(II) ions, in particular, may exhibit a higher affinity for these functional groups due to their specific electronic structure, ionic radius and coordination chemistry compared to other investigated PTEs.

3.2. Adsorption isotherms

Fig. 3 presents the adsorption isotherms of Cd(II), Zn(II), or Pb(II) ions on both b-BC and n-BC. All the isotherms exhibited an inverted "L" shape, with saturation plateaus observed at high equilibrium concentrations. These findings indicate that the adsorption of Cd(II), Zn(II), or Pb(II) ions was initially rapid at low concentrations due to the abundance of available sites on the adsorbents (Qian et al., 2024; Xiao et al., 2020). However, as these sites became occupied, the adsorption rate decreased and eventually stabilized, demonstrating saturation (Chandarana et al., 2021).

The adsorption isotherms for Cd(II), Zn(II), or Pb(II) ions were fitted using Langmuir and Freundlich models (Table S5). The Langmuir model provided superior fitting for the isotherms of both b-BC and n-BC, with R^2 values surpassing 0.95. This suggests that monolayer adsorption significantly contributed to the adsorption for these PTEs (Zhou et al., 2024; Khan et al., 2020). The findings are consistent with prior research (Iamsaard et al., 2022; Wu et al., 2021; Li et al., 2020; Cao et al., 2019; Song et al., 2014), which also demonstrated that the Langmuir model more accurately represents the experimental data for PTEs adsorption on BC compared to the Freundlich model. Importantly, both b-BC and n-BC showed a substantially higher affinity for Pb(II) ions relative to Cd(II) and Zn(II) ions (Fig. 3).

3.2.1. Effect of feedstock on PTEs adsorption

The affinity of PTEs towards individual b-BCs varied depending on their type and the feedstock used (Fig. 3a-c). Evaluating the adsorption capacities of various b-BCs (based on Langmuir fitting Table S5) for Cd

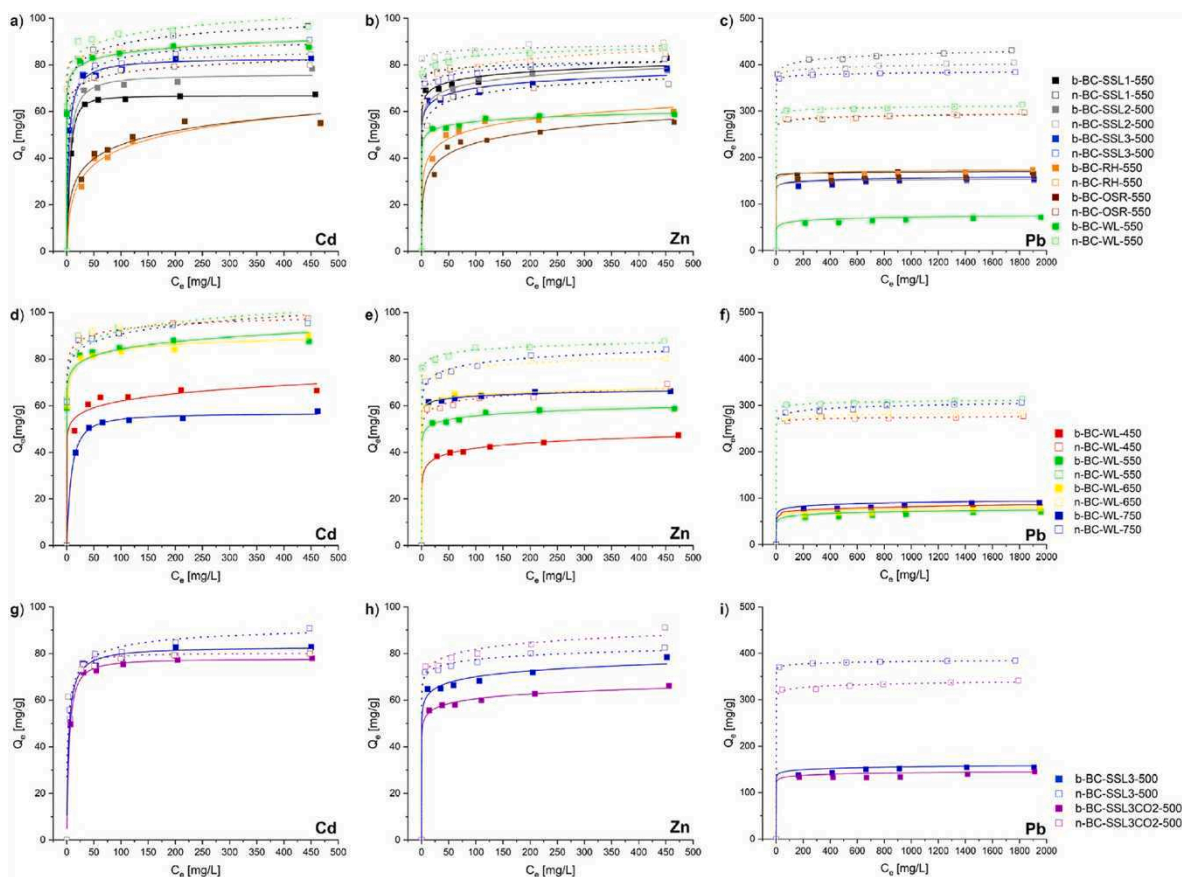


Fig. 3. Adsorption isotherms of Cd(II), Zn(II) and Pb(II) ions onto b-BC and n-BC. Positions a–c represent a comparison of different feedstocks, while d–f illustrate comparison of different pyrolysis temperatures and g–i illustrate comparison of different carrier gases.

(II) ions revealed that the highest capacities were achieved by WL- (b-BC-WL-550) (87.9 mg/g) and SSL-derived b-BCs (b-BC-SSL3-500) (91.4 mg/g). Within the different SSL-derived b-BCs, certain discrepancies were noted depending on the type of sludge. However, all SSL-derived b-BCs adsorbed Cd(II) at comparable levels (Table S5). In contrast, the lowest adsorption capacities for Cd(II) were recorded for b-BCs derived from agricultural residues (b-BC-RH-550 and b-BC-OSR-550) (from 57.4 to 57.8 mg/g). The differences in adsorption capacity between tested b-BC results from the structural differences between b-BCs (Table 1). The b-BC with the highest specific surface area (S_{BET}) and total pore volume (V_p) (b-BC-WL-550 and b-BC-SSL3-500) demonstrated greater adsorption capacities, which correlated significantly with the Langmuir isotherm constants (K_L and Q_{max}) and the experimental Q_e values for Cd (II) (Fig. S1a).

All SSL-derived b-BCs showed the highest adsorption capacities for Zn(II) ions ranging from 78.6 to 83.0 mg/g. In contrast the lowest values were noted for WL- and agricultural residues-derived b-BCs ranging from 56.6 to 60.9 mg/g. The adsorption capacity of Zn(II) ions on b-BC correlated with the O/C and (O + N)/C ratios, as well as the ash content in these BC (Fig. S1b). Higher O/C and (O + N)/C ratios indicate the presence of a greater number of oxygen-containing functional groups on the b-BC surface, which may serve as active adsorption sites for PTEs ions. Furthermore, variations in adsorption capacities may also be influenced by the standard reduction potentials of the PTEs. A more negative standard potential of a specific PTEs enhances the removal of hydrogen from surface groups, thereby increasing its binding capacity (Guzel et al., 2008). Among the ions studied, Zn(II) exhibited the most negative standard potential (Table S6), which likely explains Zn(II) superior ability to displace hydrogen and bind to dissociated groups on the surface of BC.

Notably, for Pb(II) ions, the b-BCs demonstrated significantly higher

adsorption capacities compared to Cd(II) and Zn(II) ions. Regarding to feedstock, the highest adsorption capacities for Pb(II) showed agricultural residues-derived b-BCs (b-BC-RH-550 and b-BC-OSR-550) and SSL-derived b-BCs (b-BC-SSL1-550, b-BC-SSL2-500, and b-BC-SSL3-500), with values ranging from 151.9 to 173.8 mg/g. Conversely, the lowest Pb(II) adsorption capacity was observed for WL-derived BCs (b-BC-WL-550) (72.7 mg/g). Statistical analysis revealed strong positive correlations between the adsorption capacities of b-BCs for Pb(II) ions and pore width and ash content (Fig. S1c). It was previously proved that the higher mineral content in b-BC (corresponding to ash) enhance the adsorption process by providing additional active sites on the BC surface that effectively bind PTEs ions (Yun et al., 2022). The larger pore widths may facilitate the diffusion of Pb(II) ions into the BC structure, improving access to the porous structure and increasing the number of active adsorption sites. Although Cd(II) and Zn(II) ions can also enter these pores, they do not bind as strongly to the BC surface as Pb(II) ions because Pb(II) tends to form stronger interactions with surface functional groups on BC, likely due to its higher electronegativity and stronger binding affinity with electron donors, leading to more efficient adsorption (Huang et al., 2019).

3.2.2. Effect of pyrolysis conditions on PTEs adsorption

The adsorption capacities of b-BC were also significantly influenced by pyrolysis conditions. An increase in adsorption capacity was observed with the rise in pyrolysis temperature from 450 to 650 °C for Cd(II) (Table S5). However, at 750 °C the adsorption capacity dropped and achieved the lowest value (58.1 mg/g) investigated WL-derived BCs produced at different temperatures. These observations may suggest that too high pyrolysis temperature is not favorable and the range of 550–650 °C is more optimal for b-BCs production for the removal of Cd (II) ions. Xian et al. (2018) found that BCs derived from spent mushroom

substrate and produced at high pyrolysis temperatures (700 °C) exhibited significantly higher adsorption capacities to Cd(II) compared to BCs produced at lower temperatures. For Zn(II) ions, an increase in adsorption capacity was observed across all temperatures, reaching the highest values for b-BCs produced at 650 °C and 750 °C (48.3–66.8 mg/g). However, no clear trend in adsorption capacity depending on temperature of pyrolysis was observed for Pb(II) ions (Table S5). The maximum adsorption capacities for Pb(II) ions were recorded at the two extreme temperatures of 450 °C and 750 °C, with respective values of 83.2 mg/g and 91.7 mg/g. A decrease in adsorption capacity for all investigated ions was observed, ranging from 6.1 % to 15.8 % (depending on the specific PTEs ion) after switching from N₂ to CO₂ (Fig. 3g–i).

3.2.3. Effect of size reduction on PTEs adsorption

Reducing the size of b-BC to the nanoscale enhanced BC's adsorption capacities for Cd(II), Zn(II), and Pb(II) ions from 2.1 % to 53.9 %, 14.3 %–48.3 %, and 71.9 %–332 %, respectively. The greatest increase in adsorption capacity for Cd(II) ions depending of feedstock used after reducing particle size was observed for n-BC-RH-550 (85.9 mg/g) and n-BC-OSR-550 (77.3 mg/g), with respective increases compared to their bulk counterparts of 48.5 % and 34.6 %. Nonetheless, the highest overall adsorption capacities among all n-BCs were achieved by n-BC-WL-550 (93.1 mg/g) and n-BC-SSL3-550 (91.8 mg/g). These samples also showed the highest adsorption capacities among their respective bulk counterparts (b-BCs). In contrast to b-BCs, the adsorption capacities for n-BCs derived from other feedstocks (n-BC-SSL2-500, n-BC-SSL3-500, n-BC-RH-550 and n-BC-OSR-550) became more uniform after size reduction, displaying adsorption capacities between 77.3 and 89.0 mg/g, while exceeding the values observed for their bulk counterparts. For Zn(II) ions, similar to the trend observed for Cd(II), a significant increase in adsorption capacity was noted with reduced particle size for n-BC-WL-550 and n-BC-RH-550, with respective increases of 48.3 % and 40.2 % compared to their bulk counterparts. However, no statistically ($p > 0.05$) significant differences were observed in the adsorption capacities of n-BCs derived from plant-based feedstocks (WL, RH, and OSR), with values ranging from 72.2 to 88.0 mg/g. In contrast, n-BCs produced from SSL1–3 exhibited adsorption capacities for Zn(II) that were 22.3 %–45.2 % higher compared to those obtained from WL, RH, and OSR. The highest increase after size reduction and simultaneously the highest adsorption values for all tested n-BCs were observed for Pb(II) ions, which showed superior adsorption capacity compared to other tested PTEs ions. Fig. S1 shows that for Pb(II) ions, the n-BC exhibited the largest number of significant positive correlations ($p \leq 0.05$) between adsorption capacities and the physicochemical parameters of the material compared to other PTEs ions. This suggests that the adsorption of Pb(II) ions is more effective because a wider range of factors influence it compared to other PTEs. In the case of Pb(II), if one parameter is ineffective, another is likely to compensate. Conversely, for other PTEs, if a particular parameter, there is often no alternative process that can

successfully facilitate adsorption. In this context, SSL-derived n-BCs (n-BC-SSL1-550, n-BC-SSL2-500, and n-BC-SSL3-500) exhibited the highest capacities ranging from 384.5 to 431.9 mg/g, which were approximately 33 % higher than those of n-BCs produced from other feedstocks. The adsorption capacity of SSL-derived n-BC increased from 148 % to 167 %, compared to the corresponding SSL-derived b-BC. However, the most significant increase in adsorption capacity (332 %) of Pb(II) to BC after ball milling was observed for n-BC-WL-550.

The enhanced adsorption capacities of n-BC compared to their bulk counterparts were correlated with their increased specific surface area (S_{BET}), total pore volume (V_p), and more negative zeta potential (Fig. 4). A higher S_{BET} provides more available adsorption sites on the surface of n-BCs, offering PTEs ions more opportunities to interact with the surface, thereby increasing adsorption capacities. Additionally, a larger V_p allows for greater diffusion of PTEs ions into the internal structure of the n-BC, not only increasing the overall capacity for ion adsorption but also ensuring that ions are effectively captured within the porous network rather than merely adhering to the external surface. Moreover, a more negative zeta potential indicates a higher surface charge density, enhancing the electrostatic attraction between the negatively charged n-BC surface and the positively charged PTEs ions such as Cd(II), Zn(II), and Pb(II). This strong electrostatic interaction promotes the binding of PTEs ions to the n-BC surface, improving adsorption efficiency. Collectively, these factors contribute to the superior adsorption performance of n-BCs by optimizing the interaction between the BC and PTEs ions, facilitating both surface adsorption and pore penetration. Upon reducing the size to the nanoscale, the number of significant correlations changed between b-BC and n-BC (Fig. S1). These variations differed across the studied PTEs, with Pb(II) ions exhibiting consistent correlations for both b-BC and n-BC. This consistency suggests that adsorption mechanisms for Pb(II) ions are similar for both forms, though these mechanisms are stronger in n-BC. Conversely, for Cd(II) and Zn(II) ions, differences in adsorption between b-BC and n-BC may stem from distinct mechanisms and influencing factors.

Regarding the effect of pyrolysis conditions on the adsorption capacities of n-BCs, no significant influence of pyrolysis temperature on the adsorption capacities for Cd(II) ions was observed. For WL-derived n-BCs across the temperature range of 450–750 °C, the adsorption capacities remained constant, ranging from 89.5 to 93.1 mg/g and were 232 %–332 % higher compared to WL-derived b-BCs. Conversely, for Zn(II) and Pb(II) ions, the highest adsorption capacities were observed for n-BC-WL-550, at 88.0 mg/g and 314.3 mg/g, respectively, which were higher by 48.3 % and 332 %, and simultaneously represented the highest increase compared to their bulk counterparts. However, like Cd(II), no distinct trend in adsorption capacities correlated with pyrolysis temperature was noted. In terms of the pyrolysis atmosphere, similar trend to b-BCs was observed, when the carrier gas was switched from N₂ to CO₂. A decrease in adsorption capacity ranging from 10 % to 16 % was noted depending on the specific PTEs ion and BC size. No statistically significant differences in the decrease in adsorption capacity were

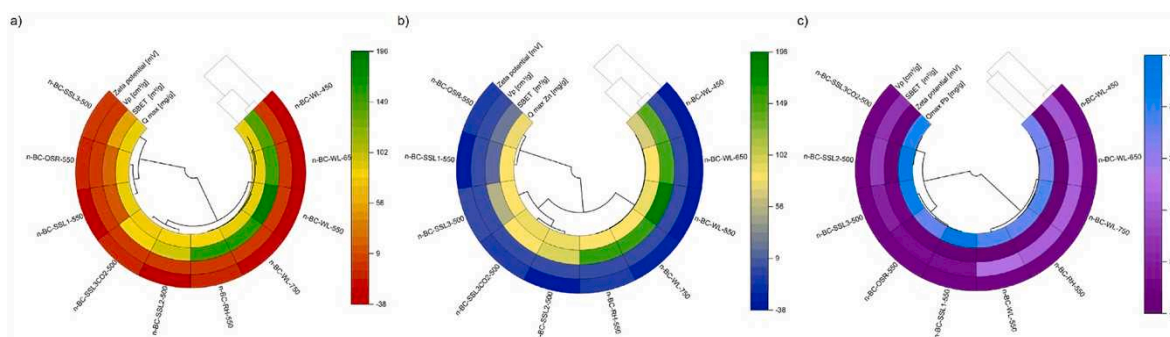


Fig. 4. Hierarchical clustering heatmap representing the physicochemical properties of n-BCs and adsorption capacities for Cd(II) (a), Zn(II) (b) and Pb(II) (c). The dendrogram highlights the similarity between n-BCs, with closer branches indicating greater similarity in their properties.

observed between b-BC and n-BC.

Based on the above discussion and previous studies, the mechanisms involved in the adsorption of PTEs such as Cd(II), Zn(II), and Pb(II) ions onto b-BC and n-BC encompass

a range of physicochemical processes. Electrostatic interactions play a significant role, where the negatively charged surfaces of BC attract positively charged Cd(II), Zn(II), and Pb(II) ions (Zhang et al., 2020). This effect is more pronounced in n-BC due to its larger specific surface area (S_{BET}) and more negative zeta potential compared to bulk counterparts. Ion exchange is another key mechanism, in which PTEs cations replace other cations, such as H^+ , Ca^{2+} , or K^+ , that are initially bound to the BC surface. The n-BC, with its higher surface reactivity, typically demonstrates greater ion exchange capacity. Complexation (chemisorption), involving the binding of PTEs ions with oxygen-containing functional groups such as -OH, -COOH etc. on the BC surface, is also evident (Yang et al., 2019). The molar ratio of O/C and (N + O)/C (Table 1), along with the FTIR spectra (Fig. S2), confirm the presence of surface oxygen-containing groups, which are more abundant in n-BC compared to b-BC. These functional groups play a crucial role in binding PTEs ions, thereby enhancing the overall adsorption capacity of the BCs for PTEs ions (Ge et al., 2024). Additionally, precipitation occurs when insoluble metal compounds form and deposit on the BC surface. This happens because the local pH near the BC surface becomes more alkaline due to the release of hydroxide ions from surface groups, which can trigger the precipitation of PTEs ions as hydroxides, thereby enhancing adsorption capacity (Ambaye et al., 2021). Furthermore, redox reactions may occur, where PTEs ions are either reduced to their metallic state or transformed into different oxidation states, with the superior catalytic properties of n-BC facilitating these reactions. Finally, pore diffusion, in which PTEs ions penetrate the porous structure of BC and are adsorbed onto internal surfaces, is more effective in n-BC due to its larger specific surface area (S_{BET}) and total pore volume (V_p), providing easier access

for PTEs ions. Collectively, these mechanisms enhance the overall adsorption capacity of both b-BC and n-BC for PTEs, with n-BC exhibiting superior performance in PTEs removal.

To elucidate the main adsorption mechanism, one representative PTEs ion was selected from each feedstock group (produced under analogous conditions: identical pyrolysis temperature and carrier gas), focusing on the most effectively adsorbed PTEs, Pb(II). In addition, for the most effective adsorbent (BC-SSL), the adsorption mechanisms were also determined for Cd(II) and Zn(II). Based on the results of XPS and FTIR analyses (Fig. 5 and S6), the predominant mechanism governing PTEs adsorption onto the investigated b-BC and n-BC is chemical complexation (chemisorption) involving oxygen-containing surface functional groups. XPS spectra in the C 1s region revealed a significant decrease in the intensity of peaks assigned to carboxylic (COOR, ~ 288.5 – 289.2 eV) and hydroxyl (C–OH, ~ 286.0 – 286.5 eV) groups after PTEs adsorption, indicating the formation of strong inner-sphere complexes such as Pb-O-C=O and Pb-OH . These findings were further supported by FTIR spectra, which showed a reduction in the characteristic vibrational bands of COO^- groups (~ 1613 and 1446 cm^{-1}) and hydroxyl groups ($\sim 3440\text{ cm}^{-1}$) (Fig. 5). The higher availability of surface functional groups in n-BC (Fig. S2), provide additional evidence of the greater surface reactivity and complexation potential of the nano-structured material. Consequently, the binding of PTEs via chemisorption is identified as the principal immobilization pathway, with n-BC exhibiting superior performance due to its higher specific surface area and greater density of active sites.

Furthermore, high-resolution XPS spectra in the Pb 4f, Cd 3d and Zn 2p region confirmed the presence of Pb 4f $7/2$, Pb 4f $5/2$, Cd 3d $5/2$, Cd 3d $3/2$, Zn 2p $3/2$ and Zn 2p $1/2$ peaks at binding energies of approximately 138.6–139.2 eV and 143.5–144.4 eV for Pb(II), 405.0–405.6 eV and 411.7–412.4 eV for Cd(II), and 1021.3–1025.0 eV for Zn(II), respectively (Figs. S5 and S6). These values are characteristic of Pb(II),

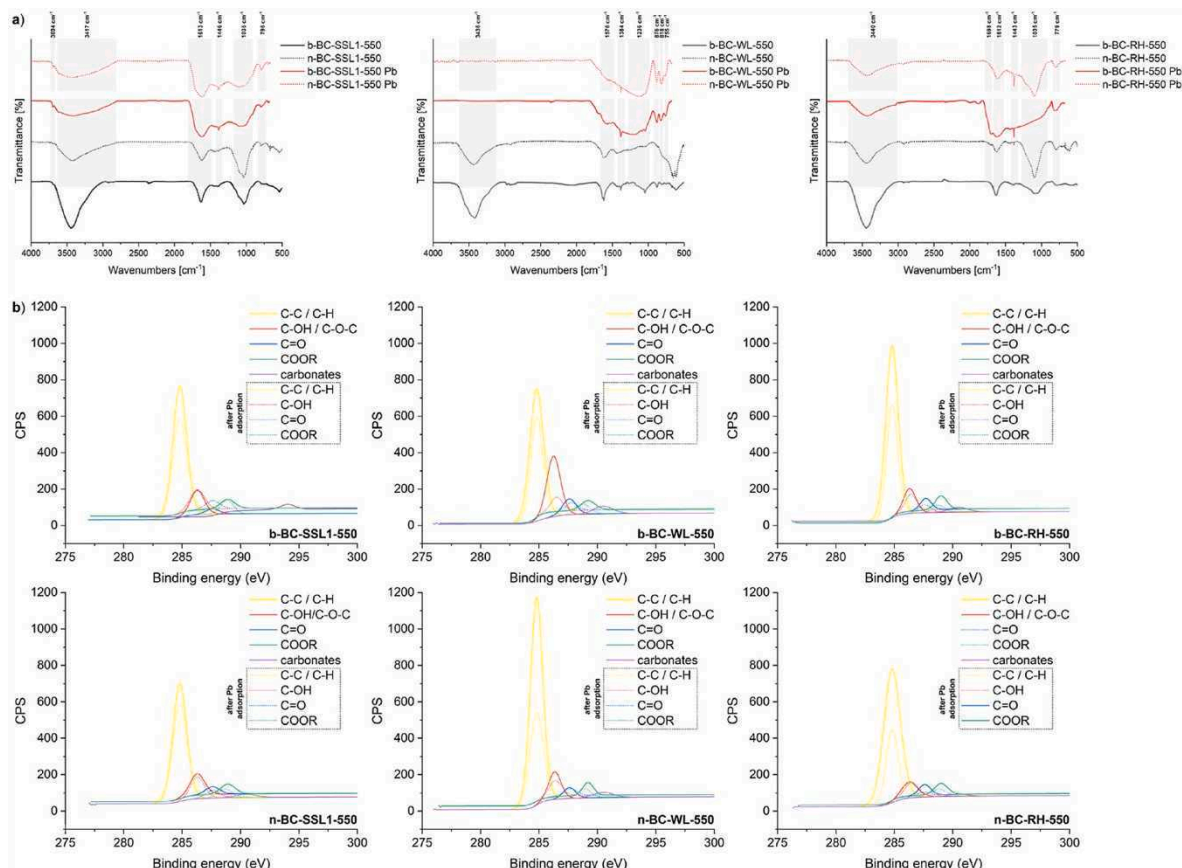


Fig. 5. FTIR and XPS for selected b-BC and n-BCs before and after adsorption of Pb (II) ions (others PTEs are presented in Supplementary Information).

Cd(II) and Zn(II) species such as $\text{Pb}(\text{OH})_2$, PbCO_3 , $\text{Cd}(\text{OH})_2$ and $\text{Zn}(\text{OH})_2$ indicating that in addition to complexation, precipitation processes contribute to the immobilization of PTEs. This likely results from localized increases in pH at the BC-solution interface, which promote the formation of poorly soluble metal compounds. Notably, the disappearance of carbonate-related signals in the C 1s spectra after Pb(II), Cd(II) or Zn(II) adsorption further supports the involvement of carbonate groups in the formation of MeCO_3 type precipitates. Therefore, while inner-sphere complexation with oxygen-containing functional groups is the dominant adsorption mechanism, the contribution of precipitation provides an additional immobilization pathway, enhancing the overall PTEs removal efficiency of the BCs.

To gain more detailed insights into the influence of the physicochemical properties of b-BC and n-BC on their adsorption capacities for Cd(II), Zn(II), and Pb(II) ions, a principal component analysis (PCA) was performed (Fig. 6). The PCA plots illustrate the relationships between the physicochemical properties of tested BCs and its ability to adsorb PTEs ions (Q_{max}), comparing b-BC (a) with n-BC (b). In both b-BC and n-BC, certain properties such as oxygen-containing functional groups ((O/C and (N + O/C)), specific surface area (S_{BET}), and pore volume (V_p) play a more significant role in enhancing adsorption efficiency. The confidence ellipses indicate some variability in the adsorption performance for Pb(II), whereas a more consistent behavior is observed for Zn(II) and Cd(II). For n-BC, the tighter clustering of data points suggests a more uniform adsorption behavior compared to b-BC, with surface properties exerting a stronger influence on the adsorption performance for PTEs ions. This may be attributed to the higher reactivity and homogeneity of nanostructured surfaces, which enhance the availability and distribution of functional groups, thereby promoting more efficient and predictable PTEs ion interactions. Although the PCA results indicate that physicochemical properties mainly specific surface area (S_{BET}), total pore volume (V_p), and ash content are strongly correlated with the maximum adsorption capacity (Q_{max}), the spatial distribution of the samples corresponding to Pb(II), Cd(II), and Zn(II) reveals a considerable overlap. The red, black, and green points representing Pb(II), Cd(II), and Zn(II), respectively, are located closely together in the PC1–PC2 space, and their 95 % confidence ellipses show significant intersection. This suggests that the physicochemical factors influencing the adsorption of these three PTEs ions are similar. Despite potential differences in ionic size or chemical affinity, the BCs studied exhibit comparable adsorption behavior toward all studied PTEs, driven by a combination of shared mechanisms such as pore-filling and electrostatic interactions. The absence of clear separation in PCA space implies that textural properties are important but do not discriminate strongly between the adsorption of Pb(II), Cd(II), and Zn(II).

This study presents a review of the most recent literature on the removal of Cd(II), Zn(II), and Pb(II) from aqueous solutions over the past few years. It compares the performance of b-BC and n-BC, as well as their

effectiveness relative to other advanced nanomaterials. Table S7 summarizes the adsorption capacities of b-BCs and n-BCs derived from different feedstocks in the context of removing Cd(II), Zn(II), and Pb(II) ions from aqueous solutions. Based on this, it can be concluded that the adsorption capacities obtained in this study for b-BCs are comparable to or exceed those reported in the literature. For example, Wang et al. (2025) reported that b-BCs produced from *P. vittata* and corn straw (pyrolyzed at 500 °C under N_2) showed varying adsorption efficiencies toward Cd(II). Corn straw b-BC demonstrated superior performance, reaching an adsorption capacity of 35 mg/g, while *P. vittata* b-BC was less effective, with a capacity of 25 mg/g. In terms of Pb(II) adsorption, the efficiencies were 40 mg/g for *P. vittata* b-BC and 45 mg/g for corn straw b-BC. Zhang et al. (2023) conducted a study on green-synthesized magnetic b-BC derived from white eta leaves to evaluate its adsorption performance toward Pb(II) and Cd(II) ions. The material exhibited notable adsorption capacities, with maximum values of 81.6 mg/g for Pb(II) and 38.6 mg/g for Cd(II). Myung et al. (2024) demonstrated that b-BC derived from *Spirulina platensis* at 400 °C and 600 °C under a N_2 atmosphere exhibited increasing PTEs removal capacities with higher pyrolysis temperature. Specifically, SP400 showed adsorption capacities of 23.1 mg/g for Pb(II) and 26.1 mg/g for Zn(II), while SP600 achieved slightly higher values of 26.9 mg/g for Pb(II) and 27.8 mg/g for Zn(II). In the case of n-BC, studies are very scarce (Table S7), and our results show that a change in size can significantly increase the efficiency of adsorption, exceeding the values observed for b-BCs. For example, Cao et al. (2019) reported that n-BC produced from wheat straw through ball milling achieved an adsorption capacity of 134.68 mg/g for Pb(II),

When comparing n-BC used in this study with other advanced nanomaterials, we can conclude that the adsorption capacities obtained are comparable to, or even higher than, those reported for various advanced materials. For instance, Ghaedi et al. (2025) reported that a UiO-67 MOF and hardwood BC composite exhibited maximum adsorption capacities of up to 121.1 mg/g for Pb(II) and 59.6 mg/g for Cd(II). Similarly, Wang et al. (2022) demonstrated that MOF@cellulose aerogels showed high Pb(II) adsorption capacities ranging from 105.2 to 123 mg/g, along with rapid adsorption kinetics. Hu and Zou (2023) found that cucurbit[7]uril-modified, magnetically recyclable carbon nanotubes followed the Langmuir isotherm well, with a maximum adsorption capacity for Pb(II) reaching 245.6 mg/g. Kończyk et al. (2019) studied raw multi-walled carbon nanotubes with maximum Pb(II) adsorption capacity of 156.25 mg/g at 313 K., while El-Sheikh et al. (2022) investigated magnetic carbon nanotubes and reported a Cd(II) adsorption capacity of 13.9 mg/g.

In our study, the b-BCs exhibited Pb(II) adsorption capacities ranging from 54.7 to 164.6 mg/g, whereas their nano-counterparts (n-BCs) demonstrated significantly enhanced performance, with capacities ranging from 235.1 to 430.7 mg/g. For Cd(II), the adsorption capacities of b-BCs ranged from 50.6 to 84.3 mg/g, while the n-BCs achieved

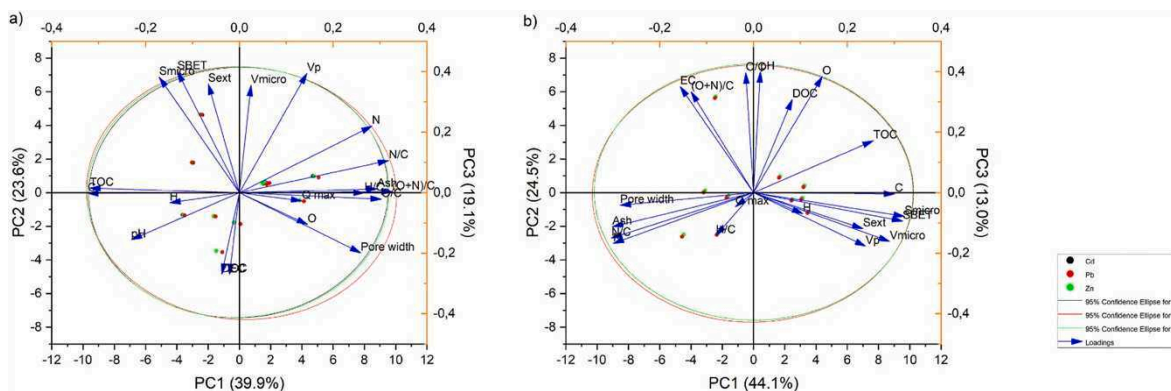


Fig. 6. Principal component analysis (PCA) illustrating the correlation between physicochemical properties of b-BC (a) and n-BC (b) and the adsorption capacities for Cd(II), Zn(II), and Pb(II) ions.

values between 71.1 and 87.8 mg/g. Similarly, for Zn(II), b-BCs showed adsorption capacities ranging from 48.3 to 83.0 mg/g, whereas n-BCs exhibited improved capacities between 69.8 and 91.9 mg/g. Although some MOF-based adsorbents have reported higher adsorption capacities for Pb(II) (Arya et al., 2025) and Cd(II), their practical application is often limited by factors such as high production costs, limited structural and chemical stability under environmental conditions, and challenges associated with regeneration and reusability (Abdollahi et al., 2020; Du et al., 2023). In contrast, n-BC offer several advantages, including low-cost synthesis from renewable biomass, environmental sustainability, and a high abundance of surface functional groups that contribute to efficient PTEs ion binding through complexation, ion exchange, and surface precipitation mechanisms. Notably, the proposed green synthesis method for n-BC—ball milling—has been widely employed in the production of various nanomaterials (Ali et al., 2025). This technique operates through mechanical forces such as collision, impact, shear, and friction, leading to particle size reduction, the introduction of structural defects, and even changes in crystal structure (Wang et al., 2023). These characteristics make ball milling especially appealing for industrial applications due to its scalability, sustainability, and cost-efficiency. Moreover, this method has already been successfully used for the large-scale production of materials such as TiS₂ (Veluswamy et al., 2022) nanoplatelets and nanoscale zero-valent iron (nZVI) (Chen et al., 2025). Despite these promising findings, the study has certain limitations that warrant further investigation. The experiments were conducted under batch conditions, which provide fundamental insight but do not fully represent the dynamic nature of adsorption in continuous flow systems. Furthermore, although the study addressed multi-ion

competition, additional research is needed to assess the performance of n-BCs in complex industrial wastewater containing competing inorganic ions and organic pollutants. The scalability and economic feasibility of the n-BC production process also require further evaluation. Future research should focus on integrating n-BCs into fixed-bed column systems, assessing their regeneration potential over multiple adsorption–desorption cycles, and verifying their long-term stability and effectiveness under real-world conditions.

3.3. TEM images with EDS mapping

Transmission electron microscopy (TEM) analysis of the selected b-BC and n-BC samples after adsorption of Cd(II), Zn(II), and Pb(II) ions confirmed the presence of these ions within the BC structure (Fig. 7 and Fig. S7). The EDS mapping in STEM mode illustrates the distribution of elements and reveals that the structure primarily consists of carbon (C), silicon (Si), oxygen (O), and adsorbed PTE ions. The cyan map shows that carbon is uniformly distributed throughout the BC particle, indicating that it is the primary component of the BC. The blue map reveals localized regions of silica, which could represent silica-based functional groups on the BC surface. The red map highlights the presence of oxygen-containing functional groups, which play a crucial role in binding PTEs ions, as confirmed by FTIR spectra. Finally, the yellow map of Cd(II), Zn(II), or Pb(II) shows successful adsorption of these PTEs ions, with concentrated areas indicating that the PTEs have penetrated the BC matrix and effectively bonded during the adsorption process, rather than precipitating as hydroxides. The combined analysis supports the conclusion that both b-BC and n-BC are effective adsorbents for Cd(II),

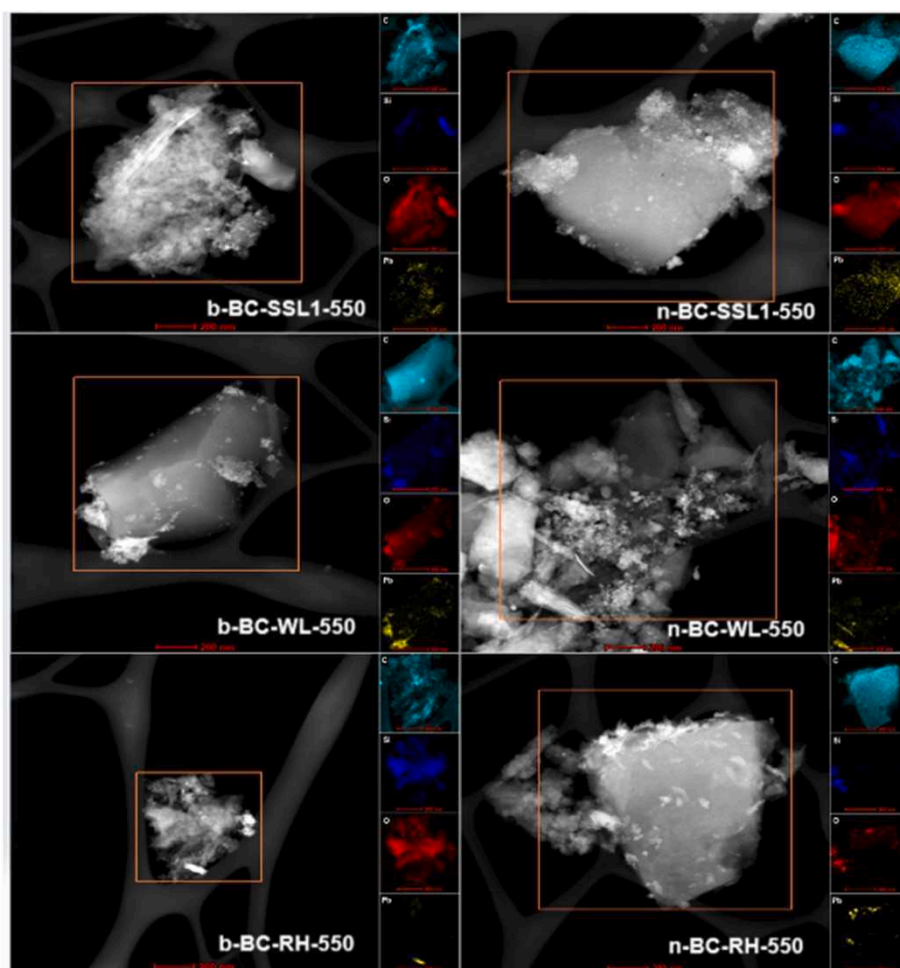


Fig. 7. TEM images with EDS mapping for selected b-BC and n-BCs with adsorbed Pb (II) ions (others are presented in Supplementary Information).

Zn(II), and Pb(II) ions, with oxygen functional groups and the BC's surface structure playing key roles in PTEs ion removal. The aggregation of Pb (Fig. 7) coinciding with clusters of Si and O suggests potential binding through mineral fractions. This spatial overlap may indicate that Pb(II) ions are interacting with silicate minerals, possibly forming surface complexes or undergoing ion exchange processes, facilitated by the oxygen-rich environments typically associated with mineral components like silica. This interaction highlights the role of mineral surfaces in the adsorption and immobilization of Pb, further contributing to its retention in such systems.

4. Conclusion

Nanoscale reduction of b-BC improves Cd(II), Zn(II), and Pb(II) adsorption through enhanced surface area, pore volume higher ash content and negative surface charge. Kinetic studies indicated that adsorption followed a pseudo-second-order (PSO) model, suggesting chemisorption as the dominant mechanism. Surface characterization using FTIR and XPS confirmed that complexation with oxygen-containing functional groups (e.g., $-\text{COOH}$, $-\text{OH}$) was the dominant mechanism of PTEs adsorption. Adsorption kinetics vary between b-BC and n-BC based on feedstock type. SSL- and WL-derived n-BC demonstrated superior adsorption performance. Optimal production parameters include pyrolysis at 550 °C under N_2 atmosphere. At this temperature, the BCs exhibited the best adsorption capacities for the Cd (II), Zn(II), and Pb(II) ions. Ball milling enhances adsorption capacity in low-performing BC, while high-performing BC maintains or improves efficiency. The results demonstrated that reducing the particle size of b-BC to the nanoscale significantly enhanced its adsorption efficiency, particularly for Pb(II). These properties establish n-BC as an effective, economical adsorbent for PTE removal from wastewater and landfill leachate. Its ease of preparation, the availability of low-cost raw materials, and the efficient removal of PTEs make n-BC a promising material for large-scale environmental applications. Importantly, these findings have strong implications for the development of cost-effective water treatment technologies and sustainable environmental remediation strategies, particularly in regions where access to advanced treatment systems is limited.

CRedit authorship contribution statement

Monika Raczkiewicz: Writing – original draft, Visualization, Validation, Methodology, Investigation, Conceptualization. **Patryk Oleszczuk:** Writing – review & editing, Supervision, Resources, Conceptualization.

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.jenvman.2025.126072>.

Data availability

Data will be made available on request.

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Supplementary material for:

Enhanced Heavy Metal Removal Using Nano-Engineered Biochars Synthesized Under Variable Pyrolysis Conditions and Feedstocks

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1. Data modeling

The capacity of the adsorbent was calculated based on the following equation (1):

$$q_e = \frac{(C_0 - C_e) \times V}{m} \quad (1)$$

where q_e is the adsorption capacity of the biochar (mg/g), c_0 is the initial concentration (mg/L) of metals, c_e is the equilibrium concentration (mg/L) of metals, V -the volume of the solution (L), and m is the mass of b-BC/n-BC (g).

Two kinetic models, the pseudo-first-order and pseudo-second-order Lagergren equations, were selected to investigate the potential sorption mechanisms. The following equations (2, 3,4) were used for modeling the adsorption rates:

$$\text{Pseudo-first-order kinetic model:} \quad \ln(q_e - q_t) = \ln q_e - k_1 t \quad (2)$$

$$\text{Pseudo-second-order kinetic model:} \quad \frac{1}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \quad (3)$$

$$\text{Intraparticle diffusion model:} \quad q_t = k_{dif} t^{\frac{1}{2}} + C \quad (4)$$

where q_e is the adsorption capacity of biochar at equilibrium time (mg/g), q_t is the adsorption capacity of biochar at any time (t) (mg/g), t is contact time t (min), k_1 (1/min) and k_2 (g/(mg·min)) represent the equilibrium rate constants of pseudo-first-order model and pseudo-second-order model, respectively.

To fit the sorption isotherms of the selected metals, two commonly used two-parameter models, Freundlich and Langmuir, were employed. The Freundlich adsorption model is based on adsorption on a monolayer heterogeneous surface with interaction between the adsorbed ions. The Langmuir model describes monolayer adsorption on a porous surface with a finite number of homogeneous sites without interaction between the adsorbed ions. The linear and nonlinear forms of these equations are (5-8) as follows:

$$\begin{array}{ll} \text{Freundlich:} & \text{linear} \end{array} \quad \log q_e = \log K_F + \frac{1}{n} \log C_e \quad (5)$$

non-linear
$$q_e = K_F C_e^n \quad (6)$$

linear
$$\frac{C_e}{q_e} = \frac{C_e}{q_{max}} + \frac{1}{q_{max} K_L} \quad (7)$$

Langmuir: non-linear
$$q_e = \frac{q_{max} K_L C_e}{1 + K_L C_e} \quad (8)$$

where q_e is the adsorption capacity of biochar at equilibrium time (mg/g), C_e is the adsorption capacity of biochar at equilibrium time (mg/g), K_F is the Freundlich constant indicative of the adsorption capacity ((mg/g)(L/mg) ^{$\frac{1}{n}$}), n is the Freundlich exponent (dimensionless), q_{max} is the maximum adsorption capacity (mg/g), K_L is the Langmuir constant related to the affinity of binding sites (L/mg).

2. Production of of bulk (b-BC) and nanobiochar (n-BC)

In our laboratory, we obtained b-BCs from willow (WL) and sewage sludge (SSL2-3), while b-BCs from other feedstocks (sewage sludge (SSL1), rice husk (RH) and oilseed rape (OSR)) were obtained from the UK Biochar Research Centre in collaboration with another research group. Briefly, feedstock, i.e., WL and SSL2-3, were dried and crushed (<2 mm) and then pyrolyzed at 450–750 °C under a nitrogen (N₂) or carbon dioxide (CO₂) atmosphere for 3 h (heating rate 10 °C·min⁻¹) [1]. SSL1, RH, and OSR biochar were produced using a pilot-scale pyrolysis unit at the UK Biochar Research Centre. The pyrolysis unit consists of a rotary kiln with a heat tube length of 2.8 m, an inner diameter of 244 mm, an angle of 0.5°, and a 1–7 rpm rotational speed. Previous studies provide further details on the production conditions used [2]. The n-BCs were obtained following a previous method [3]. Six grams of each b-BC was placed in a planetary ball mill (PM100, Retsch Corporation, German) in a 125 mL corundum jar with 7 corundum balls (ball diameter was 20 mm) (biochar-to-ball mass ratio was 1:100). The planetary ball mill was operated at room temperature with a speed of 440 rpm for 1.5 h, and the rotation direction was altered every 5 min with short intervals to control the temperature boost.

Table S1. Process parameters of obtaining bulk-biochar (b-BC).

Feedstock material	Biochar	Temperature [°C]	Heating time [min]	Heat rate [°C/min]	Gas flow [L/min]	Gas type
willow	b-BC-WL-450	450	180	10	1	N ₂
willow	b-BC-WL-550	550	180	10	1	N ₂
willow	b-BC-WL-650	650	180	10	1	N ₂
willow	b-BC-WL-750	750	180	10	1	N ₂
rice husk	b-BC-RH-550	550	15	98	10	N ₂
oilseed rape	b-BC-OSR-550	550	12	78	10	N ₂
sewage sludge						
SCOTLAND						
EDINBURGH	b-BC-SSL1-550	550	13	86	10	N ₂
55°56' N, 3°14' W						
sewage sludge						
POLAND ZAMOŚĆ	b-BC-SSL2-500	500	180	7	1	N ₂
50°42' N, 23°13' E						

sewage sludge					
POLAND LUBLIN	b-BC-SSL3-500	500	180	10	1
51°14' N, 22°35' E					N₂
sewage sludge					
POLAND LUBLIN	b-BC-SSL3-CO2-500	500	180	10	1
51°14' N, 22°35' E					CO₂

3. Characterization of bulk (b-BC) and nanobiochar (n-BC)

3.1. Physicochemical properties

Briefly, the porous structure of n- and b-BCs was determined using low-temperature (-195.8 °C) nitrogen adsorption-desorption isotherms (ASAP 2420 V2.09, Micromeritics, USA). The specific and micropore surface area (S_{BET}/S_{micro}), total pore and micropore volume and average pore width were calculated based on the nitrogen desorption data [4]. The pH and electrical conductivity (EC) were determined using the procedure described previously[5]. One gram of each BC was dispersed into 10 mL of deionized water (solid/liquid ratio 1:10 (w/v)), and the pH/EC of the suspensions was measured using a digital pH and EC meter (HQ430d Benchtop Single Input, HACH, USA or CP – 401, ELMETRON, Poland). The ash content of n- and b-BC samples was determined using the ASTM standard technique by burning 1 g of each sample at 760 °C for 6 hours and weighing the residue after ashing[6]. The ash content was calculated using the following equation:

$$\% Ash = \frac{\text{weight after ashing} - \text{weight of crucible}}{\text{dry weight of BC sample}} \cdot 100\% \quad (1)$$

The composition of elements (C, H, N) was determined using an elemental analyzer (CHN Analyzer EuroEA3000, EuroVector, Italy). The average results of each triplicate sample were reported. The total content of O was determined by the following equation:

$$\% O = 100\% - \% Ash - \% C - \% H - \% N \quad (2)$$

The functional groups of BCs were determined by FTIR-ATR spectra in the region of 400-4000 cm^{-1} with a resolution of 1 cm^{-1} (Nicolet 8700A, Thermo Scientific, USA).

3.2. Electrokinetic properties (zeta potential)

The zeta potential of the n-BC suspensions was calculated from the electrophoretic mobility measurements and the Smoluchowski equation (Zetasizer Nano ZS, Malvern Instruments) [7]. Briefly, 100 mg L^{-1} b-BC/n-BC suspensions were prepared from the background electrolyte solution (10^{-3} M NaCl or deionized water) and treated with ultrasound for 1 minute. Then, the zeta potential was measured in the pH=5, adjusted by adding 0.1 M HCl or NaOH solution. All measurements were performed 10 times, and the average values are reported.

The zeta potential analysis (Table 1) revealed significant variations in surface charge among biochars, depending on their feedstock type and particle size. The n-BCs consistently exhibited more negative zeta potential values at pH=5.0 compared to their corresponding bulk counterparts (b-BCs), suggesting a higher density of ionizable surface functional groups (Fig. S2) and improved colloidal stability. Among the different feedstocks, biochars derived from rice husk (RH) and oilseed rape (OSR) showed the most negative zeta potential at pH 5.0, indicating a high abundance of oxygen-containing functional groups. In contrast, sewage sludge SSL-based b-BC and n-BC (SSL1–SSL3) exhibited less negative zeta potential, likely due to their higher ash content, which may reduce the availability of dissociable surface groups. For SSL-derived biochars, b-BC samples showed a general trend of decreasing (i.e., less negative) zeta potential with increasing pyrolysis temperature from 450 °C to 750 °C, with the most negative value occurring at 550 °C. In the case of n-BCs, zeta potential remained relatively stable between 450 °C and 650 °C, but became noticeably less negative at 750 °C. This shift is likely due to thermal degradation of surface oxygenated groups at elevated temperatures, resulting in a reduced density of dissociable acidic functionalities. The effect of carrier gas was also evident. Both b-BC and n-BC produced from SSL3 under a N_2 atmosphere at 500 °C exhibited slightly more negative zeta potentials compared to those obtained using CO_2 . This difference may be attributed to the mild activation effect of CO_2 , which could promote structural changes or alter the nature of surface functionalities.

Overall, the observed differences in zeta potential across feedstocks, particle sizes, pyrolysis temperatures, and carrier gases underscore the significant role of biochar structure and surface chemistry in determining colloidal behavior and environmental performance, particularly with respect to stability and contaminant adsorption.

3.3. Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) analysis

The quantification of Cd, Zn, and Pb in solutions after adsorption experiments were performed using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) model iCAP 7400 Duo (Thermo Scientific, USA). Argon (99.9999%, AirLiquide, Poland) was used for the plasma generation and sample nebulization at flow rates of 12 L/min and 0.5 L/min, respectively. The quantification of metals was performed based on the six-point calibration curve prepared for selected metals in the range from 0.01 to 5 mg/L. Solutions for the calibration curve were prepared by diluting the multi-element standard (concentration of metals 1 g/L) with MiliQ- water and acidified using HNO₃ (1 %, v/v). Metal quantification on ICP-OES was carried out at wavelength on radial system: 228.802 nm, 213.856 nm, and 220.353 nm, respectively for Cd, Zn and Pb.

Table. S2. Parameters of the calibration curves for the metals analyzed.

Element	Emission wavelength (nm)	Calibration curve equation	Correlation coefficient (r)	Limit of detection (µg/L)
Cd	228.802	y = 1940.61x + 9.40	1	0.6
Zn	213.856	y = 43715.1x + 1130.19	0.9998	3.62
Pb	220.353	y = 4293.64x - 10.92	0.9998	1.56

Table S3. The fitting parameters of kinetic models simulated by different equations (pseudo-first-order (PFO) and pseudo-second-order (PSO)).

Metal	Biochar	PFO				PSO			
		Q _e [mg/g]	K _i	R ²	Q _e [mg/g]	K ₂	R ²		
Cd	b-BC-WL-450	56.39 ± 3.572	-0.0108 ± 0.0031	0.9483	61.40 ± 4.005	0.000193 ± 0.0000679	0.9578		
	n-BC-WL-450	78.07 ± 7.667	-0.0173 ± 0.0065	0.8636	84.31 ± 8.204	0.00021 ± 0.000104	0.8897		

b-BC-WL-550	77.91 ± 8.154	-0.0225 ± 0.0082	0.7834	82.24 ± 8.199	0.000316 ± 0.000156	0.8693
n-BC-WL-550	81.03 ± 9.172	-0.0225 ± 0.0089	0.7586	85.06 ± 9.601	0.000315 ± 0.000176	0.8381
b-BC-WL-650	69.62 ± 6.813	-0.0181 ± 0.0067	0.8278	74.72 ± 6.479	0.000256 ± 0.000112	0.9079
n-BC-WL-650	71.42 ± 8.906	-0.0159 ± 0.0079	0.7621	78.23 ± 8.513	0.000192 ± 0.000109	0.8730
b-BC-WL-750	45.98 ± 4.682	-0.0083 ± 0.0044	0.8829	50.56 ± 4.930	0.000191 ± 0.000103	0.9169
n-BC-WL-750	78.44 ± 6.662	-0.0190 ± 0.0061	0.8907	83.96 ± 6.967	0.000243 ± 0.000101	0.9124
b-BC-SSL1-550	72.48 ± 5.629	-0.0020 ± 0.0006	0.9558	84.27 ± 9.171	0.000029 ± 0.0000147	0.9583
n-BC-SSL1-550	80.56 ± 7.774	-0.0026 ± 0.001	0.9156	87.87 ± 10.09	0.0000473 ± 0.0000285	0.9290
b-BC-SSL2-500	67.54 ± 4.754	-0.0025 ± 0.0007	0.9569	75.74 ± 6.190	0.0000459 ± 0.0000191	0.9685
n-BC-SSL2-500	71.441 ± 6.375	-0.0027 ± 0.0009	0.9257	77.34 ± 7.981	0.0000571 ± 0.0000313	0.9393
b-BC-SSL3-500	64.55 ± 6.095	-0.0025 ± 0.0009	0.9178	70.23 ± 7.651	0.0000586 ± 0.0000335	0.9345
n-BC-SSL3-500	79.32 ± 7.023	-0.0025 ± 0.0008	0.9279	86.49 ± 8.816	0.0000464 ± 0.0000247	0.9433
b-BC-SSL3CO2-500	67.22 ± 5.068	-0.0038 ± 0.0012	0.9415	69.33 ± 4.785	0.000126 ± 0.0000481	0.9572
n-BC-SSL3CO2-500	66.56 ± 6.005	-0.0041 ± 0.0016	0.9111	71.08 ± 4.693	0.0000996 ± 0.0000367	0.9665
b-BC-RH-550	56.30 ± 3.773	-0.0041 ± 0.0012	0.9565	60.88 ± 4.119	0.000104 ± 0.0000391	0.9689
n-BC-RH-550	75.78 ± 6.513	-0.0193 ± 0.0061	0.8889	81.03 ± 6.918	0.000256 ± 0.000109	0.9077
b-BC-OSR-550	50.52 ± 4.366	-0.0032 ± 0.0011	0.9310	54.70 ± 5.408	0.0000946 ± 0.0000512	0.9422
n-BC-OSR-550	77.07 ± 6.337	-0.0031 ± 0.0011	0.9378	83.94 ± 8.024	0.0000572 ± 0.0000295	0.9481
b-BC-WL-450	52.17 ± 3.879	-0.0286 ± 0.0071	0.9047	54.44 ± 4.414	0.0000663 ± 0.0000269	0.9159
n-BC-WL-450	61.58 ± 2.285	-0.0565 ± 0.0079	0.9679	62.88 ± 1.935	0.00171 ± 0.000355	0.9793
b-BC-WL-550	65.08 ± 2.931	-0.0359 ± 0.0054	0.9588	66.99 ± 3.452	0.000787 ± 0.000215	0.9592
n-BC-WL-550	74.38 ± 3.695	-0.0674 ± 0.0141	0.9417	76.16 ± 2.848	0.00182 ± 0.000523	0.9689
b-BC-WL-650	60.07 ± 2.947	-0.0339 ± 0.0055	0.9519	62.00 ± 3.243	0.000779 ± 0.000212	0.9588
n-BC-WL-650	73.08 ± 2.868	-0.0783 ± 0.0144	0.9624	74.83 ± 2.082	0.00233 ± 0.000564	0.9825
b-BC-WL-750	57.75 ± 3.962	-0.0195 ± 0.0049	0.9269	60.98 ± 4.688	0.000372 ± 0.000142	0.9358
n-BC-WL-750	69.72 ± 6.369	-0.0314 ± 0.0095	0.8646	72.39 ± 7.707	0.000566 ± 0.000305	0.8625
b-BC-SSL1-550	66.47 ± 3.061	-0.0552 ± 0.0095	0.9508	67.86 ± 2.449	0.00154 ± 0.000373	0.9717
n-BC-SSL1-550	67.46 ± 3.299	-0.0606 ± 0.0116	0.9435	68.92 ± 2.185	0.00175 ± 0.000397	0.9776
b-BC-SSL2-500	57.29 ± 4.326	-0.0458 ± 0.0120	0.8774	58.83 ± 3.838	0.00132 ± 0.000501	0.9146
n-BC-SSL2-500	69.64 ± 2.969	-0.0505 ± 0.0078	0.9582	71.12 ± 2.389	0.00128 ± 0.000271	0.9758
b-BC-SSL3-500	62.74 ± 2.519	-0.0542 ± 0.0081	0.9624	63.91 ± 2.07	0.00162 ± 0.000348	0.9771

Zn

n-BC-SSL3-500	66.79 ± 2.569	-0.0481 ± 0.0065	0.9659	68.23 ± 1.789	0.00124 ± 0.000223	0.9852
b-BC-SSL3CO2-500	52.64 ± 1.936	-0.0575 ± 0.0081	0.9684	53.70 ± 1.688	0.00207 ± 0.000446	0.9783
n-BC-SSL3CO2-500	71.39 ± 2.463	-0.0643 ± 0.009	0.9717	72.90 ± 2.047	0.00184 ± 0.000374	0.9824
b-BC-RH-550	42.95 ± 2.899	-0.0069 ± 0.0024	0.9493	46.77 ± 2.426	0.000195 ± 0.0000561	0.9762
n-BC-RH-550	71.56 ± 2.777	-0.0579 ± 0.0086	0.9652	73.20 ± 2.640	0.00149 ± 0.000365	0.9717
b-BC-OSR-550	44.84 ± 2.601	-0.0099 ± 0.0029	0.9577	48.93 ± 2.899	0.000225 ± 0.0000723	0.9662
n-BC-OSR-550	61.88 ± 2.759	-0.0413 ± 0.0062	0.9571	63.54 ± 2.747	0.00103 ± 0.000248	0.9628
b-BC-WL-450	66.97 ± 1.353	-0.0227 ± 0.0016	0.9923	69.64 ± 1.614	0.000423 ± 0.0000485	0.9932
n-BC-WL-450	275.6 ± 10.34	-0.0065 ± 0.0013	0.9844	299.8 ± 4.844	0.0000288 ± 0.00000258	0.9977
b-BC-WL-550	55.65 ± 1.325	-0.0676 ± 0.0068	0.9645	56.67 ± 2.282	0.00251 ± 0.000786	0.9867
n-BC-WL-550	252.5 ± 18.90	-0.0216 ± 0.0057	0.9041	268.4 ± 17.11	0.0000908 ± 0.0000287	0.9400
b-BC-WL-650	61.53 ± 1.941	-0.0485 ± 0.0054	0.9782	62.81 ± 3.053	0.00134 ± 0.000395	0.9885
n-BC-WL-650	245.3 ± 11.96	-0.0131 ± 0.0028	0.9643	265.6 ± 9.052	0.0000533 ± 0.00000953	0.9863
b-BC-WL-750	73.78 ± 3.188	-0.0288 ± 0.0041	0.9425	76.27 ± 3.899	0.000514 ± 0.000133	0.9557
n-BC-WL-750	257.1 ± 18.19	-0.0154 ± 0.0044	0.9222	279.8 ± 14.33	0.0000553 ± 0.0000147	0.9671
b-BC-SSL1-550	155.7 ± 4.907	-0.0981 ± 0.0178	0.9743	159.6 ± 3.232	0.00153 ± 0.000335	0.9906
n-BC-SSL1-550	410.7 ± 23.75	-0.0232 ± 0.0046	0.9391	430.7 ± 22.09	0.0000663 ± 0.0000168	0.9585
b-BC-SSL2-500	138.3 ± 3.6245	-0.1455 ± 0.0353	0.9798	140.9 ± 3.764	0.00365 ± 0.00184	0.9839
n-BC-SSL2-500	399.2 ± 19.85	-0.0208 ± 0.0037	0.9566	420.4 ± 18.78	0.0000593 ± 0.0000131	0.9752
b-BC-SSL3-500	141.1 ± 5.693	-0.0990 ± 0.0233	0.9581	145.1 ± 3.867	0.00165 ± 0.000466	0.9838
n-BC-SSL3-500	334.9 ± 14.52	-0.02 ± 0.0031	0.9665	353.8 ± 10.20	0.0000668 ± 0.00000956	0.9875
b-BC-SSL3CO2-500	134.7 ± 4.606	-0.0737 ± 0.0113	0.9715	137.6 ± 2.867	0.00119 ± 0.000208	0.9901
n-BC-SSL3CO2-500	350.5 ± 11.32	-0.0265 ± 0.0029	0.9793	362.6 ± 10.57	0.0000995 ± 0.0000145	0.9852
b-BC-RH-550	161.3 ± 4.845	-0.0719 ± 0.0095	0.9779	164.6 ± 1.996	0.000964 ± 0.0000957	0.9966
n-BC-RH-550	292.7 ± 18.25	-0.0397 ± 0.0083	0.9167	301.1 ± 15.84	0.000206 ± 0.0000597	0.9453
b-BC-OSR-550	138.6 ± 4.268	-0.0793 ± 0.0116	0.9765	141.7 ± 2.552	0.00127 ± 0.000204	0.9926
n-BC-OSR-550	229.25 ± 13.13	-0.0463 ± 0.0092	0.9282	235.1 ± 12.23	0.000332 ± 0.000101	0.9448

Pb

Where, b-BC- bulk biochar, n-BC-nano-biochar, WL-willow, SSL 1-3-different sewage sludges, RH-rice husk, OSR-oilseed rape, 450,500,550,650,750-temperature of pyrolysis; CO2- change from N₂ to CO₂ as carrier gas.

n-BC-WL-750	0	1.486	1	-102.9	28.06	0.9876	52.44	0.4878	0.9333	63.06	0.1812	1
b-BC-SSL1-550	0	11.13	1	25.25	4.613	0.3887	53.74	0.4461	0.9961	75.65	-0.137	1
n-BC-SSL1-550	0	12.51	1	41.2	sty.87	0.2682	53.29	0.454	0.9194	63.08	0.1683	1
b-BC-SSL2-500	0	9.111	1	25.35	2.564	0.3854	36.63	0.6947	0.9377	68.48	-0.1078	1
n-BC-SSL2-500	0	10.94	1	20.39	5.676	0.4783	55.66	0.441	0.9505	68.19	0.0901	1
b-BC-SSL3-500	0	10.32	1	21.68	4.722	0.416	53.34	0.3224	0.6882	67.08	-0.0841	1
n-BC-SSL3-500	0	10.30	1	19.96	5.148	0.6526	50.45	0.5147	0.9227	65.84	0.07855	1
b-BC-SSL3CO2-500	0	8.617	1	13.75	5.067	0.542	45.60	0.2158	0.8562	49.41	0.09736	1
n-BC-SSL3CO2-500	0	12.36	1	21.99	6.684	0.6724	61.30	0.3195	0.9988	69.00	0.1141	1
b-BC-RH-550	0	0.6242	1	-20.64	5.954	0.7289	13.11	0.8647	0.9343	40.62	0.0925	1
n-BC-RH-550	0	11.27	1	9.721	8.761	0.993	55.11	0.4934	0.9891	65.7	0.225	1
b-BC-OSR-550	0	0.7697	1	-21.66	6.361	0.6002	14.23	0.7893	0.9959	31.78	0.3164	1
n-BC-OSR-550	0	7.567	1	-5.28	sie.93	0.739	45.56	0.5232	0.9996	66.77	-0.0333	1
b-BC-WL-450	0	5.453	1	-6.193	7.052	0.7979	37.63	0.8934	0.6739	63.40	0.08675	1
n-BC-WL-450	0	8.871	1	-30.62	16.78	0.7394	64.82	6.087	0.8531	245.3	0.8115	1
b-BC-WL-550	0	8.636	1	-5.854	10.15	0.9365	51.44	0.1067	0.9839	52.19	0.08983	1
n-BC-WL-550	0	19.60	1	-35.66	28.30	0.6541	114.4	4.363	1	279.4	0.01672	1
b-BC-WL-650	0	7.203	1	-23.54	13.28	0.9043	54.17	0.2215	0.5539	62.53	-0.03962	1
n-BC-WL-650	0	13.25	1	-17.86	17.86	0.4786	88.34	4.899	0.8995	268.9	-0.1962	1
b-BC-WL-750	0	5.576	1	-33.25	14.16	0.7351	52.87	0.6316	0.7361	70.98	0.07669	1
n-BC-WL-750	0	16.36	1	-10.38	19.40	0.8874	78.13	5.622	0.9802	289.2	-0.1043	1
b-BC-SSL1-550	0	33.08	1	103.00	6.491	0.5445	143.3	0.4012	0.9044	134.8	0.5965	1
n-BC-SSL1-550	0	34.84	1	-23.83	41.00	0.4013	229.9	5.622	0.9532	420.4	0.3466	1
b-BC-SSL2-500	0	32.22	1	107.1	4.563	0.1716	143.9	0.0931	0.2011	142.3	-0.0884	1
n-BC-SSL2-500	0	27.sie	1	-80.57	48.61	0.5434	219.2	5.422	0.8796	363.9	1.196	1
b-BC-SSL3-500	0	30.92	1	114.1	1.451	0.03164	131.5	0.4374	0.5478	144.9	0.002863	1
n-BC-SSL3-500	0	26.cze	1	-0.7694	26.80	0.7465	153.5	5.461	0.872	296.9	1.254	1
b-BC-SSL3CO2-500	0	25.94	1	72.21	7.294	0.4903	120.3	0.5308	0.6598	140.8	-0.0872	1
n-BC-SSL3CO2-500	0	34.41	1	7.440	32.49	0.581	214.5	4.208	0.75	349.2	0.1611	1
b-BC-RH-550	0	30.75	1	84.80	8.855	0.9066	138.6	0.7964	0.6086	167.0	-0.0851	1
n-BC-RH-550	0	40.84	1	77.54	20.82	0.5338	188.6	3.327	0.9944	311.1	0.1508	1
b-BC-OSR-550	0	27.lis	1	72.09	8.50	0.6763	122.1	0.5823	0.9666	141.8	0.0411	1

Pb

n-BC-OSR-550 0 32.76 1 32.74 24.31 0.5299 172.7 1.791 0.9473 234.0 0.2600 1

Where, b-BC- bulk biochar, n-BC-nano-biochar,WL-willow, SSL 1-3-different sewage sludges, RH-rice husk, OSR-oilseed rape, 450,500,550,650,750-temperature of pyrolysis; CO2- change from N₂ to CO₂ as carrier gas.

Table S5. The isotherm parameters simulated by different equations.

Metal	Biochar	Freundlich					Langmuir				
		linear		non-linear		K_F [mg/g]	linear		non-linear		R^2
		$1/n$	R^2	$1/n$	R^2		K_L [L/mg]	Q_{max} [mg/g]	K_L [L/mg]	Q_{max} [mg/g]	
Cd	b-BC-WL-450	85.42	0.0178	0.0184	0.9084	85.26	0.0527	92.54	80.24	67.01	
		±	±	±	0.9119	±	±	±	±	±	
		7.3875	0.0014	0.0013	0.0048	6.7274	0.0048	8.1762	7.0082	3.9583	0.9524
	n-BC-WL-450	80.97	0.0112	0.0115	0.8741	80.9	0.6232	87.94	140.8	85.09	
		±	±	±	0.8790	±	±	±	±	±	
		6.7299	0.001	0.001	0.0441	7.0886	0.0441	6.7059	13.9457	5.4929	0.9735
	b-BC-WL-550	85.48	0.0188	0.019	0.7462	85.43	0.3465	93.13	78.0	87.88	
		±	±	±	0.7470	±	±	±	±	±	
		7.6737	0.0015	0.0014	0.0301	6.563	0.0301	6.4612	6.9328	7.5802	0.9751
	n-BC-WL-550	77.85	0.0169	0.0178	0.9882	77.6	0.1763	87.55	16.28	83.99	
		±	±	±	0.9888	±	±	±	±	±	
		5.8126	0.0014	0.0015	0.0126	6.4659	0.0126	7.9379	1.217	5.9042	0.9631
Cd	b-BC-WL-650	87.1	0.0137	0.0139	0.6143	87.06	0.118	92.67	115.4	90.86	
		±	±	±	0.6129	±	±	±	±	±	
		7.4672	0.0012	0.001	0.0096	6.1977	0.0096	8.2016	10.1993	5.9593	0.9692
	n-BC-WL-650	43.65	0.0438	0.0442	0.8798	43.56	0.1417	58.13	0.444	55.43	
		±	±	±	0.8813	±	±	±	±	±	
		3.0393	0.0035	0.0039	0.0134	2.5796	0.0134	4.7738	0.0304	4.2395	0.9685
	b-BC-WL-750	84.37	0.0181	0.0185	0.9579	84.26	0.039	89.46	91.54	58.12	
		±	±	±	0.9563	±	±	±	±	±	
		6.8413	0.0012	0.0012	0.0029	7.1099	0.0029	6.0598	7.5784	4.3073	0.9727
	n-BC-WL-750	59.11	0.0215	0.0215	0.9238	59.1	0.4141	67.6	1.679	66.03	
		±	±	±	0.9273	±	±	±	±	±	
		4.7596	0.0013	0.0018	0.0359	4.9847	0.0359	5.2989	0.1574	5.2538	0.9705
b-BC-SSL1-550		77.97	0.0291	0.0309	0.9618	77.45	0.0943	88.97	9.11	66.67	
		±	±	±	0.9609	±	±	±	±	±	
		7.3487	0.0025	0.0027	0.0084	5.2409	0.0084	5.9901	0.7893	5.5896	0.9692

n-BC-SSL1-550	62.08	0.0332	58.38	0.0506	0.1422	78.77	0.916	75.03
	±	±	±	±	±	±	±	±
b-BC-SSL2-500	5.4053	0.0021	0.7476	0.0042	0.7612	5.3183	0.9998	0.9656
	±	±	±	±	±	±	±	±
n-BC-SSL2-500	63.78	0.0463	63.5	0.0474	0.1059	80.42	1.034	78.64
	±	±	±	±	±	±	±	±
b-BC-SSL3-500	4.9637	0.0037	5.7419	0.0034	0.0098	5.6203	0.0969	6.7083
	±	±	±	±	±	±	±	±
n-BC-SSL3-500	68.74	0.0397	68.15	0.0418	0.1268	91.38	1.601	83.36
	±	±	±	±	±	±	±	±
b-BC-SSL3-500	4.5971	0.0027	5.3603	0.0039	0.0099	7.9783	0.1275	6.688
	±	±	±	±	±	±	±	±
n-BC-SSL3CO2-500	66.91	0.0353	66.8	0.0357	0.1364	91.79	1.311	79.9
	±	±	±	±	±	±	±	±
b-BC-SSL3CO2-500	6.4568	0.0021	4.6133	0.0033	0.0126	7.0189	0.116	7.1454
	±	±	±	±	±	±	±	±
n-BC-SSL3CO2-500	65.27	0.0297	65.24	0.0298	0.3388	77.83	1.339	75.94
	±	±	±	±	±	±	±	±
b-BC-RH-550	4.564	0.0025	3.6215	0.0025	0.0302	4.816	0.1059	5.3915
	±	±	±	±	±	±	±	±
n-BC-RH-550	68.62	0.0275	68.66	0.0274	0.2787	78.39	1.555	78.94
	±	±	±	±	±	±	±	±
b-BC-OSR-550	5.7375	0.002	5.4237	0.0022	0.0236	6.9745	0.1325	6.8982
	±	±	±	±	±	±	±	±
n-BC-OSR-550	21.98	0.1563	22.27	0.1539	0.0461	57.82	273.4	45.97
	±	±	±	±	±	±	±	±
b-BC-WL-450	1.5604	0.0137	2.039	0.0137	0.0033	3.0821	20.7869	2.8286
	±	±	±	±	±	±	±	±
n-BC-WL-450	82.46	0.009	82.43	0.0092	0.0206	85.86	1590.0	58.73
	±	±	±	±	±	±	±	±
b-BC-SSL1-550	7.4997	0.0005	6.721	0.0009	0.0016	7.973	11.1328	5.5348
	±	±	±	±	±	±	±	±
n-BC-SSL1-550	26.31	0.1264	26.53	0.1249	0.060	57.4	0.076	55.76
	±	±	±	±	±	±	±	±
b-BC-OSR-550	2.0548	0.0107	2.2293	0.011	0.0043	3.3086	0.0066	4.4054
	±	±	±	±	±	±	±	±
n-BC-OSR-550	69.11	0.0235	69.0	0.024	0.0242	77.25	4.688	58.16
	±	±	±	±	±	±	±	±
b-BC-WL-450	5.2484	0.0015	5.9295	0.0019	0.0023	6.4265	0.4362	3.9579
	±	±	±	±	±	±	±	±
n-BC-WL-450	29.31	0.0763	29.17	0.0774	0.0721	48.32	0.1464	45.7
	±	±	±	±	±	±	±	±
b-BC-SSL2-500	2.1196	0.006	2.2991	0.007	0.005	3.831	0.0115	3.4289
	±	±	±	±	±	±	±	±
n-BC-SSL2-500	51.5	0.0437	51.13	0.0454	0.1168	69.81	0.7539	64.47
	±	±	±	±	±	±	±	±
b-BC-SSL3-500	4.4431	0.0038	3.8343	0.0045	0.0104	5.5556	0.0697	5.0889
	±	±	±	±	±	±	±	±
n-BC-SSL3-500	45.88	0.0418	45.89	0.0417	0.2074	59.34	0.3787	57.94
	±	±	±	±	±	±	±	±
b-BC-SSL3-500	2.8312	0.0036	3.2648	0.0036	0.0131	4.3425	0.0298	4.121
	±	±	±	±	±	±	±	±
n-BC-SSL3-500	62.08	0.0332	58.38	0.0506	0.1422	78.77	0.916	75.03
	±	±	±	±	±	±	±	±
b-BC-SSL2-500	5.4053	0.0021	0.7476	0.0042	0.7612	5.3183	0.9998	0.9656
	±	±	±	±	±	±	±	±
n-BC-SSL2-500	63.78	0.0463	63.5	0.0474	0.1059	80.42	1.034	78.64
	±	±	±	±	±	±	±	±
b-BC-SSL3-500	4.9637	0.0037	5.7419	0.0034	0.0098	5.6203	0.0969	6.7083
	±	±	±	±	±	±	±	±
n-BC-SSL3-500	68.74	0.0397	68.15	0.0418	0.1268	91.38	1.601	83.36
	±	±	±	±	±	±	±	±
b-BC-SSL3-500	4.5971	0.0027	5.3603	0.0039	0.0099	7.9783	0.1275	6.688
	±	±	±	±	±	±	±	±
n-BC-SSL3CO2-500	66.91	0.0353	66.8	0.0357	0.1364	91.79	1.311	79.9
	±	±	±	±	±	±	±	±
b-BC-SSL3CO2-500	6.4568	0.0021	4.6133	0.0033	0.0126	7.0189	0.116	7.1454
	±	±	±	±	±	±	±	±
n-BC-SSL3CO2-500	65.27	0.0297	65.24	0.0298	0.3388	77.83	1.339	75.94
	±	±	±	±	±	±	±	±
b-BC-RH-550	4.564	0.0025	3.6215	0.0025	0.0302	4.816	0.1059	5.3915
	±	±	±	±	±	±	±	±
n-BC-RH-550	68.62	0.0275	68.66	0.0274	0.2787	78.39	1.555	78.94
	±	±	±	±	±	±	±	±
b-BC-RH-550	5.7375	0.002	5.4237	0.0022	0.0236	6.9745	0.1325	6.8982
	±	±	±	±	±	±	±	±
n-BC-RH-550	21.98	0.1563	22.27	0.1539	0.0461	57.82	273.4	45.97
	±	±	±	±	±	±	±	±
b-BC-OSR-550	1.5604	0.0137	2.039	0.0137	0.0033	3.0821	20.7869	2.8286
	±	±	±	±	±	±	±	±
n-BC-OSR-550	82.46	0.009	82.43	0.0092	0.0206	85.86	1590.0	58.73
	±	±	±	±	±	±	±	±
b-BC-WL-450	7.4997	0.0005	6.721	0.0009	0.0016	7.973	11.1328	5.5348
	±	±	±	±	±	±	±	±
n-BC-WL-450	26.31	0.1264	26.53	0.1249	0.060	57.4	0.076	55.76
	±	±	±	±	±	±	±	±
b-BC-SSL1-550	2.0548	0.0107	2.2293	0.011	0.0043	3.3086	0.0066	4.4054
	±	±	±	±	±	±	±	±
n-BC-SSL1-550	69.11	0.0235	69.0	0.024	0.0242	77.25	4.688	58.16
	±	±	±	±	±	±	±	±
b-BC-SSL2-500	5.2484	0.0015	5.9295	0.0019	0.0023	6.4265	0.4362	3.9579
	±	±	±	±	±	±	±	±
n-BC-SSL2-500	29.31	0.0763	29.17	0.0774	0.0721	48.32	0.1464	45.7
	±	±	±	±	±	±	±	±
b-BC-SSL3-500	2.1196	0.006	2.2991	0.007	0.005	3.831	0.0115	3.4289
	±	±	±	±	±	±	±	±
n-BC-SSL3-500	51.5	0.0437	51.13	0.0454	0.1168	69.81	0.7539	64.47
	±	±	±	±	±	±	±	±
b-BC-SSL3-500	4.4431	0.0038	3.8343	0.0045	0.0104	5.5556	0.0697	5.0889
	±	±	±	±	±	±	±	±
n-BC-SSL3-500	45.88	0.0418	45.89	0.0417	0.2074	59.34	0.3787	57.94
	±	±	±	±	±	±	±	±
b-BC-SSL3-500	2.8312	0.0036	3.2648	0.0036	0.0131	4.3425	0.0298	4.121
	±	±	±	±	±	±	±	±

Zn

b-BC-WL-550	74.56	0.0254	74.45	0.0258	0.8930	±	0.8940	0.3157	88.02	5.9225	84.02
	±	±	±	±	0.8930	±	0.8940	±	±	±	±
	6.0403	0.0022	6.5499	0.0024	0.0246	5.533	0.0246	5.533	0.5243	6.2129	0.9712
n-BC-WL-550	57.53	0.0253	57.63	0.0249	0.9398	±	0.9400	0.4779	66.77	0.8473	66.04
	±	±	±	±	0.9398	±	0.9400	±	±	±	±
	4.109	0.0015	4.9249	0.0019	0.0347	6.468	0.0347	6.468	0.0574	5.4466	0.9644
b-BC-WL-650	66.98	0.0294	66.83	0.030	0.8880	±	0.8853	0.3115	80.72	1.9586	77.4
	±	±	±	±	0.8880	±	0.8853	±	±	±	±
	3.9223	0.0017	5.6091	0.0027	0.0293	6.7731	0.0293	6.7731	0.178	4.2634	0.9683
n-BC-WL-650	57.69	0.0228	57.66	0.0229	0.8082	±	0.8158	0.3709	66.58	1.1111	65.09
	±	±	±	±	0.8082	±	0.8158	±	±	±	±
	3.6528	0.0016	4.961	0.0021	0.0289	4.5109	0.0289	4.5109	0.0826	6.0506	0.9695
b-BC-WL-750	63.21	0.0454	62.94	0.0464	0.9337	±	0.9345	0.1619	84.91	0.8842	79.58
	±	±	±	±	0.9337	±	0.9345	±	±	±	±
	4.9053	0.0035	4.8518	0.0041	0.0141	6.7984	0.0141	6.7984	0.0703	6.7263	0.9633
n-BC-WL-750	61.38	0.0425	60.76	0.045	0.9508	±	0.9535	0.1078	82.95	1.0761	75.88
	±	±	±	±	0.9508	±	0.9535	±	±	±	±
	5.347	0.0032	4.3098	0.004	0.0082	6.8817	0.0082	6.8817	0.0753	4.7514	0.9676
b-BC-SSL1-550	72.44	0.0192	72.35	0.0195	0.8151	±	0.8218	0.2769	83.54	3.4944	79.54
	±	±	±	±	0.8151	±	0.8218	±	±	±	±
	6.2227	0.0017	6.704	0.0016	0.0258	5.8432	0.0258	5.8432	0.2476	4.5339	0.9682
n-BC-SSL1-550	55.26	0.058	55.3	0.0579	0.9012	±	0.8979	0.1763	78.59	0.4499	75.15
	±	±	±	±	0.9012	±	0.8979	±	±	±	±
	4.172	0.0052	4.0957	0.0042	0.0157	5.2614	0.0157	5.2614	0.0393	4.8081	0.9678
b-BC-SSL2-500	81.57	0.0127	81.46	0.0131	0.9246	±	0.9709	0.4363	89.8	28.67	86.32
	±	±	±	±	0.9246	±	0.9709	±	±	±	±
	7.7472	0.0009	4.74	0.0009	0.034	7.5352	0.034	7.5352	2.7676	5.8584	0.9689
n-BC-SSL2-500	55.08	0.0516	54.39	0.0545	0.6828	±	0.9883	0.092	79.34	0.5916	71.92
	±	±	±	±	0.6828	±	0.9883	±	±	±	±
	3.7926	0.0034	4.5605	0.0041	0.0067	5.0121	0.0067	5.0121	0.0468	4.2848	0.9710
b-BC-SSL3-500	66.13	0.0339	65.85	0.0349	0.8358	±	0.8452	0.189	91.94	1.5162	78.19
	±	±	±	±	0.8358	±	0.8452	±	±	±	±
	4.7721	0.0025	3.8082	0.0026	0.0168	6.2307	0.0168	6.2307	0.1349	7.3385	0.9691
n-BC-SSL3-500	48.19	0.0493	47.95	0.0504	0.8898	±	0.8961	0.122	66.82	0.4509	62.78
	±	±	±	±	0.8898	±	0.8961	±	±	±	±
	4.6352	0.0039	2.5018	0.0042	0.0112	3.8292	0.0112	3.8292	0.0354	5.1455	0.9724
b-BC-SSL3CO2-500	65.25	0.0486	64.51	0.0513	0.9522	±	0.9541	0.1046	83.12	0.9313	83.17
	±	±	±	±	0.9522	±	0.9541	±	±	±	±
	5.1527	0.0043	5.572	0.0048	0.0095	4.6121	0.0095	4.6121	0.0825	6.4783	0.9699

n-BC-SSL3CO2-500	39.9	0.0654	39.72	0.0664	0.0959	60.94	0.2333	57.56
	±	±	±	±	±	±	±	±
	2.8497	0.0055	2.0283	0.0052	0.0077	4.0959	0.017	3.7262
								0.9700
b-BC-RH-550	69.85	0.0299	69.67	0.0305	0.2169	85.44	2.0811	80.88
	±	±	±	±	±	±	±	±
	6.257	0.0016	5.8985	0.0022	0.0189	6.8428	0.1519	5.3731
	32.38	0.085	32.14	0.0869	0.0658	56.62	0.1464	52.83
n-BC-RH-550	±	±	±	±	±	±	±	±
	2.6221	0.0048	2.6494	0.0067	0.005	3.8801	0.0129	3.2031
								0.9775
b-BC-OSR-550	59.05	0.0319	59.02	0.0321	0.2657	72.16	0.8987	69.82
	±	±	±	±	±	±	±	±
	3.7151	0.0027	4.2393	0.0027	0.0219	5.3878	0.0827	5.877
	48.32	0.0771	48.51	0.0764	0.0418	83.21	0.0644	80.42
n-BC-OSR-550	±	±	±	±	±	±	±	±
	3.8574	0.0044	4.132	0.0065	0.0032	6.534	0.0049	5.4649
								0.9700
b-BC-WL-450	256.4	0.0096	256.5	0.0096	0.1416	276.4	0.3748	274.8
	±	±	±	±	±	±	±	±
	5.8403	0.0007	3.3576	0.0008	0.0122	7.9976	0.0268	7.5064
	42.59	0.0776	42.97	0.0761	0.048	72.73	0.0593	71.51
n-BC-WL-450	±	±	±	±	±	±	±	±
	3.5105	0.0067	3.0354	0.005	0.0042	1.5887	0.0054	1.8946
								0.9734
b-BC-WL-550	283.8	0.0121	282.9	0.0125	0.0755	314.3	0.4451	308.6
	±	±	±	±	±	±	±	±
	5.1871	0.0008	2.4715	0.0009	0.0061	8.0884	0.0424	7.4139
	49.37	0.0683	49.85	0.0666	0.0559	78.96	0.0714	77.73
n-BC-WL-550	±	±	±	±	±	±	±	±
	3.868	0.0053	3.9291	0.0061	0.0046	2.1212	0.0059	2.0701
								0.9633
b-BC-WL-650	255.2	0.0142	255.0	0.0143	0.0909	286.0	0.3508	281.8
	±	±	±	±	±	±	±	±
	3.8137	0.0008	2.428	0.001	0.0061	8.1103	0.0344	6.6337
	58.97	0.0643	59.16	0.0637	0.0628	91.7	0.0891	89.6
n-BC-WL-650	±	±	±	±	±	±	±	±
	4.7424	0.0052	4.0277	0.0052	0.0055	2.0229	0.0073	2.1383
								0.9676
b-BC-WL-750	255.5	0.023	255.0	0.0234	0.0525	307.7	0.2275	300.0
	±	±	±	±	±	±	±	±
	9.122	0.0013	1.3124	0.0019	0.0041	8.055	0.0188	7.8937
	158.2	0.0094	158.1	0.0095	0.6018	168.9	5.8259	166.4
n-BC-WL-750	±	±	±	±	±	±	±	±
	3.975	0.0007	1.820	0.0007	0.0526	3.9107	0.5626	4.358
								0.9691

Pb

b-BC-SSL1-550	359.2	0.0236		359.0	0.0237		0.0702	431.9		0.9066	420.4
	±	±	0.5297	±	±	0.5327	±	±	1.0000	±	±
	2.1708	0.0019		2.4931	0.0017		0.0048	6.4132		0.0741	6.4413
n-BC-SSL1-550	131.3	0.0221		131.3	0.022		0.3003	151.9		0.8613	149.2
	±	±	0.9821	±	±	0.9808	±	±	0.9997	±	±
	1.0303	0.0018		1.4377	0.0017		0.0288	4.4637		0.0633	3.5106
b-BC-SSL2-500	363.5	0.0132		363.0	0.0135		0.0835	406.5		1.5996	396.6
	±	±	0.8360	±	±	0.9625	±	±	1.0000	±	±
	9.3462	0.001		4.1939	0.0011		0.0067	7.734		0.1276	5.1378
n-BC-SSL2-500	127.9	0.030		128.3	0.0294		0.2798	155.0		0.5658	152.5
	±	±	0.8054	±	±	0.9731	±	±	0.9999	±	±
	1.7718	0.0025		1.6221	0.0024		0.0212	4.012		0.0515	4.3435
b-BC-SSL3-500	360.5	0.0085		360.5	0.0085		0.2478	384.5		1.3943	381.9
	±	±	0.8843	±	±	0.8798	±	±	1.0000	±	±
	2.8658	0.0006		3.8113	0.0008		0.0179	8.8396		0.1272	8.6767
n-BC-SSL3-500	120.7	0.0234		120.2	0.0242		0.1009	145.5		0.9259	137.7
	±	±	0.9990	±	±	0.9989	±	±	1.0000	±	±
	1.3654	0.002		8.6061	0.002		0.0078	3.3201		0.0774	3.6801
b-BC-SSL3CO2-500	300.2	0.0158		299.7	0.0161		0.066	342.6		0.5206	334.3
	±	±	0.7062	±	±	0.7186	±	±	0.9997	±	±
	3.7112	0.0013		2.8943	0.0013		0.006	9.1724		0.0386	8.296
n-BC-SSL3CO2-500	148.4	0.021		148.2	0.0213		0.1549	173.8		2.5773	166.2
	±	±	0.8021	±	±	0.8060	±	±	0.9998	±	±
	1.2265	0.002		1.5927	0.0019		0.0095	4.754		0.2017	4.6551
b-BC-RH-550	262.7	0.0148		262.2	0.0151		0.0551	298.8		0.4114	290.9
	±	±	0.8872	±	±	0.8889	±	±	0.9997	±	±
	4.2515	0.001		2.2564	0.0014		0.0045	7.2831		0.0303	7.5459
n-BC-RH-550	147.2	0.0122		147.0	0.0125		0.1821	162.9		4.0917	157.0
	±	±	0.6751	±	±	0.9999	±	±	0.9997	±	±
	3.5784	0.0008		1.7105	0.001		0.0147	3.8422		0.2911	4.1266
b-BC-OSR-550	259.4	0.0163		258.6	0.0167		0.0552	297.5		0.2888	290.6
	±	±	0.5019	±	±	0.5142	±	±	0.9997	±	±
	3.3808	0.0013		2.8186	0.0012		0.0047	7.1477		0.0203	7.407
n-BC-OSR-550	85.42	0.0178		85.26	0.0184		0.0527	92.54		80.24	67.01
	±	±	0.7636	±	±	0.7652	±	±	0.9997	±	±
	7.3875	0.0014		6.7274	0.0013		0.0048	8.1762		7.0082	3.9583

Where, b-BC- bulk biochar, n-BC-nano-biochar,WL-willow, SSL 1-3-different sewage sludges, RH-rice husk, OSR-oilseed rape, 450,500,550,650,750-temperature of pyrolysis; CO2- change from N₂ to CO₂ as carrier gas.

Table S6. Selected properties of studied metals.

Metal (Me)	Ionic radius [Å]	K_{sp} of Me(OH)₂	Normal potential [V]	Hydration energy [kJ/mol]
Cd	0.95	7.2·10 ⁻¹⁵	-0.40	-1750
Zn	0.74	3.0·10 ⁻¹⁷	-0.76	-2046
Pb	1.19	1.4·10 ⁻²⁰	-0.13	-1480

Table S7. The comparison of adsorption capacity of bulk biochars and nano-biochars on metals (Cd, Zn, Pb) based on Langmuir isotherm model.

Adsorbed metal	Feedstock used for BC production	Pyrolysis conditions	Adsorption capacity [mg/g]	References
Bulk biochar				
Cd	Rice hulk	350°C for 4 h, N ₂ atmosphere	21.7	(Xiang et al., 2018)
	Peanut husk	450 °C for 2 h, N ₂ atmosphere	29.0	(Cheng et al., 2016)
	Pinecone	350-400 °C, N ₂ atmosphere	92.7	(Teng et al., 2020)
	Willow	450-750°C for 3 h, N ₂ atmosphere	57.7-88.1	This work
	Sewage sludge	500-550°C for 3 h, N ₂ atmosphere	66.5-84.6	This work
	Sewage sludge	500°C for 3 h, CO ₂ atmosphere	77.9	This work
	Rice husk	550°C for 0.25 h, N ₂ atmosphere	55.7	This work
	Oilseed rape	550°C for 0.2 h, N ₂ atmosphere	55.7	This work
	Nano-biochar			
	Cow bone	300°C for 2 h, N ₂ atmosphere + ball-milling	66.3	(Xiao et al., 2020)
	Wood	n.a + disc mill grinding	922	(Ramanayaka et al., 2020)
	Willow	450-750°C for 3 h, N ₂ atmosphere + ball-milling	94.5-97.5	This work
	Sewage sludge	500-550°C for 3 h, N ₂ atmosphere + ball-milling	82.8-96.6	This work
	Sewage sludge	500°C for 3 h, CO ₂ atmosphere + ball-milling	80.9	This work
	Rice husk	550°C for 0.25 h, N ₂ atmosphere + ball-milling	88.2	This work

Oilseed rape	550°C for 0.2 h. N ₂ atmosphere + ball-milling	79.9	This work
Bulk biochar			
Wheat straw	650-700°C for 4h. N ₂ atmosphere	40.2	(Bogusz et al., 2015)
Melon seed shells	500-900°C for 1h. N ₂ atmosphere	53.2	(Cui et al., 2024)
Bamboo wood	550°C for 3h. oxygen limited atmosphere	7.62	(Van Hien et al., 2020)
Willow	450-750°C for 3h. N ₂ atmosphere	47.4-66.5	This work
Sewage sludge	500-550°C for 3 h. N ₂ atmosphere	77.7-82.9	This work
Sewage sludge	500°C for 3 h . CO ₂ atmosphere	66.1	This work
Rice husk	550°C for 0.25 h. N ₂ atmosphere	60.0	This work
Oilseed rape	550°C for 0.2 h. N ₂ atmosphere	55.4	This work
Nano-biochar			
Zn			
Willow	450-750°C for 3 h. N ₂ atmosphere + ball-milling	69.4-87.7	This work
Sewage sludge	500-550°C for 3 h. N ₂ atmosphere + ball-milling	82.8-89.4	This work
Sewage sludge	500°C for 3 h . CO ₂ atmosphere + ball-milling	81.1	This work
Rice husk	550°C for 0.25 h. N ₂ atmosphere + ball-milling	85.0	This work
Oilseed rape	550°C for 0.2 h. N ₂ atmosphere + ball-milling	71.8	This work
Bulk biochar			
Banana peel	600°C for 2 h. N ₂ atmosphere	247.1	(Ahmad et al., 2018)
Sewage sludge+CaSO₄ *	600°C for 2 h. oxygen limited atmosphere	125.7	(Wang et al., 2024)
Maize straw powder	500°C for 4h. N ₂ atmosphere	72.9	(Li et al., 2023)
Willow	450-750°C for 3h. N ₂ atmosphere	71.2-90.1	This work
Sewage sludge	500-550°C for 3 h. N ₂ atmosphere	151.4-168.6	This work
Sewage sludge	500°C for 3 h . CO ₂ atmosphere	144.6	This work
Rice husk	550°C for 0.25 h. N ₂ atmosphere	173.5	This work
Oilseed rape	550°C for 0.2 h. N ₂ atmosphere	162.7	This work
Nano-biochar			
Cowe bone	300°C for 2 h. N ₂ atmosphere + ball-milling	329.3	(Xiao et al., 2020)
Pb			

Wheat straw	600°C for 1 h. N ₂ atmosphere + ball-milling	134.7	(Cao et al., 2019)
Bovine bone+Fe ₃ O ₄ *	500°C for 2h. N ₂ atmosphere + ball-milling	339.9	(Qu et al., 2023)
Willow	450-750°C for 3 h. N ₂ atmosphere + ball-milling	276.8-313.7	This work
Sewage sludge	500-550°C for 3 h. N ₂ atmosphere + ball-milling	384.0-431.3	This work
Sewage sludge	500°C for 3 h . CO ₂ atmosphere + ball-milling	341.5	This work
Rice husk	550°C for 0.25 h. N ₂ atmosphere + ball-milling	297.5	This work
Oilseed rape	550°C for 0.2 h. N ₂ atmosphere + ball-milling	296.9	This work
*-modified biochar			

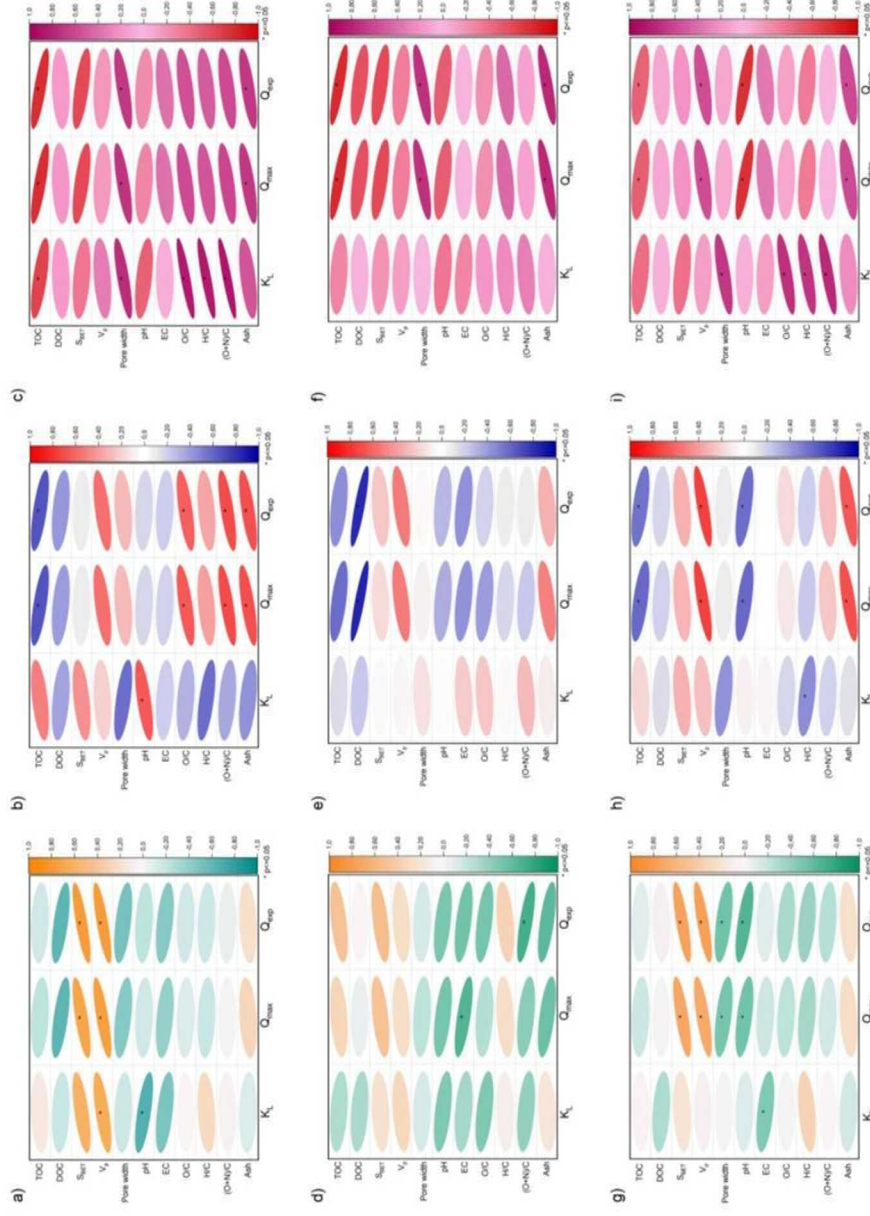


Figure S1. Correlation plot presenting the values of Pearson correlation factor between selected physicochemical properties and the Langmuir isotherm constants (K_L and Q_{max}) and the experimental Q_{exp} values calculated for b-BC (a) for Cd (II) ions and (c) Pb (II) ions, for n-BC (d) for Cd (II) ions (e) for Zn (II) ions and (f) Pb (II) ions and for b-BC and n-BC together g) for Cd (II) ions (h) for Zn (II) ions and (i) Pb (II) ions. The significant correlations were indicated by * $p \leq 0.05$.

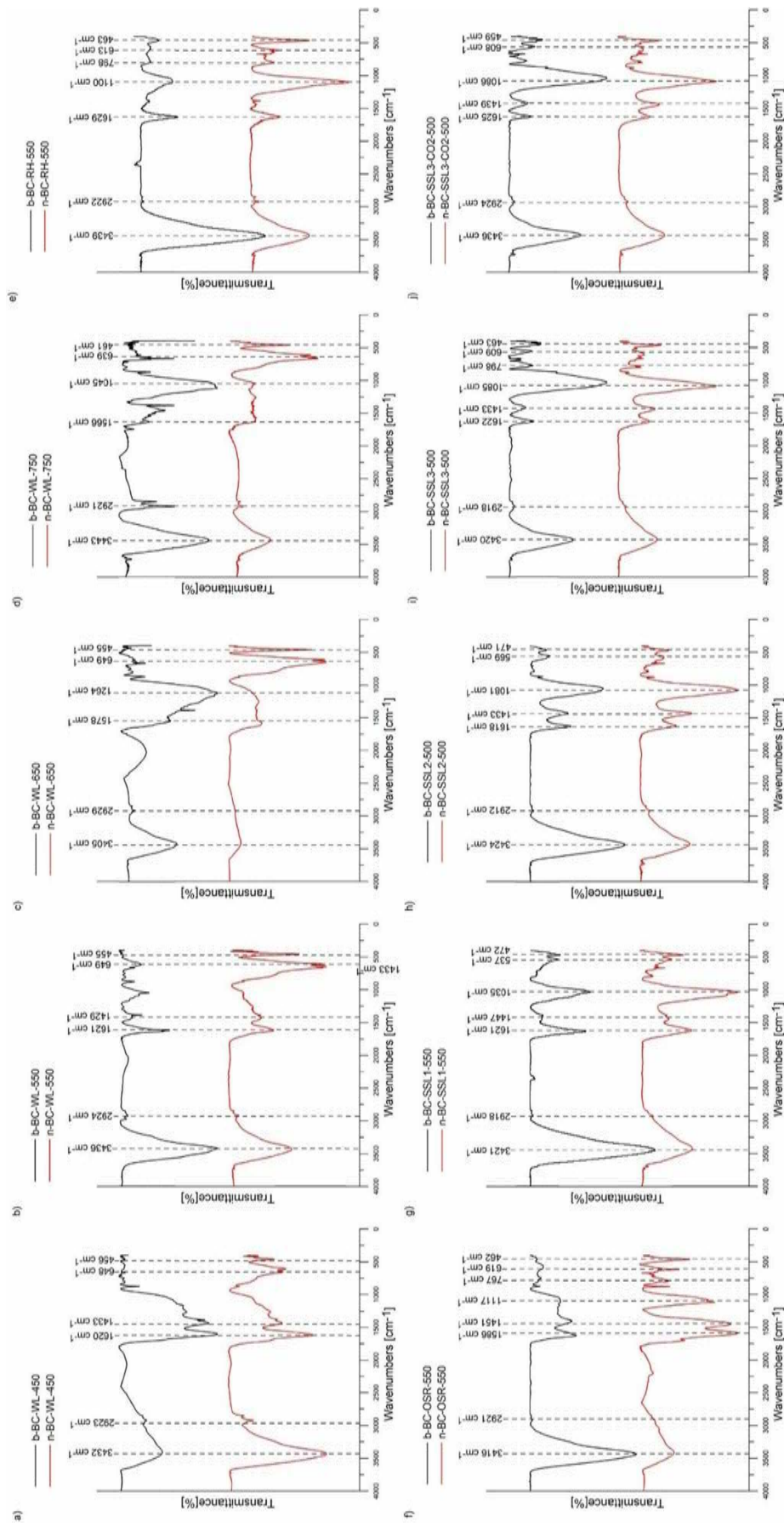


Figure S2. FT-IR spectra of obtained b-BC and n-BC.

Where, b-BC- bulk biochar, n-BC-nano-biochar, WL-willow, SSL 1-3-different sewage sludges, RH-rice husk, OSR-oilseed rape, 450,500,550,650,750-temperature of pyrolysis; CO₂- change from N₂ to CO₂ as carrier gas.

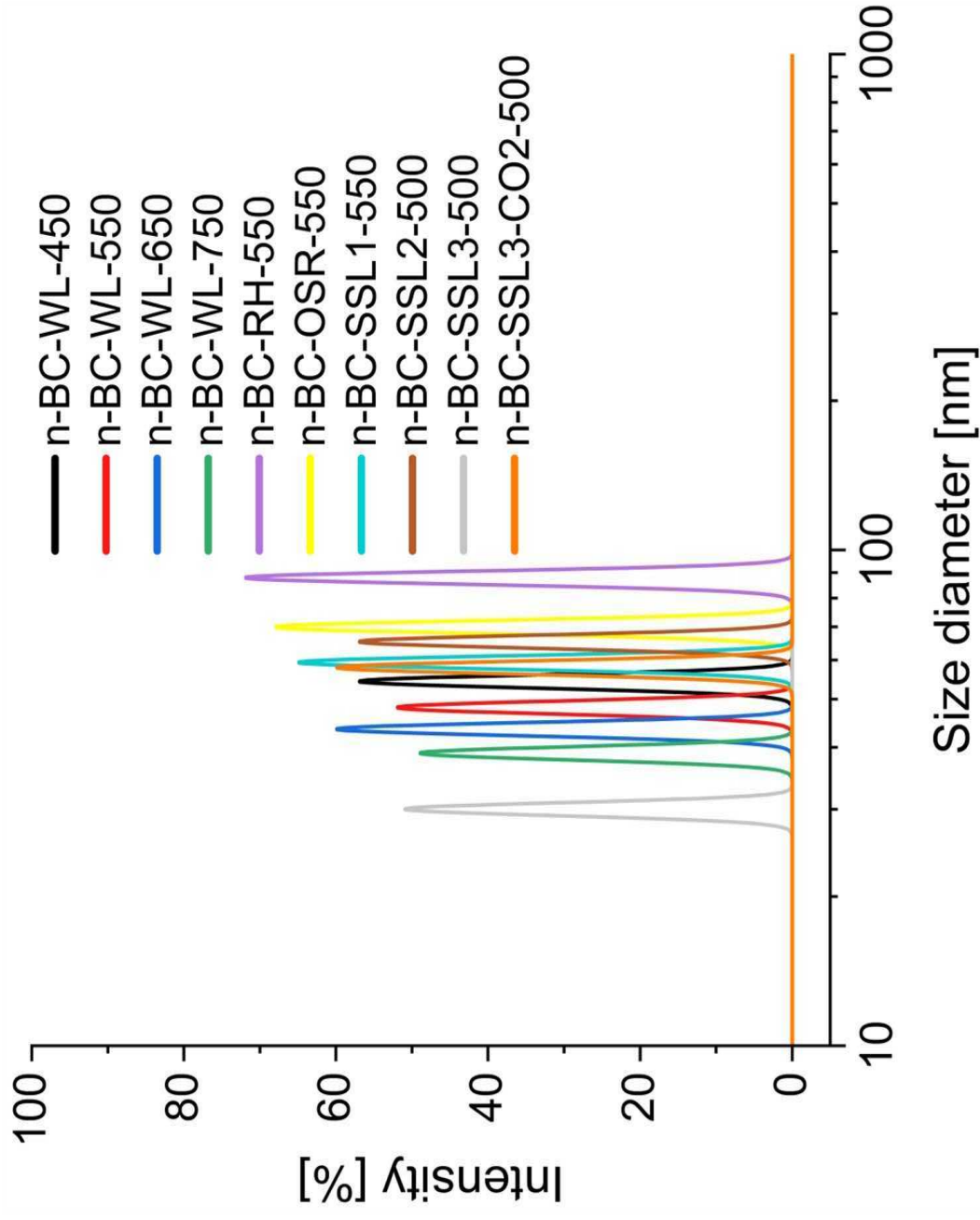
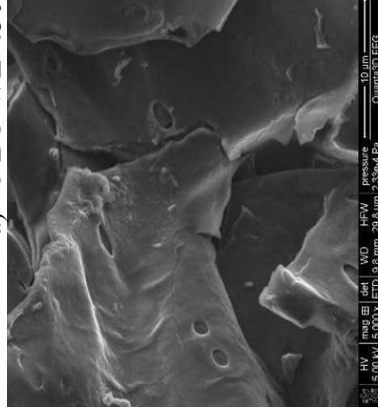


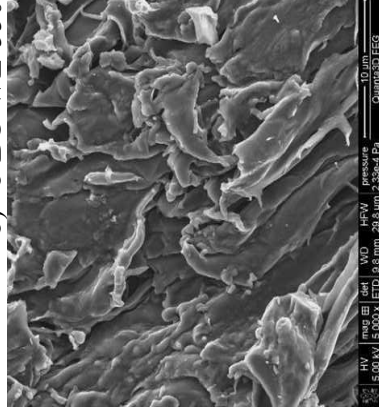
Figure S3. Size distribution of n-BC derived from different feedstocks and under different pyrolysis conditions.

Where, n-BC-nano-biochar, WL-willow, SSL 1-3-different sewage sludges, RH-rice husk, OSR-oilseed rape, 450,500,550,650,750-temperature of pyrolysis; CO₂- change from N₂ to CO₂ as carrier gas.

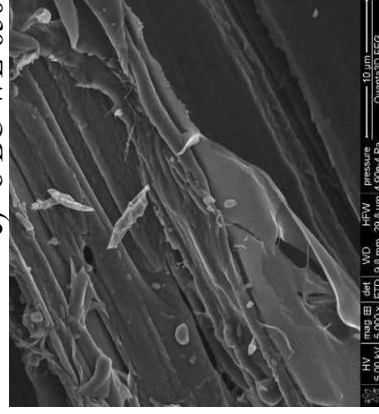
a) b-BC-WL-450



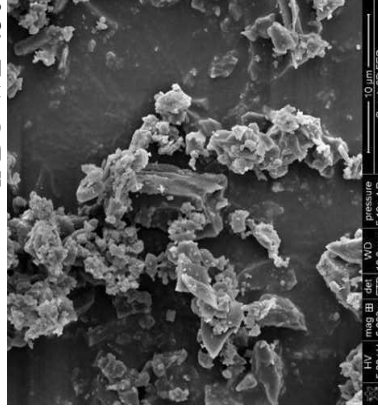
b) b-BC-WL-550



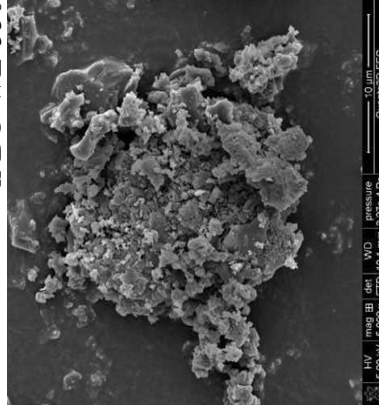
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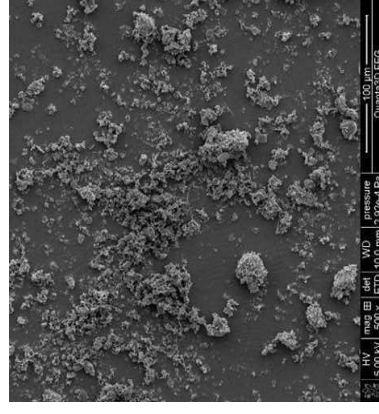
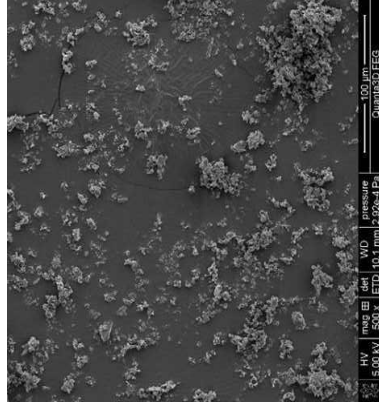
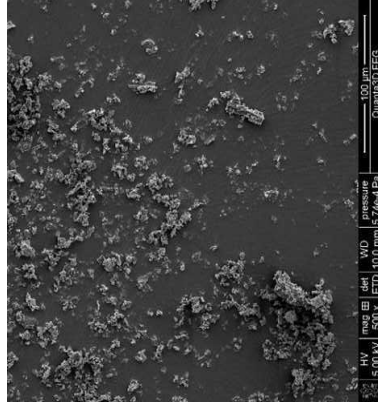
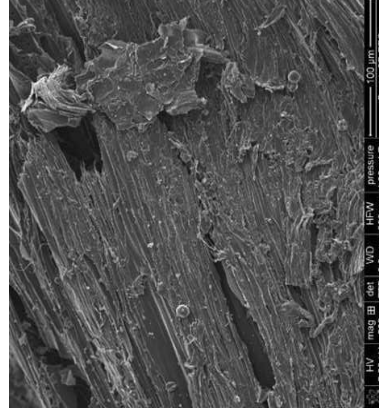
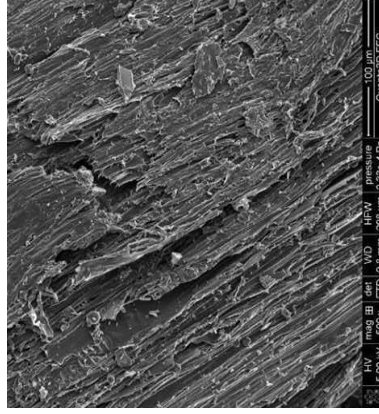
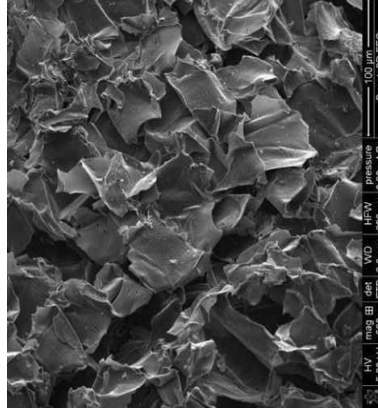
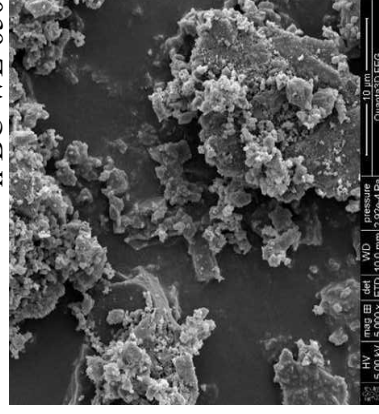
n-BC-WL-450



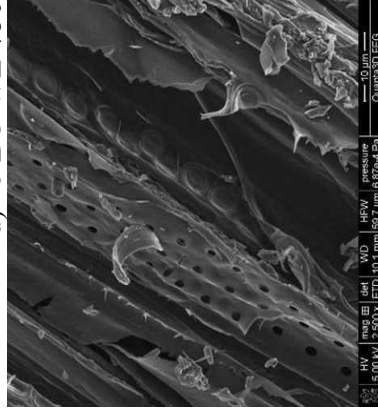
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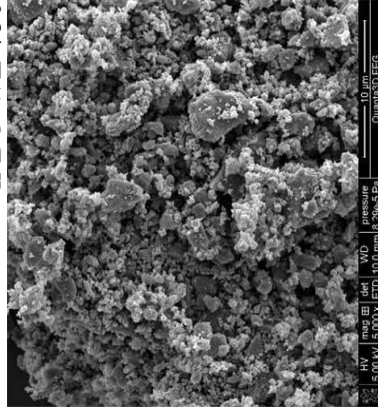
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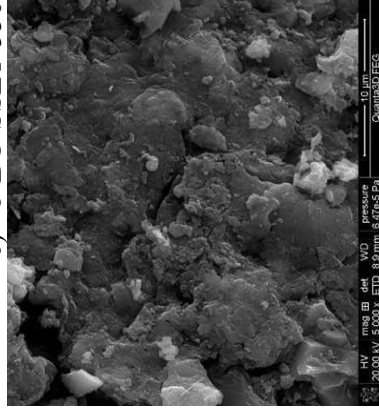
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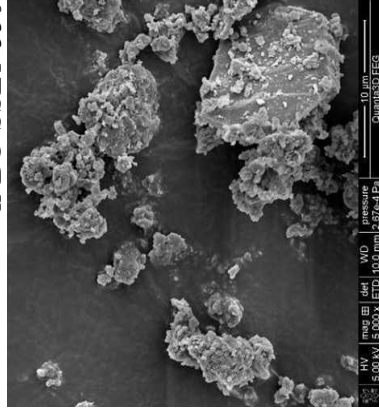
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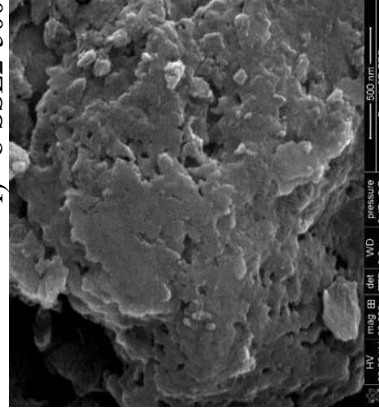
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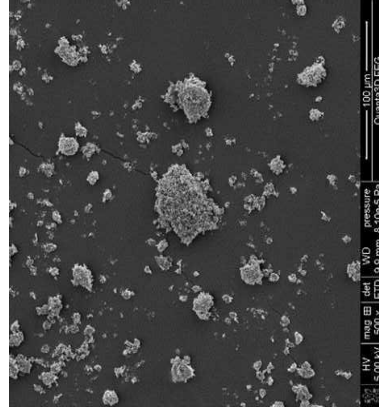
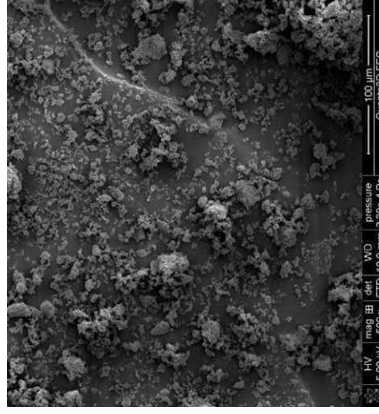
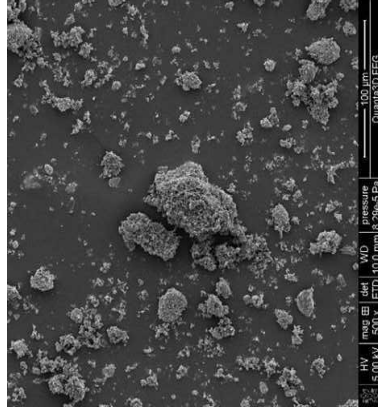
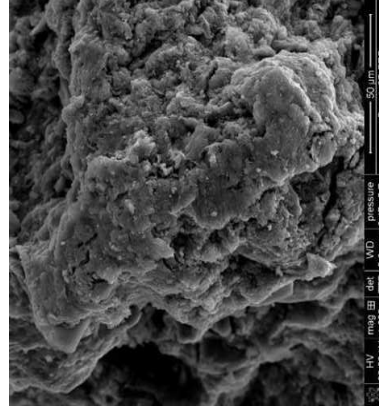
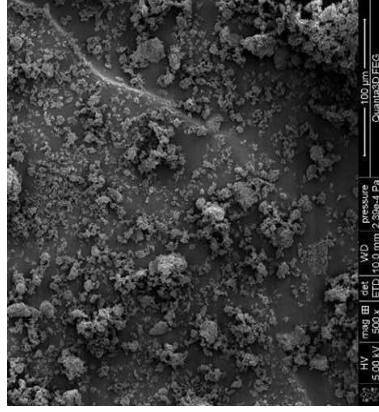
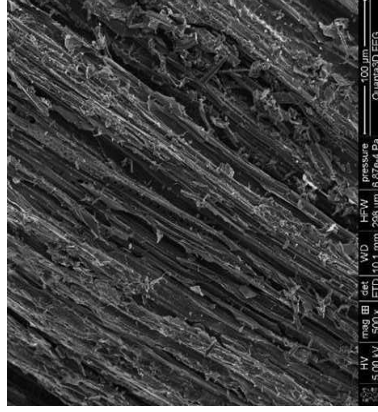
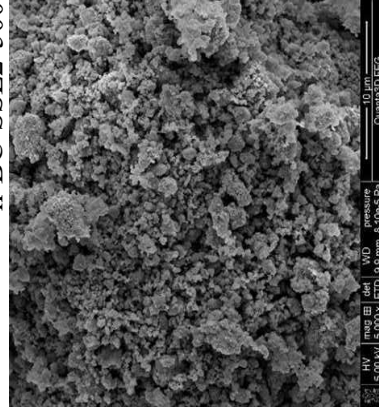
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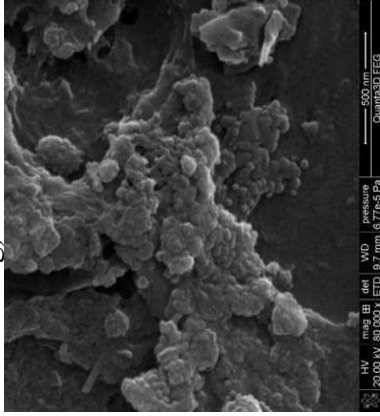
f) b-SSL2-500



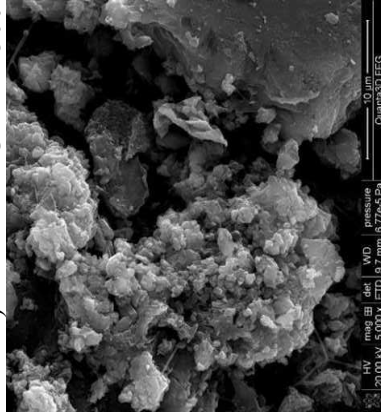
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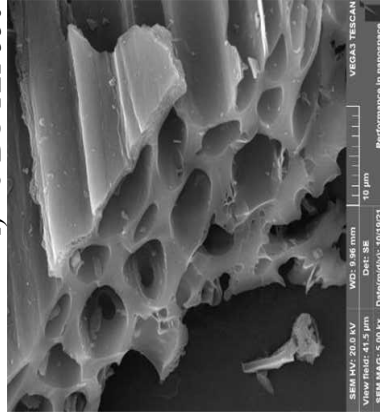
g) b-BC-SSL3-500



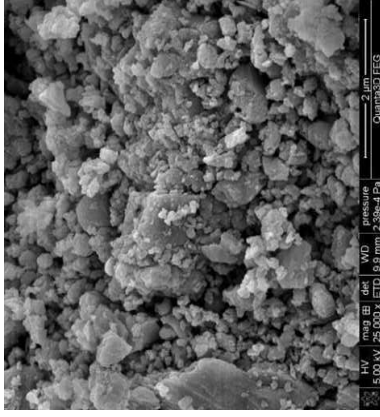
h) b-BC-SSL3-CO2-500



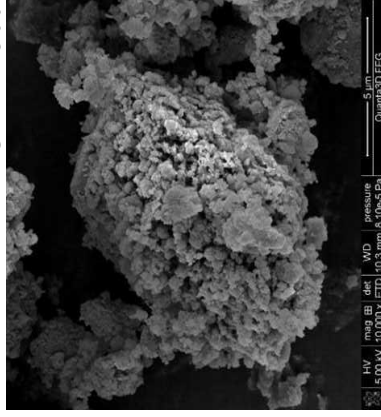
i) b-BC-RH-550



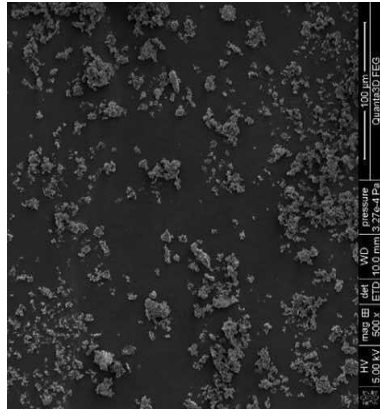
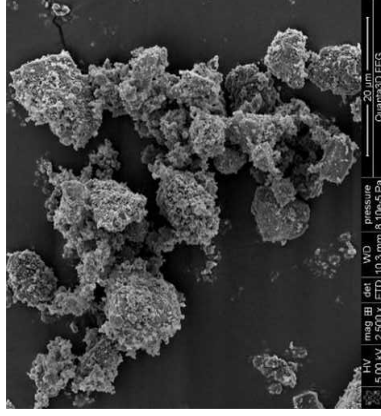
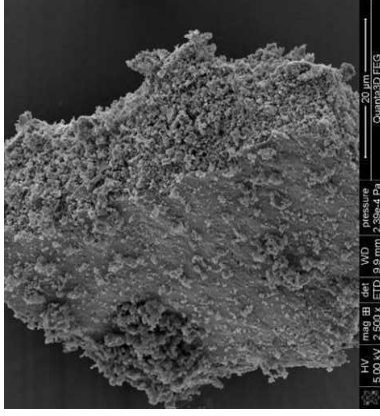
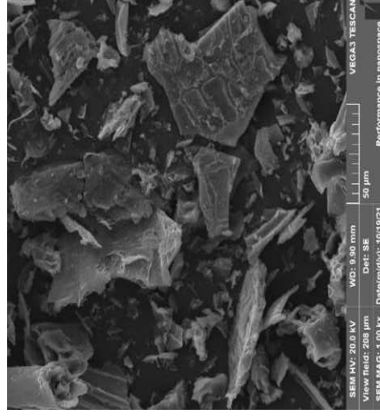
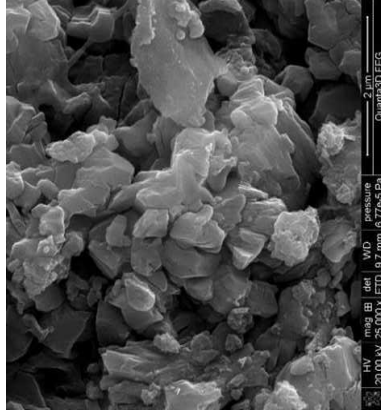
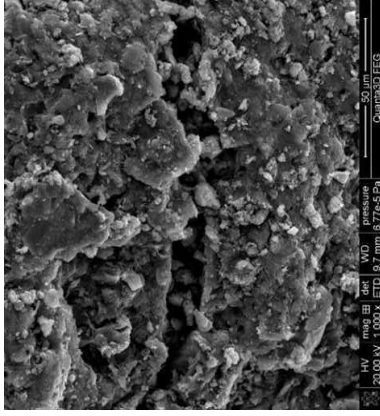
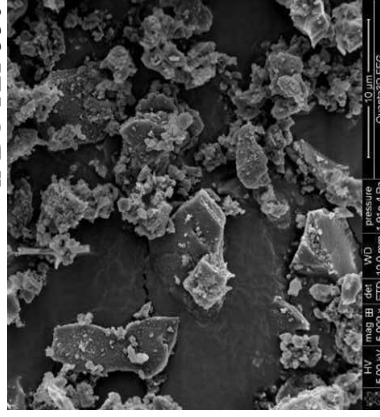
n-BC-SSL3-500



n-BC-SSL3-CO2-500



n-BC-RH-550



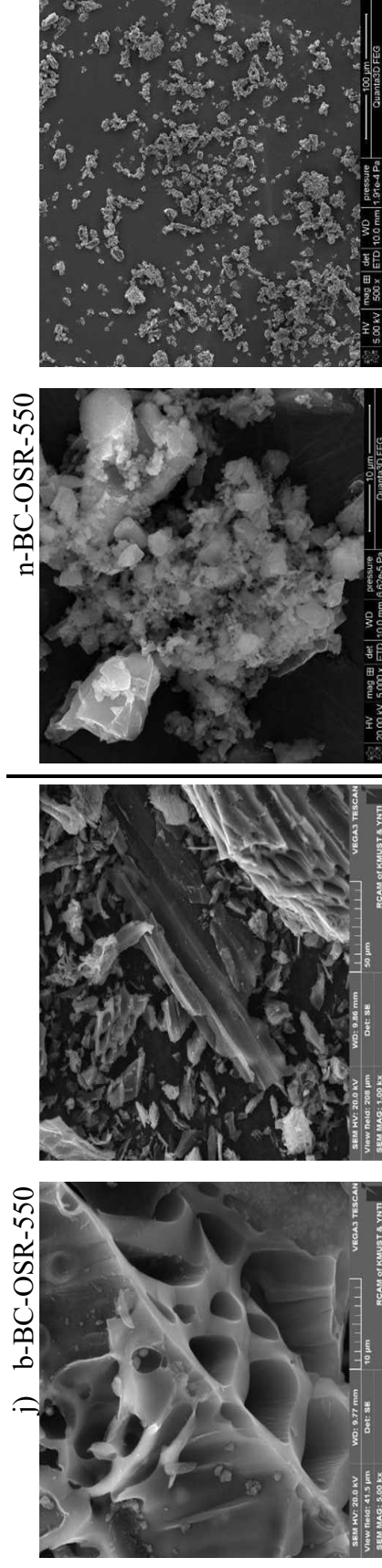


Figure S4. SEM images of b-BC and n-BC.

Where, b-BC- bulk biochar, n-BC-nano-biochar, WL-willow, SSL 1-3-different sewage sludges, RH-rice husk, OSR-oilseed rape, 450,500,550,650,750-temperature of pyrolysis; CO₂- change from N₂ to CO₂ as carrier gas.

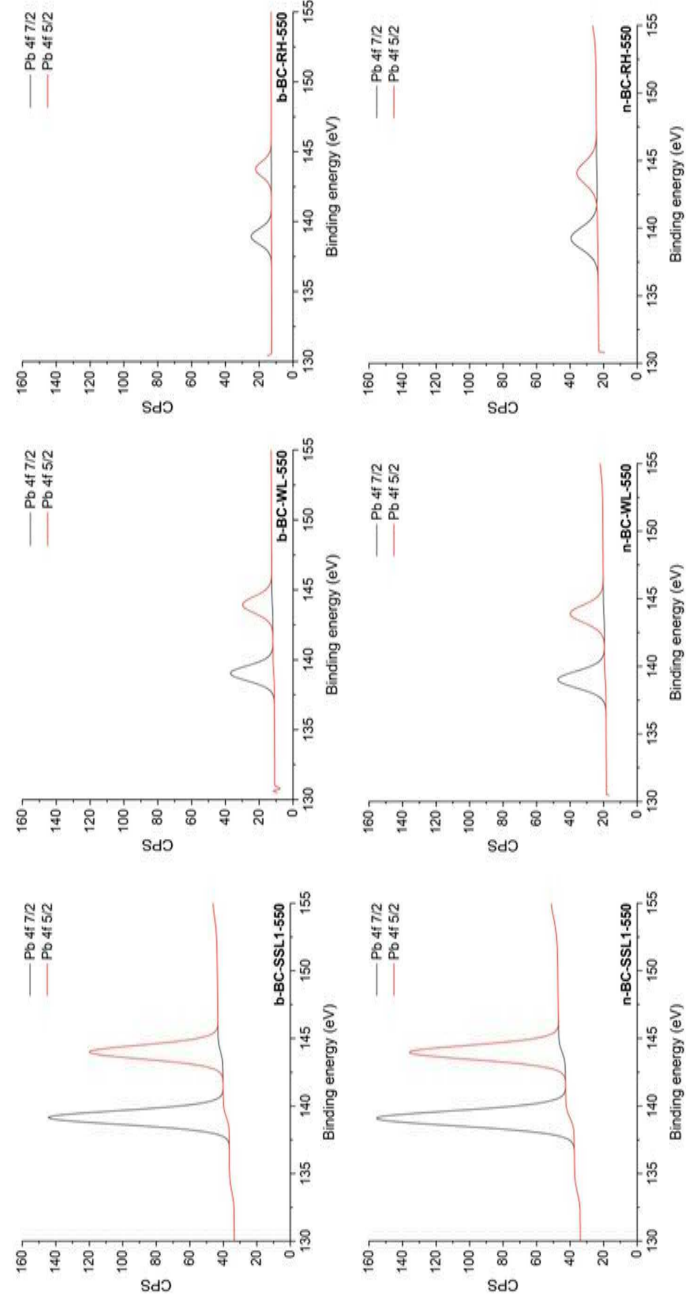


Figure S5. XPS for selected b-BC and n-BCs before and after adsorption of Pb(II) ions.

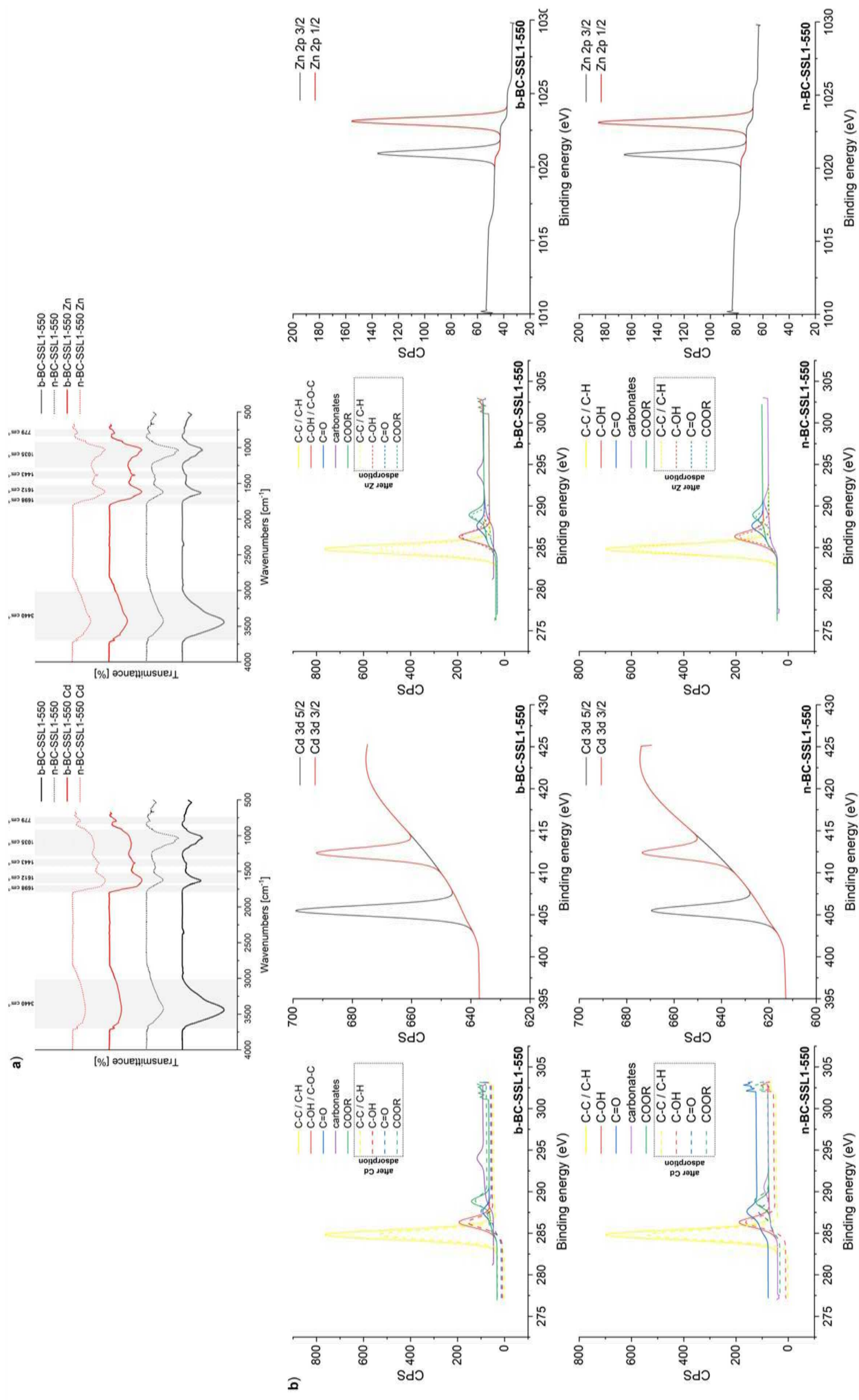
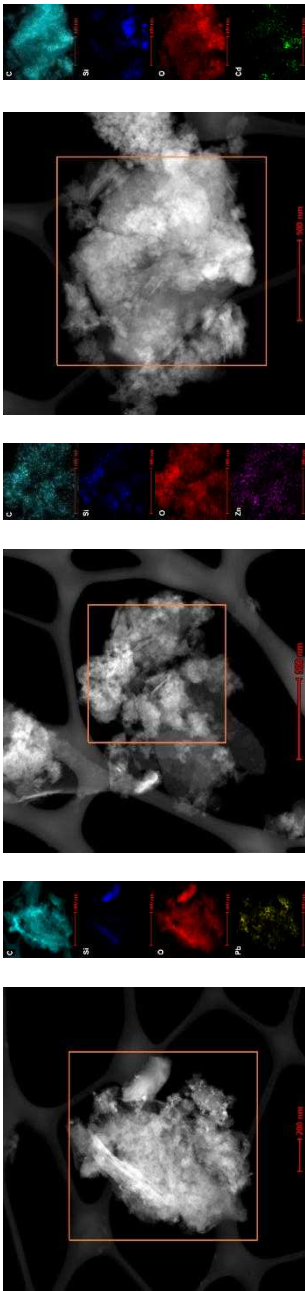
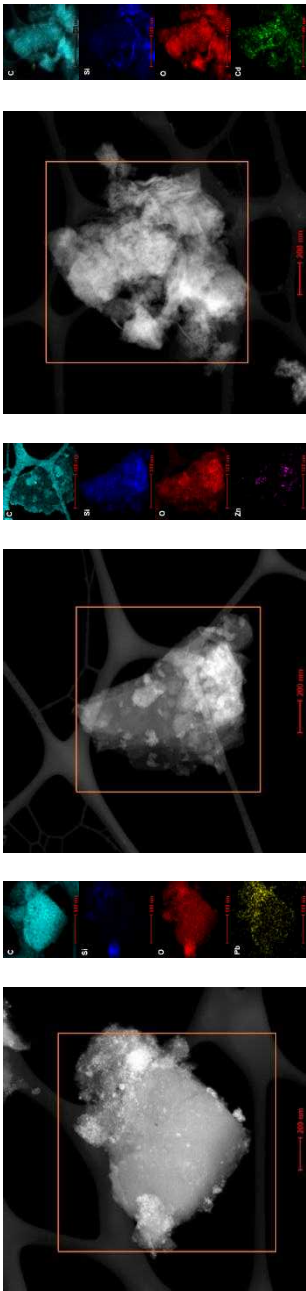


Figure S6. FTIR (a) and XPS (b) for selected b-BC and n-BCs before and after adsorption of Cd(II) or Zn(II) ions.

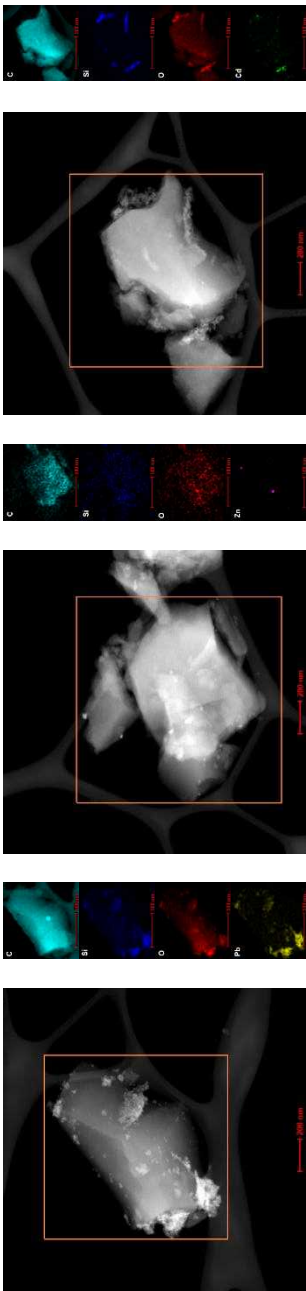
b-BC-SSL1-550



n-BC-SSL1-550



b-BC-WL-550



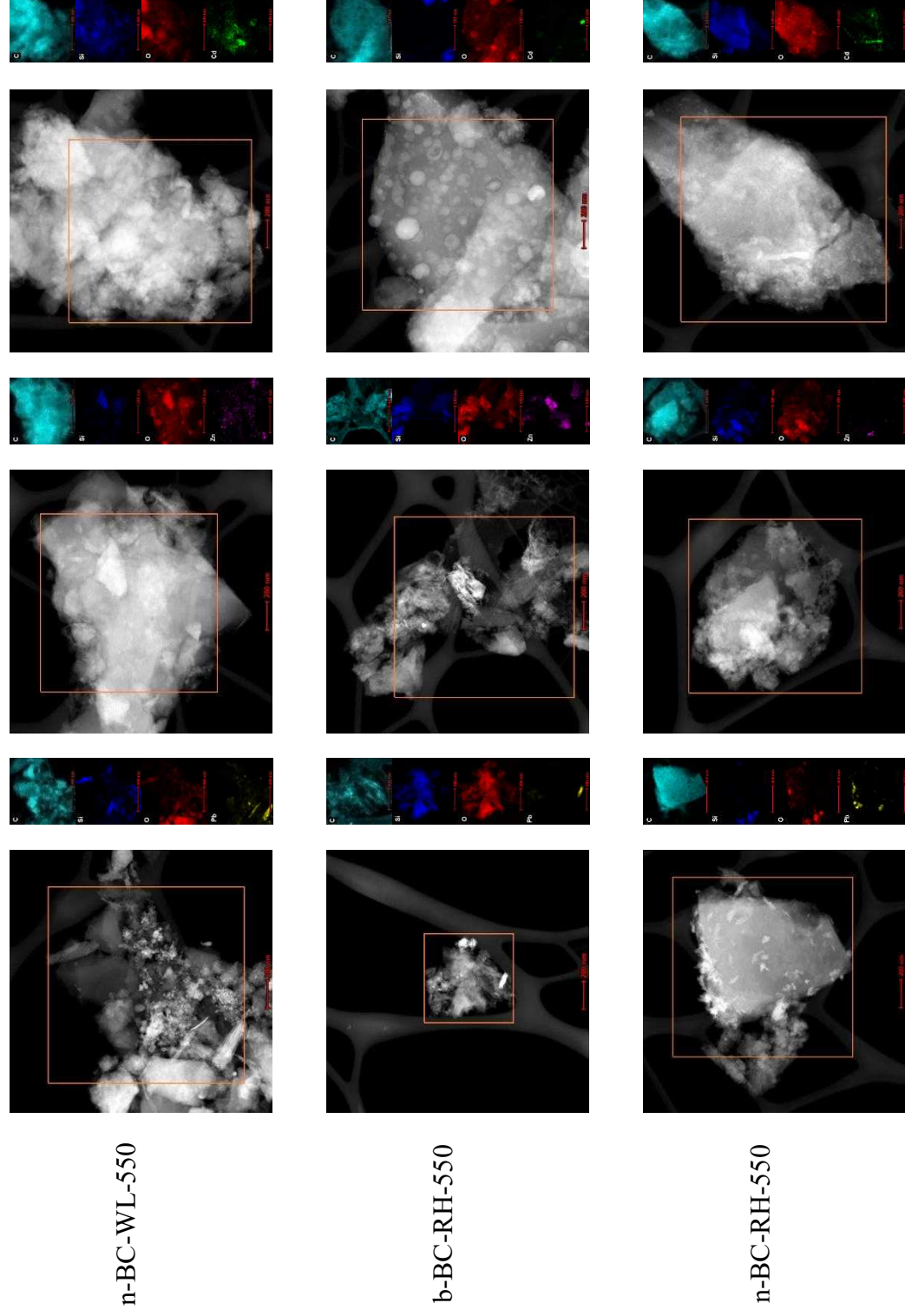


Figure S7. TEM images with EDS mapping for selected b-BC and n-BCs with adsorbed Cd (II), Zn (II) and Pb (II) ions.

Where, b-BC-bulk biochar; n-BC-nano-biochar- WL-willow, SSL 1-sewage sludge, RH-rice husk, 550-temperature of pyrolysis.

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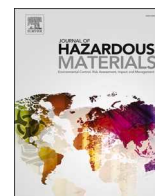
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Publikacja D5

M. Raczkiewicz, P. Oleszczuk

“Boosting sewage sludge safety with nano–biochar for polycyclic aromatic hydrocarbons immobilization and ecotoxicity reduction”

Journal of Hazardous Materials, 492, 138224



Boosting sewage sludge safety with nano-biochar for polycyclic aromatic hydrocarbons immobilization and ecotoxicity reduction

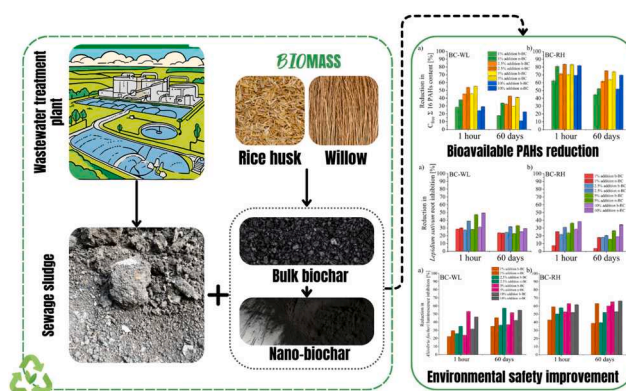
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HIGHLIGHTS

- Nano-biochar significantly outperforms bulk biochar in PAH stabilization.
- Optimal PAH reduction achieved at 2.5%–5% nano-biochar dosing levels.
- Rice husk-derived biochar outperformed willow-derived biochar in efficiency.
- Nano-biochar reduces SSL toxicity, enhancing reuse potential and safety.
- Ash content in biochar plays a key role in immobilizing PAHs effectively.

GRAPHICAL ABSTRACT



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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are common pollutants in sewage sludge (SSL), posing environmental and health risks. Stabilizing these contaminants is crucial for improving the ecological safety and reuse potential of SSL. This study investigated the effectiveness of nano-biochars (n-BC) and bulk biochars (b-BC) derived from willow (WL) and rice husk (RH) in reducing freely dissolved (C_{free}) PAHs in SSL and mitigating its ecotoxicity. RH-derived biochars demonstrated superior performance, achieving 1.5- to 4.7-fold greater reductions in C_{free} PAH content compared to WL-derived variants. Notably, n-BC proved more effective than b-BC, with n-BC-RH and n-BC-WL showing up 1.2- to 2.0-fold greater reductions, respectively. The optimal BC's dose range was 2.5–5%, with diminishing returns observed at higher concentrations (10%). The n-BC treatment also showed enhanced toxicity reduction, improving *Aliivibrio fischeri* luminescence and *Lepidium sativum* root growth by up to 109% and 369% compared to b-BC. Analysis revealed that inorganic minerals (present in ash) in both BC types played a key role in PAH immobilization. These results highlight n-BC's potential as an innovative solution for sustainable SSL management. This research addresses a critical gap in sludge management by proposing practical, scalable, and sustainable solutions for PAH contamination.

Abbreviations: SSL, sewage sludge; B-BC, bulk biochar; N-BC, nano-biochar; BC, generally biochar including b-BC and n-BC.

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1. Introduction

Sewage sludge (SSL), a by-product of wastewater treatment processes, often disposed of through landfilling, incineration or agricultural application. SSL is abundant in essential nutrients, including nitrogen, phosphorus, and potassium [1], which are advantageous for plant growth and development [2]. This is very important particularly in scenarios where SSL is utilized as soil fertilizers or soil improvement [3]. However, these conventional disposal methods pose notable environmental challenges due to its to the presence of various pollutants in SSL, including polycyclic aromatic hydrocarbons (PAHs) [4,5]. To address these challenges, alternative upgrading technologies have been developed to convert SSL into less contaminated products. Techniques such as composting or stabilization are utilized to diminish the pathogen load, enhancing the safety of the SSL during land application [6,7]. However, these methods have several limitations. Composting, while effective in reducing pathogens, requires long processing times and careful control of temperature and aeration to ensure complete sanitization [8]. Additionally, it may not effectively degrade persistent organic pollutants, such as PAHs, which can remain in the treated sludge [9]. Stabilization methods, including lime or chemical treatments, can reduce microbial contamination but do not remove organic contaminants and may lead to increased salinity or potentially toxic elements (PTEs) mobilization in the SSL [10]. Furthermore, both approaches may result in nutrient losses, reducing the agronomic value of the treated sludge [11]. Other methods for the removal of PAHs from SSL have been developed, including advanced oxidation processes (AOPs), which can be used for PAH elimination [12]. However, these techniques often necessitate the use of chemical reagents, specific reaction conditions, and may result in the generation of secondary waste, posing additional environmental risk. Despite these efforts, SSL remains a significant carrier of organic pollutants, particularly PAHs, which present long-term environmental risks. PAHs are organic compounds characterized by their high toxicity, mutagenicity, and carcinogenicity [13], making their presence in SSL seriously threatening both environmental and public health. PAHs tend to accumulate in organic-rich matrices such as SSL, where their mobility can increase over time during organic matter mineralization [14], potentially leading to soil and groundwater contamination. Considering the persistence and potential for bioaccumulation of PAHs and other organic contaminants, there is an urgent requirement for advanced methodologies to mitigate their release, thereby reducing their leaching potential and environmental harm. These challenges highlight the need for alternative treatment strategies, such as the n-BC approach, which could enhance pollutant removal while maintaining the beneficial properties of SSL for land application.

In recent years, biochar (BC) has gained attention as a promising material for the remediation of contaminated environments, including soil and SSL [15–17]. BC, typically produced through biomass (or other residues) pyrolysis, has a highly porous structure and large surface area, which endows it with excellent adsorption properties for both organic and inorganic pollutants [18,19]. These exceptional properties of BCs can be used for the immobilization of various contaminants such as trace metals, organic pollutants, and nutrients, reducing their bioavailability and leaching potential [20]. Nano-biochar (n-BC), a refined form of BC (<100 nm), may offer more tremendous potential for binding contaminants due to its enhanced properties related to adsorption capacity [21, 22]. While extensive research has been conducted on the effectiveness of bulk biochar (b-BC) for contaminants immobilization [23,24], there is a pronounced lack of understanding regarding the nano-scale variant of BC. The reduced particle size of n-BC results in an increased specific surface area for adsorption [21,25], thereby enhancing its ability to immobilize PAHs [26,27]. Additionally, n-BC's distinct physicochemical properties, including surface functional groups and high porosity, facilitate more effective interactions with PAHs, reducing their solubility and mobility within the sludge matrix. This reduction in PAHs mobility mitigates environmental contamination risk and stabilizes the

sludge, potentially improving its safety for land application and other waste management practices. Furthermore, the integration of n-BC in sludge treatment extends benefits beyond PAHs immobilization such as enhancing sludge structure, increasing nutrient retention, and reducing odors and greenhouse gas emissions during storage and handling [28]. Nonetheless, comprehensive studies on the specific behavior, stability, and environmental impacts of n-BC in pollutant immobilization are still limited, highlighting the necessity for further research in this emerging field.

The aim of this study was to evaluate the effectiveness of n-BC as an amendment to SSL in reducing the concentration and mobility of freely dissolved PAHs (C_{free} PAHs). By investigating the immobilization PAHs by n-BC, as well as its impact on the ecotoxicological properties of the sludge, this research seeks to provide new insights into sustainable sludge management practices. Justification for analyzing contaminants like PAHs lies in their diverse chemical characteristics, which range from relatively water-soluble to practically insoluble, each exhibiting different affinities for organic matter. By studying this broad group of compounds, we can obtain data that serve as representative indicators for other contaminants, enhancing our understanding of their environmental impact and health risks. The results of this study could contribute to the development of novel waste treatment technologies that minimize the environmental impact of SSL disposal and promote the safe reuse of treated sludge in agriculture or other applications.

2. Materials and methods

2.1. Sewage sludge and biochar characterization

The sewage sludge (SSL) was collected in the summer of 2023 from a municipal wastewater treatment plant situated in the southwestern region of Poland (Opole, 50.6751° N, 17.9213° E). After the SSL fermentation process, a sample weighing approximately 5 kg was collected at the final stage. The fermentation treatment step is a standard practice in wastewater treatment facilities to improve the quality of the resulting sludge and energy recovery. For experimental purposes, 10 representative subsamples (each weighing about 500 g) were collected. Subsequently, the SSL was air-dried for one week and then further dried in a laboratory oven at 60°C for two days. Then dried SSL were ground in a laboratory grinder to a particle size < 2 µm (TESTCHEM, Poland) and thoroughly mixed for homogenization. The physicochemical properties of SSL used in the experiment can be found in [Supplementary Information \(Table S1\)](#).

In this study, two bulk biochars (b-BC) and their corresponding nano-biochars (n-BC) obtained from willow (WL) and rice husk (RH) were used. The BCs were produced at a temperature of 550°C (3 h, heating rate 10 °C·min⁻¹) in an N₂ atmosphere (flow rate 1 L·min⁻¹). Please refer to our previous publications for comprehensive details regarding the synthesis protocols and physicochemical characterization of both b-BC and n-BC [25]. The physicochemical properties (including elemental analysis (CHNO), ash content, pH, electrical conductivity (EC), size measurements, total organic carbon (TOC) content in solid b-BC/n-BC samples, dissolved organic carbon (DOC) content in b-BC/n-BC's leachates and porous structure analysis) and freely dissolved PAHs (C_{free} Σ16 PAHs) content in b-BC and n-BC used in the experiment can be found in [Supplementary Information \(Table S2 and S3\)](#).

2.2. Preparation of sewage sludge-biochar/nano-biochar mixtures

The mixing of SSL with b-BC or n-BC for all tests was conducted in the laboratory using a batch experiment setup following a previous protocol [29]. Briefly, dried SSL (4 g) and b-BC or n-BC (at 1 %, 2.5 %, 5 %, and 10 %) were added to 50 mL glass flasks and pre-mixed for 2 hours on a horizontal shaker (Model 358 A, ELPINSC+, Poland). Dosage was calculated based on the dry weight of the materials. Then, 2 mL of sodium azide solution (200 mg/L) was added as a biocide (for inhibition of

microbiological processes) to each mixture, forming a slurry. Biocide prevents any potential influence of biodegradation on PAHs content. The mixtures were thoroughly homogenized using a glass spatula. The mixtures were shaken on a rotary shaker at 10 rpm (Rotax 6.8, VELP, Usmate Velate, Italy) for 1 hour (short-term effect) and 60 days (long-term effect) in darkness at room temperature of $24 \pm 2^\circ\text{C}$. The justification for selecting specific time points in research on immobilization processes is to address a gap in existing studies, which often focus on long-term effects while neglecting the initial stages [30,31]. Comparing measurements at 1 hour and 60 days reveals both rapid adsorption and long-term stabilization processes, showing which stage contributes most to immobilization. This approach allows for a better understanding of which stage plays the most significant role in the immobilization process, providing new insights into the dynamics of immobilization and helping to clarify which stage is most effective and responsible for overall stabilization.

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

The content of freely dissolved PAHs (C_{free} $\Sigma 16$ PAHs) was determined according to the method previously described by Cornelissen and Gustafsson [32]. In brief, one gram of each SSL-b-BC/n-BC mixture was placed into a 50 mL flask, along with two polyoxymethylene (POM) passive samplers (40 x 40 mm). As a biocide, a 40 mL sodium azide solution (200 mg/L) was added to each flask. The flasks were then agitated for 30 days using a rotary shaker (Rotax 6.8, VELP, Usmate Velate, Italy) to reach equilibrium according to previous work [33]. After the shaking period, the POM strips were retrieved and rinsed with Milli-Q water to remove any adhering solid particles. The POM samplers were subsequently dried and extracted using a mixture of acetone and n-heptane (1:4, v/v) over a 48-hour period. The resulting extract was concentrated to 1 mL using a rotary vacuum concentrator (RVC 2-25CD plus, Martin Christ, Germany). Qualitative and quantitative analysis of the PAHs in the extract was performed using a gas chromatograph coupled with a mass spectrometer (GC-MS) (Trace 1300, ThermoFisher Scientific, USA) equipped with a Restek Rxi-5 ms column (Restek, Glaston, France), which had a length of 30 m, an internal diameter of 0.25 mm, and a film thickness of 0.25 μm . Detailed information about the PAH's limit of detection can be found in [Supplementary Information \(Table S4\)](#).

2.4. Ecotoxicological test

To assess the ecotoxicity of the SSL-b-BC/n-BC mixtures, two liquid-phase tests were conducted with the bacteria *Aliivibrio fischeri* and the plant *Lepidium sativum*. The leachates were prepared following the EN 12457-2 protocol [34]. Each of the SSL-b-BC/n-BC mixture was mixed with Milli-Q-water in a ratio of 1:10 (w/v) and shaken on a horizontal shaker for 24 h (Model 358 A, ELPINSC+, Poland). The resulting leachates were filtered using a 0.45 μm hydrophilic syringe filter. Liquid phase (leachates) tests allow for determining the potential toxicity of components and/or contaminants leached from SSL and/or b-BC/n-BC-SSL mixtures under environmental conditions. The test with *A. fischeri* was based on the Microtox® protocol, using a Microtox M500 analyzer (Modern Water, UK) following the standard test procedure [35]. Luminescence inhibition was measured after 5 and 15 minutes of bacterial exposure to the leachates. The data were analyzed using the Microtox Omni software. The elongation test with *L. sativum* was performed according to the OECD guidelines [36]. The endpoints (seed germination and root elongation) were conducted in glass Petri dishes (90 x 20 mm), each lined with a thin layer of filter paper. Fifteen seeds were placed in each dish, along with 5 mL of leachate or 5 mL of Milli-Q-water for the control. The seed germination and root growth were measured after three days of exposure. The measurements and calculations were performed using Image Tool 1.28 software (Image Tool 1.28, CMEIAS, USA).

2.5. Statistical analysis

The data were subjected to statistical analysis using OriginPro 2024 (OriginLab Corporation, USA) software. Mean values were obtained from a triple set of data. To examine the relationships between physicochemical properties of b-BC/n-BC immobilization of C_{free} PAHs and ecotoxicological test results in OriginPro 2024 (OriginLab Corporation, USA) software. Pearson's and Kendall's correlation coefficient test was conducted. Significance was established at $*p \geq 0.05$.

3. Results and discussion

3.1. C_{free} $\Sigma 16$ PAHs content in sewage sludge

The content of the sum of 16 C_{free} PAHs (C_{free} $\Sigma 16$ PAHs) in SSL used in the experiment was 251 ± 2.4 ng/L and increased during incubation to 391 ± 2.9 ng/L (Fig. 1). The increase was caused by increasing the contact time between the liquid phase (during POM extraction) and the solid phase (SSL or SSL-b-BC/n-BC mixtures) containing PAHs. When water molecules have extended contact time, they penetrate more deeply into the SSL structure. This enhanced penetration facilitates the release of PAHs from the solid to the liquid phase. These findings suggest that prolonged exposure of SSL to water in natural environments may increase PAHs bioavailability, potentially elevating environmental risks. The contribution of C_{free} $\Sigma 16$ PAHs based on the number of aromatic rings was dominated by 2-, 3-, and 4-ring PAHs, accounting for 59–61 %, 20–21 %, and 19–20 %, respectively. The 5- and 6-ring PAHs accounted for only 0.3 % and 0.1 % of the total C_{free} $\Sigma 16$ PAHs, respectively. The distribution of specific PAHs groups remained consistent over incubation periods (Fig. 2), although the relative percentages of naphthalene (NAP), fluoranthene (FLA), and phenanthrene (PHE) showed slight variations (please see SI). Notably, after 60 days, there was a 5–6 % decrease in the contribution of 3- and 4-ring PAHs, with a corresponding increase in the proportion of 2-ring PAHs (Fig. 2).

3.2. Changes in C_{free} $\Sigma 16$ PAHs content in sewage sludge-b-BC/n-BC mixtures

The amendment of b-BC or n-BC to SSL effectively decreased C_{free} $\Sigma 16$ PAHs content (Fig. 1). The reduction varied based on the feedstock type, dosage, particle size, and incubation time. Notably, regardless the feedstock type, dosage and particle size, the reduction in C_{free} $\Sigma 16$ PAHs content was more pronounced in the initial phase (1 hour) of the experiment compared to an extended incubation period (60 days). This suggests that BC might rapidly immobilize PAHs initially, but its effectiveness could diminish over time as equilibrium is reached or as desorption processes become more significant. The competitive sorption phenomenon may also explain the temporal variations in PAHs binding behavior when BC is present in the system. The initial adsorption capacity of BC for PAHs is primarily driven by the strong affinity of these hydrophobic compounds for its surface [37]. This hypothesis is supported by the finding that, after 60 days of incubation, a more pronounced reduction in the 6-ring PAHs content was observed compared to the measurements taken after 1 hour (Table S5). This indicates that 6-ring PAHs, being more hydrophobic, exhibit a greater affinity for the surface of BC. However, over time, competitive adsorption processes may result in the displacement of PAHs by other constituents present in SSL as was confirmed in other matrices [38,39]. SSL is a complex matrix containing a wide array of contaminants, including surfactants, and oils or other hydrophobic substances, including dissolved organic matter (DOM). These components may exhibit a greater affinity for BC, thereby replacing previously immobilized PAHs. Elevated dissolved organic carbon (DOC) in SSL can interact with BC, influencing PAHs immobilization in two ways. Higher DOC levels may enhance PAHs mobilization by forming soluble complexes facilitating the desorption of PAHs and diminishing their immobilization efficiency. Conversely, DOC can also

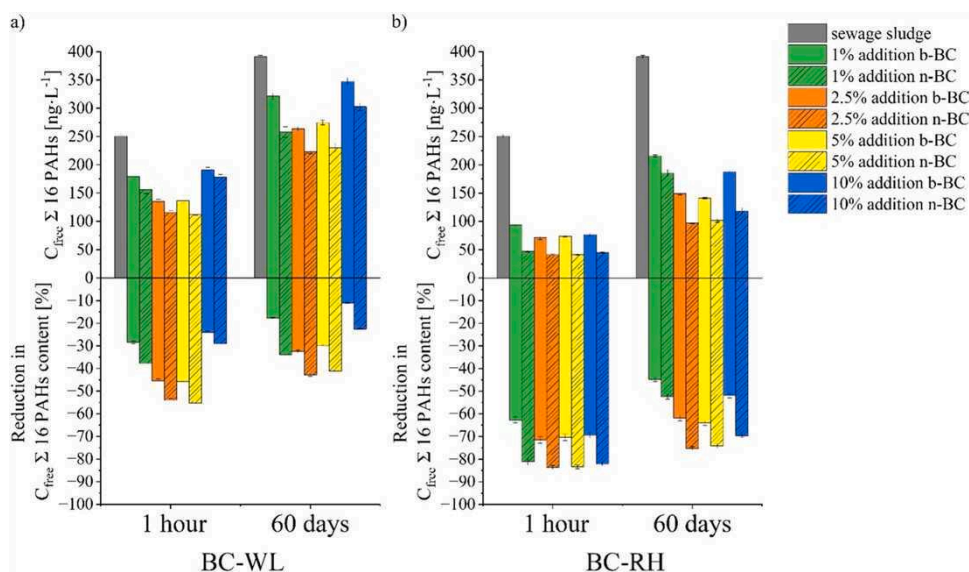


Fig. 1. The C_{free} $\Sigma 16$ PAHs content and percentage reduction in sewage sludge treated with b-BC-WL/n-BC-WL (a) and b-BC-RH/n-BC-RH (b). The top panel shows the C_{free} $\Sigma 16$ PAHs content, while the bottom panel displays the percentage reduction. Error bars represent the standard deviation of the mean ($n = 3$).

create additional adsorption sites on BC, strengthening interactions with hydrophobic contaminants [40]. The overall effect depends on the balance between these opposing mechanisms and the specific DOC levels in the SSL amended with BC. Surfactants and oils present in SSL may further enhance this process by modifying the surface chemistry of BC and increasing PAHs mobility. This reduction's magnitude varied based on the BC type and application rate (Fig. 1). After 60 days of incubation, the reduction in 2-ring PAHs was less pronounced compared to the reduction observed after just 1 hour (Table S4). After 60 days, the reduction in 2-ring PAHs for WL-derived b-BC was 1.3- to 6.7-fold lower, while for WL-derived n-BC, it was 1.1- to 1.7-fold lower compared to the 1 hour. A similar pattern was observed for RH-derived BC, with b-BC showing a 1.3- to 1.4-fold decrease and n-BC a 1.3- to 1.6-fold decrease relative to the 1 hour term. These findings point to a key insight that BC's effectiveness in reducing low-molecular-weight PAHs diminishes over time, this decline likely stems from their weaker interactions with the BC's matrix due to competitive effects [41]. The data suggest that the BC works best at reducing C_{free} $\Sigma 16$ PAHs in SSL immediately after application, with a gradual decline in efficacy potentially due to saturation or changing binding dynamics over more extended incubation periods. This interplay between various SSL constituents and PAHs highlights the complex mechanisms governing contaminant dynamics in BC-amended systems.

3.2.1. Effect of feedstock and size on C_{free} $\Sigma 16$ PAHs content

The effectiveness of BC in reducing C_{free} PAHs content varied significantly based on feedstock type, as shown in Fig. 1. RH-derived BC outperformed WL-derived BC reducing C_{free} $\Sigma 16$ PAH by 51.9–71.5 % compared to 11.0–45.7 %. The performance gap was most notable at 10 % dosage, where RH-derived BC was up to 4.7 times more effective (Fig. 1). Both types of BCs primarily reduced 2-ring PAHs, with contributors around 42–73 % (Fig. 2). RH-derived BC showed superior reduction across most PAHs groups, reducing 2-ring PAHs by up to 665 %, 3-ring PAHs by up to 13,379 %, 4-ring PAHs by up to 259 %, and 6-ring PAHs by up to 723 % more effectively than WL-derived BC. However, both BC increased 5-ring PAHs compared to untreated SSL (Table S5), though RH-derived BC showed a smaller increase due to its lower initial 5-ring PAHs content (Table S3).

The n-BCs showed varying efficiency based on their source feedstock. RH-derived n-BCs outperformed WL-derived n-BC in reducing C_{free} $\Sigma 16$ PAHs by 1.5- to 3.1-fold, depending on the dose and incubation time. WL-derived n-BCs achieved notably higher reductions in C_{free} $\Sigma 16$ PAHs

compared to their bulk counterparts, showing improvements of 18.6–33.1 % after 1 hour and 91.3–103.8 % after 60 days of incubation (Fig. 1a). Similarly, the reduction in particle size for RH-derived n-BCs led to enhanced reduction efficiency, with increases of 16.9–29.2 % after 1 hour and 15.9–33.4 % after 60 days compared to RH-derived b-BCs. However, even RH-derived n-BCs were more effective in the reduction of C_{free} PAHs content in SSL, the size reduction had a greater impact on WL-derived n-BCs, with these differences especially pronounced after 60 days of incubation. The most significant improvements in reduction efficiency emerged under specific conditions. For both feedstock types, peak efficiency occurred at 1 % dose for 1-hour incubation and 10 % dose for 60-day incubation, confirming n-BCs' superior performance overall.

Previous studies [44–46] have demonstrated that the affinity of hydrophobic organic contaminants (HOC) to BC may be influenced by the feedstock material. This connection often relates to specific surface area, carbon content and the presence of the functional groups on the BC's surface. The b-BC produced from RH exhibit greater effectiveness in reducing C_{free} $\Sigma 16$ PAHs content in SSL than those derived from WL. This is likely due to distinct physicochemical properties of these BCs. Notably, the surface area of WL-derived b-BCs was significantly (52.8-fold) larger than that of RH-derived b-BCs (Table S2), suggesting that mechanisms other than pore-filling based only on surface area may be involved in the binding of contaminants by the tested BCs. This is very important finding showing that surface area in some cases is not a crucial in effectiveness of binding of organic contaminants, especially during its immobilization in complex matrices. Other factors may play a key role in this process. The higher pH of RH-derived b-BCs creates an alkaline environment that may stabilize PAHs by neutralizing acidic components in the SSL and enhancing chemical interactions on the BC's surface [47]. Additionally, the larger pore width in RH-derived b-BCs (Table S1) provides improved access for various PAHs molecules, including those with higher molecular weight, enabling effective immobilization despite a lower surface area. The elevated electrical conductivity (EC) of RH-derived b-BCs may further promote PAHs adsorption by enhancing electrostatic interactions within the sludge matrix [48]. A higher O/C ratio indicates a greater presence of oxygenated functional groups, whose presence is confirmed by FT-IR spectra (Fig. S2), on the RH-derived b-BCs surface, facilitating PAHs adsorption through hydrogen bonding and polar interactions with the aromatic rings of PAHs. [49]. The increased ash content (Table S2), especially with minerals such as silica and metal oxides, provides

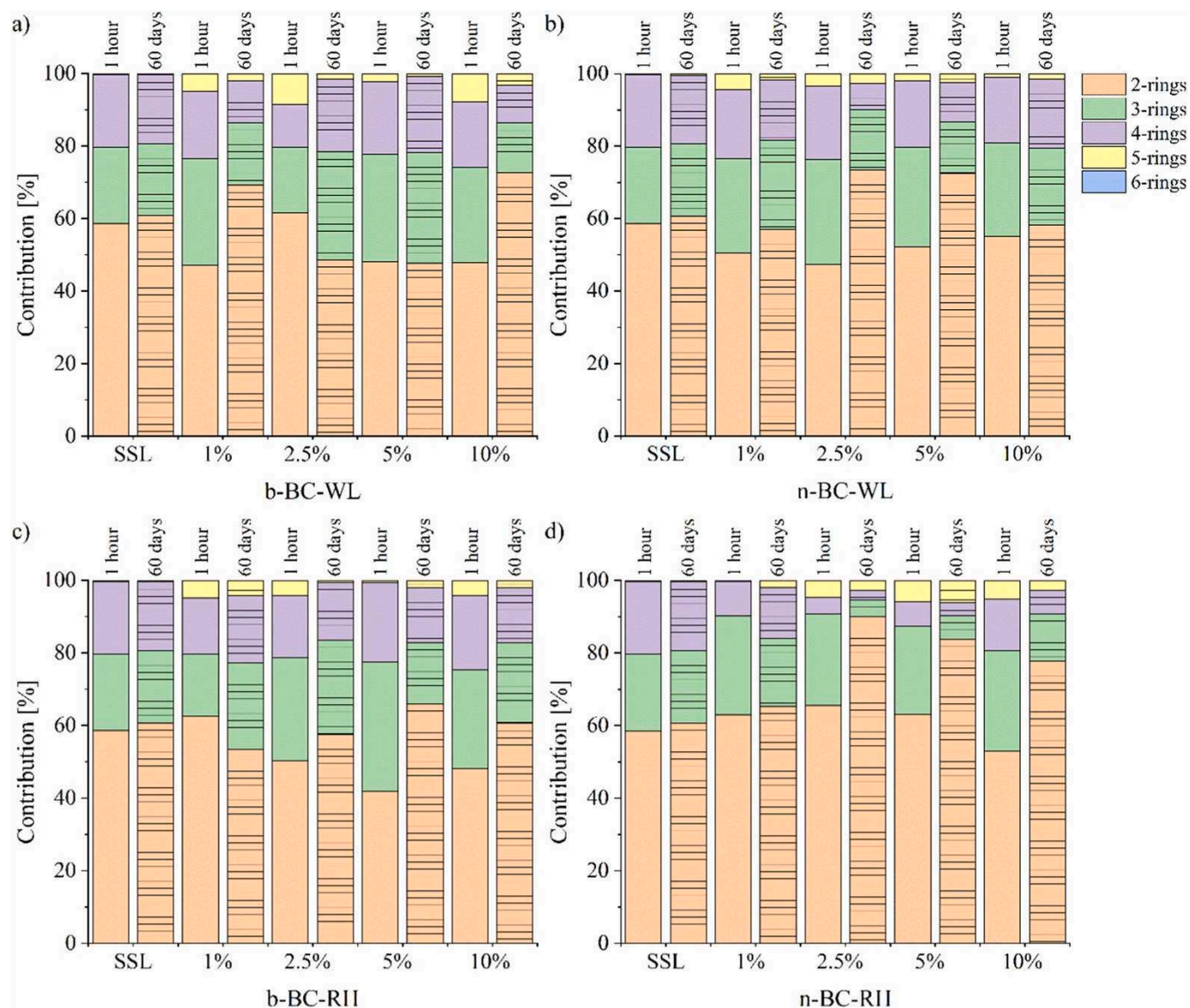


Fig. 2. Changes in ring contribution in C_{free} $\Sigma 16$ PAHs content in sewage sludge with addition of b-BC-WL-550 (a), n-BC-WL-550 (b), b-BC-RH-550 (c) and n-BC-RH-550 (d).

additional adsorption sites and may reduce PAHs bioavailability via physical encapsulation or complexation [50,51]. Collectively, these attributes make RH-derived b-BCs particularly effective in lowering the bioavailability and mobility of C_{free} PAHs in SSL compared to WL-derived b-BCs. Moreover, this is further confirmed by observations that although b-BC-RH have a similar ash content to n-BC-WL, which plays a key role in PAHs immobilization, b-BC-RH more effectively immobilizes C_{free} $\Sigma 16$ PAHs than n-BC-WL, highlighting that other factors influence the immobilization capacity. The principal component analysis (PCA) biplots (Fig. S3) further confirmed the distinct effect of BC type, dose, and contact time on C_{free} $\Sigma 16$ PAHs content in SSL-b-BC/n-BC mixtures at 1 hour (a) and 60 days (b) of incubation, highlighting mentioned key trends. The loading vectors indicate that S_{BET} , V_p and C_{free} $\Sigma 16$ PAHs content of BCs positively correlate with PC1, whereas O/C ratio, ash, and EC show negative correlations, reinforcing the role of oxygenated functional groups, mineral composition, and electrostatic interactions in PAHs immobilization. The pH values and pore width contribute more to PC2. Over 60 days, treatment clusters shift, suggesting progressive changes due to BC interactions, possibly via leaching, oxidation, or microbial processes. The observed separation

along PC1 highlights the strong impact of BC's modifications on C_{free} $\Sigma 16$ PAHs content in SSL, reinforcing its evolving environmental role over time.

Furthermore, it was observed that n-BCs exhibit greater effectiveness in reducing C_{free} $\Sigma 16$ PAHs content in SSL compared to their bulk counterparts. Smaller BC's particles tend to have higher adsorption capacities for organic contaminants due to increased specific surface area, total pore volume, and enhanced surface properties [25,42,43,52]. Similar to b-BCs, surface area was not the primary factor influencing the reduction of C_{free} $\Sigma 16$ PAHs content in n-BCs. Only for RH-derived n-BCs, the significant (42.9-fold) increase in surface area contributed to improved reduction efficiency. However, the surface area of the more effective RH-derived n-BCs was still smaller than that of WL-derived n-BCs, for which reduction efficiency improved despite no change in surface area. Notably, all n-BCs demonstrated enhanced some properties compared to b-BCs, such as increased EC, O/C molar ratio, and ash content, all of which positively influenced their effectiveness in reducing C_{free} $\Sigma 16$ PAHs content. The only exception was pH, which was lower in n-BCs compared to b-BCs. However, this did not significantly affect their performance, as the improvements in other properties outweighed the

influence of pH, making n-BCs more effective overall.

It is worth noting that a more pronounced improvement in C_{free} $\Sigma 16$ PAHs reduction was observed for WL-derived n-BC compared to RH-derived n-BC following particle size reduction. This indicates that, for WL-derived n-BC, changes in other parameters, such as increased pore diameter, external surface area, ash content, O/C molar ratio and EC had a greater influence on its enhanced performance than surface area alone. These findings emphasize that, particularly for n-BCs, factors other than surface area—such as pore structure, chemical composition, and surface functionality—are critical in improving the sorption and immobilization of PAHs. They highlight the importance of evaluating a combination of physicochemical properties to optimize the performance of BCs in complex matrices like SSL. Statistical analysis using Pearson's correlation confirmed this observation (Fig. 3). An evaluation of correlations between C_{free} $\Sigma 16$ PAHs content and the physicochemical properties of the b-BC and n-BC reveals significant relationships ($p \leq 0.05$) between C_{free} $\Sigma 16$ PAHs in mixtures and C_{total} $\Sigma 16$ PAHs, C_{free} $\Sigma 16$ PAHs, ash content, pH, EC and O/C molar ratio in b-BC/n-BC (Fig. 3).

3.2.2. Effect of dose on C_{free} $\Sigma 16$ PAHs content

The reduction of C_{free} $\Sigma 16$ PAHs content in SSL following the addition of b-BC/n-BC was also significantly influenced by the applied dose. Specifically, adding 2.5–5 % of b-BC/n-BC to SSL resulted in the most notable decrease in C_{free} $\Sigma 16$ PAHs content. There were no statistically significant differences ($p > 0.05$) in the reduction of C_{free} $\Sigma 16$ PAHs content between these two doses. The PCA plots (Fig. S3) also reveal and confirmed that 1 % and 10 % BC's doses are distinctly separated from 2.5 % and 5 % doses, suggesting that extreme doses were less effective in PAHs immobilization. The reduction effectiveness varied with contact time duration and BC form. WL-derived b-BC at 2.5–5 % doses reduced C_{free} $\Sigma 16$ PAHs by $45.6 \pm 0.07 \%$ after 1 hour of incubation and $31.1 \pm 1.38 \%$ after 60 days of incubation. These reductions were about 2-fold higher compared to the reductions observed for the 1 % and 10 % doses. RH-derived n-BCs showed no significant dose-dependent differences ($p > 0.05$) in C_{free} $\Sigma 16$ PAHs immobilization after 1 hour of incubation (Fig. 1), though 2.5–5 % doses were 1.2–1.4-fold more effective than other doses after 60 days. For PAHs groups (Tables S4),

2.5–5 % WL-derived b-BC was superior at 1 hour, achieving 1.3–103-fold greater reduction across most types, except 5-ring PAHs, which exhibited a 1.7-fold lower increase relative to untreated SSL. After 60 days, this pattern persisted only for 2- and 5-ring PAHs, with approximately 7-fold higher and 5-fold lower reductions, respectively. For RH-derived b-BC, the 2.5–5 % dose proved most effective only for 2- and 6-ring PAHs after 1 hour, showing 1.1–1.3-fold and 1.2–2.3-fold greater reductions, respectively, while simultaneously minimizing the increase in 5-ring PAHs with a 3.2-fold lower increase compared to other doses.

The applied dose also influenced the differences between b-BC and n-BC in immobilizing C_{free} $\Sigma 16$ PAHs. At 1 % dose after 1 hour, nanoscale particles increased immobilization by 33.1 % (WL-derived n-BC) and 29.2 % (RH-derived n-BC) (Fig. 1). The 10 % dose showed larger increases after 60 days: 103 % for WL-derived n-BC and 34.3 % for RH-derived n-BC. RH-derived n-BC doses had similar effects across time-points, except for 1.4-fold decrease at 1 % dose over 60-days.

WL-derived n-BC doses of 2.5–5 % reduced most individual PAHs groups effectively showing 1.3–52.9-fold decreases after 1 hour and 1.1–3.7-fold 60 days. Compared to b-BC, significant differences ($p \leq 0.05$) emerged for select groups (3- and 6-ring PAHs after 1 hour, and for 2-, 3-, 4-, and 6-ring PAHs after 60 days). However, 5-ring PAHs showed 1.5–4.9-fold lower increases versus untreated SSL, with particle size reduction showing minimal impact. RH-derived n-BC exhibited no initial C_{free} PAH reduction differences across doses at 1 hour, but 2.5–5 % doses proved 1.1–1.7-fold more effective for most ring groups after 60 days. The 5-ring PAHs showed no significant changes with RH-derived n-BC treatment.

Interestingly, the application of the highest dose of BC led to similar (WL-derived BC) or even poorer (RH-derived BC) reductions in C_{free} $\Sigma 16$ PAHs compared to the lowest dose (Fig. 1). A dose of 2.5–5 % b-BC or n-BC is likely the most effective for reducing C_{free} $\Sigma 16$ PAHs. This observation aligns with our previous findings [29], where we reported that a statistically significant ($p \leq 0.05$) difference in C_{free} $\Sigma 16$ PAHs content was observed only for the addition to SSL of maize stover-derived b-BC at doses of 2 % and 5 %. This effectiveness results from an optimal balance between the available adsorption sites and the distribution of BC's particles within the SSL matrix. At these doses, the

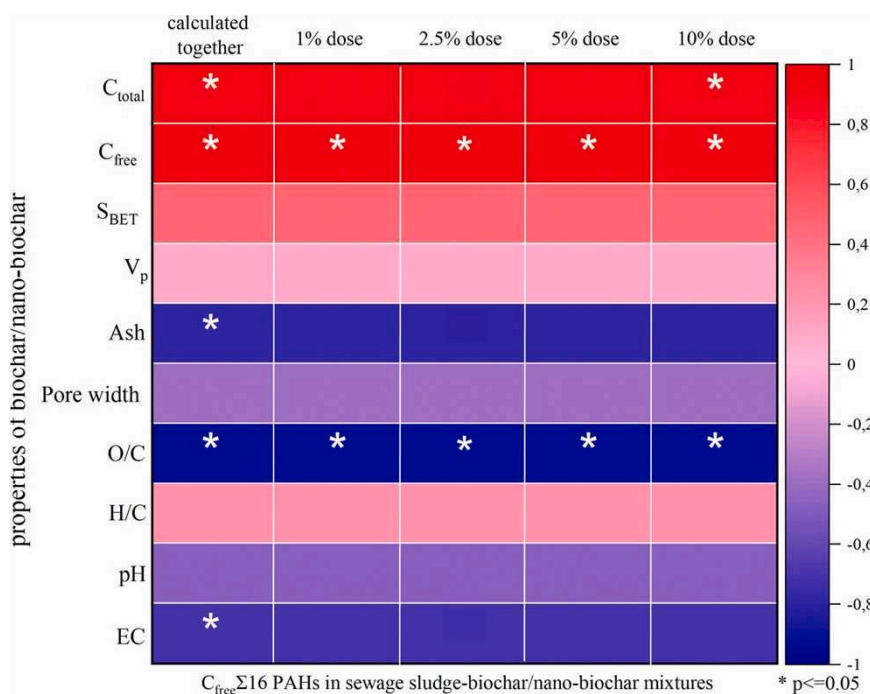


Fig. 3. Correlation plot presenting the values of Pearson's correlation coefficients between physicochemical properties of biochars and nano-biochars and C_{free} $\Sigma 16$ PAHs content in sewage sludge-biochar/nano-biochar mixtures. * means statistically significant correlation ($p \leq 0.05$).

BC's surface area, pore structure, and active functional groups are highly accessible to PAHs molecules, allowing for effective immobilization. This process avoids the saturation or aggregation effects that can decrease BC's efficiency at higher doses. We suppose that when the dose is excessively high, several factors may diminish the effectiveness of BC. Higher doses of BCs may promote particle aggregation, reducing the accessible surface area and blocking pores, which limits the availability of active adsorption sites for PAHs. Furthermore, an increased amount of BC may alter the physical properties of the sludge, potentially interfering with the binding efficiency of PAHs by restricting BC dispersion throughout the matrix or creating zones of low PAH-BC interaction. Additionally, higher BC's concentrations may reach a saturation point for available binding sites, resulting in diminishing returns in PAHs immobilization efficiency. Moreover, with increased BC's doses (like 10 % dose), there is a heightened risk of C_{free} Σ 16 PAHs leaching from the BC itself [53,54], which can reduce the overall efficiency of PAHs reduction in the SSL. This phenomenon may be associated with the presence of surface-active compounds in the sludge [55,56], which, while facilitating the adsorption of PAHs (notably heavier ones) onto b-BC/n-BC, can also promote the desorption of other PAHs from the BC matrix [57,58].

3.2.3. The contribution of C_{free} Σ 16 PAHs in sewage sludge-b-BC/n-BC mixture

Evaluating the input of distinct PAHs groups following the treatment of SSL with b-BC or n-BC is crucial. This process enables the analysis of potential hazards linked to various PAHs, each possessing unique toxicological characteristics. The data indicate that both the dose and type of b-BC or n-BC significantly influence the distribution of PAHs by ring structure in SSL, with distinct trends emerging over short (1 hour) and prolonged (60 days) incubation periods (Fig. 2). Generally, 2-ring PAHs tend to increase in contribution over time, particularly for the 2.5 % dose, while heavier PAHs (3-, 4-, and 6-ring) typically decrease in response to this dose after prolonged incubation. The 5-ring PAHs consistently show substantial increases across b-BC and n-BC types and doses, although the magnitude varies, generally diminishing over time. The observed increase in the contribution of 2-ring PAHs over time, coupled with a decrease in 3-, 4-, and 6-ring PAHs, can be explained by their differing properties and interactions with SSL and BC's matrix. Lighter PAHs, such as 2-ring compounds, exhibit lower hydrophobicity and higher water solubility, enhancing their mobility in the SSL-b-BC/n-BC mixtures. Moreover, dissolved organic carbon (DOC) in SSL can enhance the mobility of lighter PAHs, further contributing to their increased relative contribution in mixtures with b-BC/n-BC. In contrast, 3-, 4-, and 6-ring PAHs, which are more hydrophobic and display stronger affinity for BC, are preferentially retained in BC, reducing their bioavailable fraction in SSL-b-BC/n-BC mixtures and contribution over time. These results suggest that the 2.5 % dose is often optimal for modifying PAHs distributions, leading to a selective increase in lighter PAHs and reduction in heavier PAHs, indicating dose-dependent and time-sensitive dynamics in PAHs stabilization and retention within the BC-treated SSL.

3.3. Ecotoxicological assessment

3.3.1. Microtox® test

Fig. 4 shows the toxicity of leachates to *A. fischeri* from untreated SSL and SSL amended with b-BC/n-BC after 1 hour (Fig. 4a) and after 60 days (Fig. 4b) of incubation. Generally, no significant differences ($p > 0.05$) were noted in the inhibition of *A. fischeri* luminescence between 5 and 15 minutes of testing, which is related to the sensitivity of the assay to different types of compounds: water-soluble substances, such as divalent metals, often require a longer exposure time (15 minutes) to elicit a toxic response [59], while organic compounds can affect the bacteria more rapidly, making the 5 minute exposure sufficient for detecting toxicity [60].

Leachates from untreated SSL showed high toxicity, with values 87.9 % and 96.6 % after 1 hour and 60 days of incubation, respectively. The addition of b-BC/n-BC markedly reduced the leachate toxicity, with toxicity reduction effects increasing with higher b-BC/n-BC dose. Depending on the type and dose of BC used, the leachates inhibition was from 15.9 to 63.1 % lower compared to untreated SSL (Fig. 4). Regarding feedstock, similar to the reduction in C_{free} Σ 16 PAHs content, b-BC and n-BC derived from RH proved more effective in reducing toxicity. Depending on the dose, the reduction in toxicity was 1.1-fold to 2.6-fold more effective compared to WL-derived b-BC and n-BC. It was observed that for the WL-derived b-BC and n-BC amendments, toxicity towards *A. fischeri* progressively decreased with longer incubation times (1 hour vs 60 days), a trend not seen with RH-derived b-BC and n-BC. For RH-derived b-BC and n-BC, the toxicity of leachates after 60 days either slightly increased (for certain doses) or remained unchanged compared to 1 hour incubation period (Fig. 4). It may suggest that with increasing of contact time between RH-derived BC and SSL the release of surface-bound or weakly adsorbed PAHs into the leachate. This is confirmed by Kendall's correlation analysis, which revealed significant ($p \leq 0.05$) correlations only for RH-derived b-BC and n-BC between the content of specific PAHs (Table S8) and luminescence inhibition after 5 minutes. The n-BCs demonstrated the highest capacity for reducing toxicity towards *A. fischeri* compared to b-BC, showing a 1.2-fold to 2.5-fold enhanced reduction in toxicity, depending on the dose used. In contrast to the content of C_{free} Σ 16 PAHs, the most effective reduction in toxicity after BC application was observed for the highest doses (5 % and 10 %) of both b-BC and n-BC. The data indicates that C_{free} Σ 16 PAHs are not the primary cause of the observed toxicity, as demonstrated by the enhanced reduction in C_{free} Σ 16 PAHs concentrations at both 2.5 % and 5 % dosage levels (Fig. 1). Importantly, the degree of toxicity reduction did not significantly differ ($p > 0.05$) between the 5 % and 10 % BC dose applications (Fig. 4). Our study reinforces the previous observation [61, 62] that the presence of BC in SSL can decrease the luminescence inhibition of *A. fischeri*, thereby lowering toxicity of this biosolid. The dose-dependent reduction in toxicity to *A. fischeri* with increasing amounts of b-BC or n-BC further underscores the profound influence of these amendments. Kończak et al. [62] have shown that adding to soil the mixture of BC with SSL effectively reduces toxicity, as indicated also by tests with *A. fischeri*. The authors reported that the addition of SSL enriched with BC to soil reduced the toxicity of leachates towards *A. fischeri* by 16.7 % (with 5 % BC content) and 38.1 % (with 10 % BC content) compared to soil enriched with SSL. In our study, we observed even greater reductions in the toxicity of SSL leachates enriched with b-BC and n-BC compared to untreated SSL, ranging from 15.9 to 61.4 % after 1 hour of incubation and from 22.2 to 63.1 % after 60 days of incubation, depending on the dose and type of BC. Notably, for the 5 % and 10 % doses, we observed higher toxicity reductions, ranging from 1.1- to 3.7-fold for b-BC and from 1.3- to 3.9-fold for n-BC, depending on the BC's type and incubation time.

3.3.2. Elongation test

Leachates from untreated SSL inhibited the germination of *L. sativum* by 46.7 % and 53.3 % after 1 hour and 60 days of incubation, respectively. However, the addition of b-BC/n-BC to the SSL eliminated this harmful effect, with no statistically significant differences ($p > 0.05$) observed between treatments with the b-BC/n-BC-amended SSL and control (Table S9). This suggests that the incorporation of b-BC/n-BC into SSL positively influences *L. sativum* germination. The addition of b-BC and n-BC likely because reduce the bioavailability of toxic compounds in the sludge [63], thereby mitigating their inhibitory effects on seed germination [64]. Additionally, the b-BC/n-BC may improve the overall environmental conditions for seed germination [65,66] as has been observed previously in BC-amended soils.

Fig. 5 illustrates the effect of b-BC/n-BC addition to SSL on root growth inhibition of *L. sativum*. For all tested doses of b-BC/n-BC added to SSL, a reduction in root growth inhibition was observed compared to

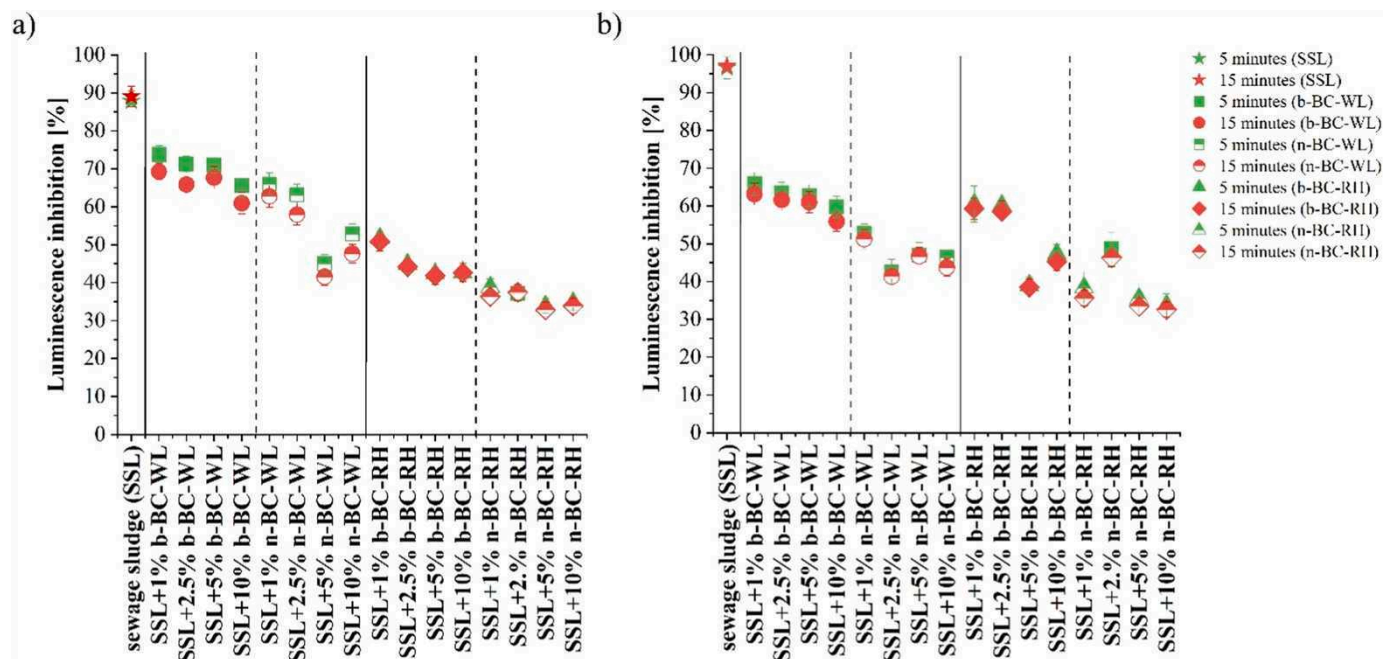


Fig. 4. Influence of leachates from SSL-b-BC/n-BC mixtures after 1 hour of incubation (a) and 60 days of incubation (b) on inhibition of *Aliivibrio fischeri* luminescence. Error bars represent the standard deviation of the mean (n = 3).

untreated SSL. These findings are consistent with previous study [67], where the application of activated carbon (AC) or BC to various SSL at doses ranging from 0.5 % to 10 % (w/w) positively impacted root growth, reducing the phytotoxic effects by 7.8–42 %, depending on the type and amount of BC used. WL-derived b-BC application to SSL demonstrated dose-dependent reduction in *L. sativum* root growth inhibition. Initial inhibition (1 hour) decreased 27–31 %, maintaining 23–25 % through 60 days of incubation. Further enhancement was observed when the particle size of WL-derived b-BC was reduced to

nano-scale. In this case, root growth inhibition was more pronounced, ranging from 30 to 49 % after 1 hour and from 23 to 33 % after 60 days of incubation (Fig. 5). In contrast to the Microtox® test and C_{free} $\Sigma 16$ PAHs content, RH-derived b-BC and n-BC demonstrated lower efficacy in reducing toxicity towards *L. sativum* than WL-derived b-BC and n-BC. This key observation indicates that, while RH-derived BC is more efficient in immobilizing C_{free} $\Sigma 16$ PAHs, WL-derived BC exhibits superior efficacy in mitigating toxicity. This suggests that toxicity may not be solely attributable to the presence of contaminants but instead results

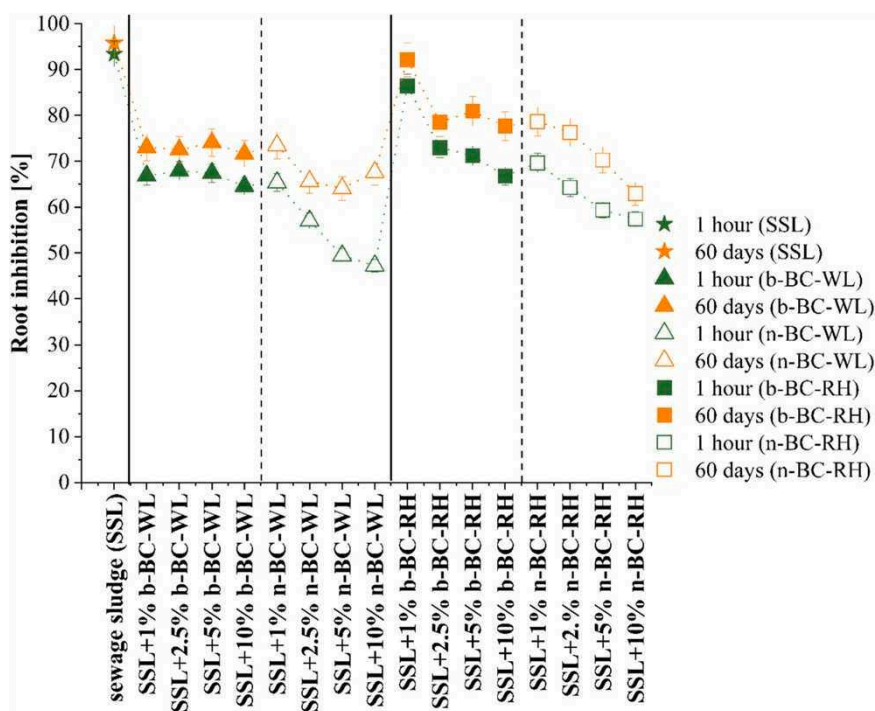


Fig. 5. Influence of leachates from SSL-b-BC/n-BC mixtures after 1 hour and 60 days of incubation on *Lepidium sativum* root growth inhibition. Error bars represent the standard deviation of the mean (n = 3).

from a complex interplay of multiple factors such as nutrient availability, microbial interactions, pH, and electrical conductivity (EC) changes, emphasizing the critical role of BCs as plant growth stimulators [68,69]. The b-BC/n-BC amendment can improve nutrient retention, enhancing the availability of essential macro- and micronutrients for plant uptake. Additionally, both b-BC and n-BC can reduce the bioavailability of other hazardous compounds, including potentially toxic elements (PTEs), thereby mitigating oxidative stress in plants. Changes in leachate pH induced by BC amendments may further influence nutrient solubility and metal toxicity, while reductions in electrical conductivity (EC) could alleviate osmotic stress. Thus, promoting optimal plant growth could help mitigate toxicity, even in the presence of pollutants, unlike the case with bacteria, where the effect is more directly correlated to contaminants. However, similar to WL-derived BC, reducing the particle size to nano-scale enhanced the efficiency of toxicity reduction. For RH-derived b-BC, the root growth inhibition was reduced by 7.4–28.4 % and from 3.8 to 18.8 % after 1 hour and 60 days, respectively. For RH-derived n-BC, the reductions in root growth inhibition ranged from 25 to 39 % after 1 hour and from 18 to 34 % after 60 days of incubation. Interestingly, the reduction in root growth inhibition of *L. sativum* was more pronounced in the SSL-b-BC/n-BC mixtures immediately after application (1 hour) than after 60 days of incubation (Fig. 5). However, this effect was diminished over the 60-day incubation period, suggesting that the initial impact may not persist in the long-term and that additional contaminants or harmful components may leach from the SSL over time or other factors, like as reduced nutrient availability, emerge that exhibit a toxic effect towards plants. However, studies demonstrate that carbon-rich materials, including BC, exhibit enhanced contaminant sorption capacity with aging [33,70,71]. While this increased binding should theoretically reduce contaminants' phytotoxic effects, the observed diminishing positive impact of BC application suggests alternative mechanisms at play. Recent research indicates that BC demonstrates dual functionality: it effectively sequesters contaminants [72, 73], while simultaneously reducing nutrient bioavailability [74]. This nutrient immobilization may explain the temporal decline in BC's effectiveness. The hypothesis is further supported by the observation of RH-derived BC, which exhibited reduced efficacy in phytotoxicity mitigation, potentially due to its heightened affinity for both contaminants and nutrients. Consequently, while b-BC/n-BC amendments initially reduce toxicity, prolonged incubation may necessitate additional treatment measures to maintain low levels of bioavailable contaminants in SSL following a 60-day incubation period.

The impact of b-BC/n-BC dosage varied depending on the feedstock type and particle size. For WL-derived b-BC, no statistically significant ($p > 0.05$) differences were observed in the reduction of *L. sativum* root growth inhibition across different doses (Fig. 5). However, when the particle size was reduced to the nanoscale (WL-derived n-BC), a clear trend emerged, showing that root growth inhibition decreased proportionally with increased dosage (Fig. 5). A similar dose-dependent pattern was observed for RH-derived BCs as well. This indicates that higher doses of b-BC and n-BC, especially those derived from specific feedstocks, enhance their effectiveness in reducing toxicity. The increased adsorption surface area and reactivity of higher doses likely improve their interaction with contaminants in the sewage sludge matrix, thereby amplifying their detoxifying effects. This interaction is not limited to PAHs but extends to other pollutants present in SSL that may exhibit phytotoxic effects. Additionally, BC itself is known to positively influence plant growth by improving soil properties and nutrient availability [75]. Studies have demonstrated that BC application can increase plant biomass and enhance soil fertility, particularly when combined with organic materials such as manure [76].

WL-derived n-BC enhanced root growth by 43–70 % (1 hour) and 17–46 % (60 days) versus b-BC. RH-derived n-BC showed more pronounced effects, with 36–241 % (1 hour) and 13–369 % (60 days) improvements. These enhanced mitigation effects stem from n-BC's reduced particle dimensions. The n-BC possesses a significantly larger

surface area (Table S2), offering more active sites for contaminant adsorption, thereby reducing the bioavailability of contaminants. Its heightened chemical reactivity enables stronger adsorption and immobilization of toxic compounds, preventing them from affecting plant growth. Additionally, n-BC disperses more evenly within the sludge matrix, ensuring uniform immobilization of contaminants. A few studies [77–80] have demonstrated the significant potential of n-BC in enhancing plant growth and mitigating environmental stress. Carbon nanoparticles improve plant growth by retaining and gradually releasing nutrients, as observed in wheat (*Triticum aestivum*) at an optimal concentration of 50 mg/L water-soluble carbon nanoparticles [81]. In rice, BC's nanoparticles have been shown to increase plant biomass and chlorophyll content while reducing cadmium phytotoxicity [82]. Additionally, BC's nanoparticles enhance physiological responses, such as chlorophyll production and antioxidant activity, which support overall plant health and resilience [83]. Thus, the impact of n-BC on plants is multifaceted, stemming not only from its ability to bind pollutants but also from its direct positive effects on plant growth and development, which are linked to its properties and nutrient content.

4. Conclusion

The application of biochar (b-BC) and nano-biochar (n-BC) to sewage sludge (SSL) significantly affects the C_{free} $\Sigma 16$ PAHs content, thus enhancing the ecotoxicological safety of the SSL. Both BC and n-BC significantly reduce the environmental risks associated with sewage sludge; however, n-BC demonstrates superior efficacy and is therefore more advantageous as a sewage sludge amendment for minimizing environmental hazards resulting from soil application of sewage sludge. Moreover, the efficiency of this immobilization also depends on factors such as BC's dose and feedstock type. The optimal b-BC/n-BC dose for reducing C_{free} $\Sigma 16$ PAHs content was identified to be within the 2.5–5 % range. Higher doses, especially with n-BC, tended to reduce the efficiency of PAHs immobilization. In contrast, for promoting root growth in *L. sativum* and reducing toxicity towards *A. fischeri*, doses within the 5–10 % range were found to be more beneficial. This study highlights the innovative application of n-BC as a highly effective material for PAHs reduction and improving ecological safety in sewage sludge treatment processes. The n-BC demonstrated superior performance compared to its bulk counterpart, showcasing its potential to achieve greater contaminant immobilization at moderate doses. However, the characteristics of SSL can vary significantly, which may influence PAH concentrations and behavior. Variations in organic content, mineral composition, and contamination levels across different sludge types can lead to different outcomes in biochar-based treatments. Despite the effectiveness of n-BC, future studies should explore a broader range of contaminants, such as potentially toxic elements (PTEs), per- and polyfluoroalkyl substances (PFAS), and other emerging pollutants in SSL, to assess the comprehensive safety of this approach. Furthermore, the potential risks of long-term n-BC use and the feasibility of large-scale n-BC production remain unresolved and warrant further investigation. By leveraging the advanced properties of n-BC, sludge management strategies can be significantly improved, offering safer reuse options and minimizing ecological risks associated with sludge contaminants.

Environmental Implication

This work addresses the critical challenge of mitigating polycyclic aromatic hydrocarbons (PAHs) in sewage sludge (SSL), a major environmental pollutant. By demonstrating the superior efficacy of nano-biochars (n-BC) compared to bulk biochars (b-BC) in reducing freely dissolved PAHs and ecotoxicity, this study advances sustainable sludge management practices. The enhanced stabilization of PAHs achieved through n-BC applications reduces environmental contamination risks and improves SSL reuse potential. Furthermore, the correlation between biochar mineral composition and PAH immobilization underscores the

importance of tailored biochar design for effective pollutant mitigation, contributing to a cleaner, healthier ecosystem.

CRedit authorship contribution statement

Oleszczuk Patryk: Writing – review & editing, Supervision, Resources, Formal analysis, Conceptualization. **Raczkiewicz Monika:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Conceptualization.

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. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jhazmat.2025.138224](https://doi.org/10.1016/j.jhazmat.2025.138224).

Data availability

Data will be made available on request.

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Supplementary material for:

Boosting sewage sludge safety with nano-biochar for polycyclic aromatic hydrocarbons immobilization and ecotoxicity reduction

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Supplementary text
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References

1. Physicochemical properties of sewage sludge and biochars

The porous structure of sewage sludge (SSL), bulk biochar (b-BC) and nano-biochars (n-BC) was analyzed using low-temperature nitrogen adsorption-desorption isotherms at 77.3 K with an ASAP 2420 analyzer (Micromeritics, USA). Key parameters, including specific surface area (S_{BET}), micropore surface area (S_{micro}), total pore volume (V_p), micropore volume (V_{micro}), and average pore diameter, were derived from nitrogen desorption data, as described elsewhere [1]. The pH and electrical conductivity (EC) of the biochar samples were assessed following the procedure [2]. Briefly, suspensions were prepared by mixing 1 g of biochar with 10 mL of deionized water, achieving a solid-to-liquid ratio of 1:10 (w/v). Measurements of pH and EC were taken using a digital meter (HQ430d Benchtop Single Input, HACH, USA or CP-401, ELMETRON, Poland). The hydrodynamic diameter of n-BCs was determined by dynamic light scattering (DLS) using a Zetasizer Nano ZS90 analyzer (Malvern Instrument Inc., UK) equipped with a universal dip cell. The intensity-weighted hydrodynamic particle size was determined assuming the Stokes-Einstein equation for spherical particles. Elemental composition, including carbon, hydrogen, and nitrogen, was determined using a Perkin–Elmer 2400 CHN analyzer (Akron, OH, USA). The ash content was measured according to the ASTM D3176 standard by combusting dried samples at 760°C for 6 hours [3]. The ash content was calculated using the following equation:

$$\% \text{ Ash} = \frac{\text{weight after ashing} - \text{weight of crucible}}{\text{dry weight of BC sample}} \cdot 100\% \quad (1)$$

The total content of O was determined by the following equation:

$$\% \text{ O} = 100\% - \% \text{ Ash} - \% \text{ C} - \% \text{ H} - \% \text{ N} \quad (2)$$

The structural properties and morphology of n- and b-BCs were characterized by a high-scanning electron microscope (FEI Quanta 3D FEG, USA). The SEM images were obtained with a resolution of 1–500 μm at 5 kV. The functional groups of BCs and n-BC were determined by FTIR-ATR spectra in the region of 400–4000 cm^{-1} with a resolution of 1 cm^{-1} (Nicolet 8700A, Thermo Scientific, USA) and X-ray photoelectron spectroscopy (XPS) (analytical system UHV Prevac, Poland).

1.1. The physicochemical characterization of SSL

The physicochemical parameters of the sewage sludge (SSL) are presented in Table S1. The pH value was measured at 6.37, while the electrical conductivity (EC) was 6.73 $\text{mS}\cdot\text{cm}^{-1}$. Elemental analysis revealed that the SSL primarily consisted of carbon (22.9%), with hydrogen (4.0%), nitrogen (2.9%), oxygen (26.4%), and ash content accounting for 43.8%. The SSL exhibited a very low specific surface area of 2.96 $\text{m}^2\cdot\text{g}^{-1}$ and a total pore volume of 0.025 $\text{m}^3\cdot\text{g}^{-1}$, classifying it as a non- or low-porosity material, consistent with findings from previous studies [4]. The total organic carbon (TOC) and dissolved organic carbon (DOC) contents

were measured at 19.2 mg·g⁻¹ and 633.3 mg·L⁻¹, respectively. The FT-IR spectra (Fig. S2) confirmed the universal composition of SSL through the identification of various functional groups. A broad band at 3416 cm⁻¹ corresponds to –OH stretching vibrations, indicative of the presence of water, acids, alcohols, or phenolic compounds [5]. The doublet peaks at 2923 cm⁻¹ and 2853 cm⁻¹ are attributed to asymmetrical and symmetrical C-H stretching vibrations in –CH₂ groups, likely originating from hydrocarbon chains associated with fats, surfactants and lipid residues. An intense band at 1640 cm⁻¹ is associated with C=O stretching vibrations of carboxyl anions, as well as C=C stretching in conjugation with other C=C or C=O bonds, or aromatic rings [6]. A sharp, peak at 1425 cm⁻¹ corresponds to –CH₃ bending vibrations and possibly O-H bending in phenolic compounds [7]. The broad peak in the 1112 cm⁻¹ range can be attributed to C–O stretching vibrations of polysaccharide-like substances and Si–O stretching from silicate and clay mineral residues [8]. This peak is strong in the SSL sample, likely due to its high ash content and mineral contributions in this spectral region.

Table S1. Physicochemical properties of sewage sludge.

Sample	S _{BET} [m ² /g]	V _p [cm ³ /g]	pH	EC [mS/cm]	C [%]	H [%]	N [%]	O [%]	Ash [%]	TOC [mg/g]	DOC [mg/L]
SSL	2.96±	0.025±	6.37±	6.73±	22.9±	4.0±	2.9±	26.4±	43.8±	19.2±	6330±
	0.1597	0.007593	0.4520	0.5514	0.7894	0.02843	0.0175	0.8796	0.7259	1.496	11.6

1.1. The physicochemical characterization of b-BC and n-BC

The analysis of the physicochemical properties of bulk biochars (b-BCs) and nanobiochars (n-BCs) revealed notable differences depending on both the feedstock and particle size (Table S2). In general, b-BCs exhibited an alkaline pH ranging from 9.02 to 9.71 and electrical conductivity (EC) values between 270 and 970 µS/cm. Biochars derived from rice husk (RH) showed higher pH and EC values—by 7.6% and 259%, respectively—compared to those obtained from willow (WL). This difference could indicate a greater mineral content or distinct chemical composition of the RH-based BCs. When the particle size was reduced to the nanoscale (n-BCs), a pH decrease of 5.97% to 17.65% was observed, while EC increased by 36.9% to 303%, depending on the feedstock used. These changes were more significant for WL-derived n-BCs than for those from RH, indicating a stronger effect of particle size reduction on the properties of WL-derived n-BC. Both b-BCs and n-BCs exhibited relatively low porosity overall (Table S2). However, the WL-derived b-BCs and n-BCs demonstrated the highest specific surface area (S_{BET}), reaching 195.2 m²/g for b-BC-WL and 195.8 m²/g for n-BC-WL—values that were 1.24 to 52.8 times higher than those of RH-derived b-BC and n-BC. This higher S_{BET} correlated with an increased pore volume (V_p), which was between 1.1- and 6.5- fold

greater, consistently favoring the WL-derived b-BC and n-BC across all measured parameters (Table S2). Elemental composition analysis revealed that both WL- and RH-derived b-BCs and n-BCs were predominantly made up of carbon (ranging from 34.9% to 79.2%) [9]. RH-derived samples, however, had a higher ash content (35.6% to 50.8%), likely due to the mineral-rich nature of the RH feedstock. The effects of ball milling on elemental composition (CHNO) and ash content were dependent on the feedstock. Similar to findings from our previous studies [9], milling led to a reduction in carbon content and an increase in ash for both WL- and RH-based biochars, possibly due to bond breakage and oxidation processes occurring during milling. Additionally, an increase in the oxygen-to-carbon (O/C) ratio was generally observed in n-BCs, suggesting a greater abundance of polar functional groups on their surfaces. This also corresponded with a reduction in aromaticity, indicated by a lower hydrogen-to-carbon (H/C) ratio compared to their bulk counterparts [10]. Regarding organic carbon content, WL-derived b-BCs and n-BCs showed higher total organic carbon (TOC), while RH-derived b-BC and n-BC had elevated dissolved organic carbon (DOC) levels (Table S2). This difference may be due to the higher bioavailable organic compound content in RH feedstock. For all b-BC samples tested, downsizing the particles to the nanoscale resulted in a TOC decrease of 28.9% to 36.6%, depending on the feedstock. In contrast, DOC levels increased after milling, possibly due to the release of previously trapped organic compounds during the mechanical breakdown of the biochar structure [11]. These findings underscore the importance of feedstock composition and biochar structure in determining surface charge characteristics, which are likely to influence both stability and adsorption performance in environmental applications.

Table S2. Physicochemical properties of bulk (b-BC) and nanobiochars (n-BC) produced at various temperatures and conditions by ball milling.

Sample	S _{BET} [m ² /g]	S _{micro} [m ² /g]	S _{ext} [m ² /g]	V _p [cm ³ /g]	V _{micro} [cm ³ /g]	Pore width [nm]	pH	EC [μS/cm]	O/C [molar ratio]	H/C [molar ratio]	Ash [%]	Average particle size [nm]	TOC [mg/g]	DOC [mg/L]
b-BC-WL	195.2± 13.50	170.5± 10.33	25.3± 1.760	0.091± 0.005287	0.075± 0.005099	1.85± 0.0720	9.02± 0.3931	270± 12.55	0.1089± 0.00445	0.4074± 0.0185	4.34± 0.5421	-	20.2± 1.121	8.50± 0.9438
n-BC-WL	195.8±10. 73	142.9± 8.136	52.4± 2.727	0.143± 0.007820	0.060± 0.003330	2.92± 0.1009	7.43± 0.2197	1089± 40.92	0.1199± 0.00454	0.3371± 0.0152	34.37± 1.610	48.1 ± 3.9	12.8± 1.6107	41.4± 3.4524
b-BC-RH	3.7± 0.2359	1.1± 0.07285	2.6± 0.1805	0.014± 0.000934	0.003± 0.000193	14.52± 0.9009	9.71± 0.4399	970± 41.18	0.2143± 0.00856	0.5178± 0.0225	35.63± 2.237	-	11.2± 0.5226	14.4± 2.441
n-BC-RH	158.6±	99.8±	58.8±	0.139±	0.042±	3.49±	9.13±	1328±	0.2683±	0.2435±	50.82±	87.9 ± 6.4	8.69±	25.6±

Where: b-BC- bulk biochar; n-BC-nanobiochar; WL-willow; RH-rice husk; 550-temperature of pyrolysis; S_{BET} -specific surface area of biochars; S_{micro} -specific surface area of micropores; S_{ext} - external surface area of biochars; V_p - pore volume; V_{micro} - micropore volume; pH- in H₂O; EC- electrical conductivity; O/C, H/C- molar ratios, TOC- total organic carbon. DOC-dissolved organic carbon. The values are mean of three repetitions.

2. C_{free} Σ16 PAHs content in biochars/nano-biochars

The type of feedstock and size influences the content of C_{free} Σ16 PAHs in biochar (b-BC) and nano-biochars (n-BC). Detailed information on the mechanism of reducing C_{free} Σ16 PAHs can be found in our previous work [9,12]. Briefly, the C_{free} Σ16 PAHs content was 99.3 ± 6.45 ng/L in b-BC-WL-550, while in b-BC-RH-500, it was notably lower, 43.1 ± 3.1 ng/L. For nanoscale counterparts the C_{free} Σ16 PAHs content was at level 97.4± 7.0 ng/L and 28.9± 1.9 ng/L, for n-BC-WL-550 and n-BC-RH-500, respectively (Table S2). Among the C_{free} Σ16 PAHs, 2- and 3-ring PAHs, specifically naphthalene (NAP) and phenanthrene (PHE), were predominant regardless of the type of feedstock in b-BC and n-BC. Specifically, in b-BC-WL-550 and n-BC-WL-550, the 2-ring PAHs accounted for 63.5% and 74.1%, respectively, while the 3-ring PAHs for 36.6% and 25.4%, respectively. For b-BC-RH-550 and n-BC-RH-550, the 2-ring PAHs represented 70.4% and 78.6%, respectively, with the 3-ring PAHs contributing 29.2% and 20.3%, respectively. This distribution indicates a general trend where 2-ring PAHs predominate in tested biochars. Additionally, after reducing the BC's particle size to the nanoscale, the contribution of 2-ring PAHs in n-BC became more pronounced at the expense of 3-ring PAHs.

Table S3. The content of individual C_{free} PAHs [ng/L] in b-BCs and n-BCs.

Sample	NAP	ACY	ACE	FLO	PHE	ANT	FLA	PYR	BaA	CHR	BbF	BkF	BaP	IcdP	DahA	BghiP	Σ16 PAHs [ng/L]
b-BC-WL	63.02± 4.51	0.19± 0.01	3.45± 0.29	12.32± 0.80	19.96± 2.14	0.1± 0.01	0.07± 0.01	0.11± 0.01	<LOD	<LOD	0.03± 0.003	<LOD	0.01± 0.005	<LOD	<LOD	<LOD	99.3± 6.45
n-BC-WL	72.2± 7.4	0.23± 0.01	1.47± 0.10	3.35± 0.28	19.7± 2.1	0.04± 0.01	0.1± 0.01	0.16± 0.01	<LOD	<LOD	0.08± 0.01	0.01± 0.00	0.08± 0.01	<LOD	<LOD	<LOD	97.4± 7.0
b-BC-RH-	30.4± 2.8	0.11± 0.01	0.48± 0.04	5.36± 0.37	6.55± 0.58	0.09± 0.01	0.05± 0.004	0.09± 0.009	<LOD	<LOD	<LOD	<LOD	0.05± 0.003	<LOD	<LOD	<LOD	43.1± 3.1
n-BC-RH	22.8± 2.6	0.06± 0.006	0.28± 0.02	1.98± 0.16	3.34± 0.31	0.24± 0.01	0.1± 0.01	0.21± 0.02	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	28.9± 1.9

Values are mean of three repetitions. <LOD – below limit of detection. NAP - naphthalene; ACY - acenaphthylene; ACE - acenaphthylene; FLO - fluorene; PHE - phenanthrene; ANT - anthracene; FLA - fluoranthene; PYR - pyrene; BaA - benz(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(g,h,i)perylene.

3. Limit of detection GC-MS

To ensure the accuracy and precision of the PAHs analysis in this investigation, a five-point calibration standard (15 to 250 ng·mL⁻¹) was prepared in solution, encompassing detection limits, procedural blanks, check standards, sample duplicates and matrix spike standards. The limit of detection (LOD) for each PAH is presented in Table S1. Extraction blanks (below 3 times LOD) were systematically analyzed within each sample batch and deducted from all sample measurements. The precision of both the extraction procedure and GC-MS analysis was evaluated by measuring three replicates of biochar samples (**b-BC-WL** and **b-BC-RH**) and sewage sludge, yielding a precision level below 20%.

Table S4. The limit of detection (LOD) of PAHs for GC-MS analysis.

Polycyclic aromatic hydrocarbons (PAHs)	LOD
Naphthalene [NAP]	0.00357
Acenaphthylene [ACY]	0.00242
Acenaphthene [ACE]	0.00256
Fluorene [FLO]	0.00217
Phenanthrene [PHE]	0.00240
Anthracene [ANT]	0.00174
Fluoranthene [FLA]	0.00124
Pyrene [PYR]	0.00073
Benzo[a]anthracene [BaA]	0.00042
Chrysene [CHR]	0.00103
Benzo[b]fluoranthene [BbF]	0.00010
Benzo[k]fluoranthene [BkF]	0.00058
Benzo[a]pyrene [BaP]	0.00192
Indeno[1.2.3-c.d]pyrene [IcdP]	0.00631
Dibenz[a,h]anthracene [DahA]	0.00815
Benzo[g,h,i]perylene [BghiP]	0.00464

4. The contribution of $C_{\text{free}} \sum 16$ PAHs in sewage sludge-biochar/nano-biochar mixture

The applied b-BC or n-BC in different dose influenced the contribution of PAHs regards to number of rings, with distinct trends observed after 1 hour and 60 days of incubation (Fig. 2). After 1 hour of incubation, the addition of WL-derived b-BC caused contribution increase of 2-rings PAHs and decrease in a 2.5% dose resulted in decreases in the contribution of 3-ring (14.3%), 4-ring (40.8%), and 6-ring (41.4%) PAHs compared to. Additionally, only this dose showed a 5.1% increase in the contribution of 2-ring PAHs untreated SSL. Notably, for 1%, 5%, and 10% doses of WL-derived b-BC, an increase in the contribution of 3- and 6-ring PAHs was observed, ranging from 24.2% to 41.5% and 34.3% to 55.4%, respectively. Furthermore, across all tested doses of WL-derived b-BC, an increase in the contribution of 5-ring PAHs was noted, with increases ranging from 567% to 2299%. The lowest increase in 5-ring PAH contribution was observed with the 5% dose of WL-derived b-BC. After 60 days of incubation, different trends were observed. For the 2.5% and 5% doses, the contribution of 2-ring PAHs decreased by 19.9% to 21.4%. Conversely, the contributions of 3- and 4-ring PAHs increased, with values ranging from 49.5% to 56.3% for 3-ring PAHs and 5.5% to 11% for 4-ring PAHs. For the remaining doses (1% and 10%), opposite trends were observed for 2-, 3-, and 4-ring PAHs compared to the 2.5% and 5% doses, indicating a dose-dependent variation in PAHs distribution over time. Also, an increase in the contribution of 5-ring PAHs was noted for all tested doses of WL-derived b-BC. However, these increases were 3- to 7-fold lower compared to the 1-hour incubation period. For 6-ring PAHs, a decrease in contribution was observed across all tested doses after 60 days. In the case of RH-derived b-BC added to SSL after 1 hour of incubation, only the 1% dose showed a 6.6% increase in the contribution of 2-ring PAHs. For the remaining doses, a decrease in the contribution of 2-ring PAHs was observed, ranging from 14.3% to 28.5%, with the highest decrease noted for the 5% dose. For 3- and 4-ring PAHs, a trend was observed where their contribution increased with higher doses compared to untreated SSL, with the highest increases occurring at the 5% dose, followed by a decrease at the 10% dose. Similar to the observations with WL--derived b-BC, the addition of RH-derived b-BC also led to an increase in the contribution of 5-ring PAHs, with the smallest increase in 5-ring PAH contribution observed at the 5% dose of RH-derived b-BC. After 60 days of incubation, the trends also shifted. The contribution of 2-ring PAHs increased with the dose, although a decline was observed at the 10% dose. For

3-ring PAHs, only the 5% dose resulted in a 14.9% decrease in their contribution, while the other doses showed increases ranging from 11.7% to 31.2%, with no clear trend. In contrast to the 1 hour incubation period, for 4- and 6-ring PAHs, all tested doses showed a decrease in contribution after 60 days. The trend in the contribution of 5-ring PAHs remained consistent with that observed for WL-derived b-BC after both 1 hour and 60 days of incubation

For n-BC, with WL-derived n-BC, after 1 hour of incubation the 2.5% dose resulted in the highest decrease (19.1%) in the contribution of 2-ring PAHs and the highest increase in the contributions of 3-, 4-, and 6-ring PAHs compared to untreated SSL. For the 1%, 5%, and 10% doses, a higher dose was associated with a lower decrease in the contribution of 2-ring PAHs. For 3-ring PAHs, the lowest increase in contribution was observed at the 1% and 10% doses. Regarding 4-ring PAHs, the 1%, 5%, and 10% doses led to a decrease in their contribution. Similar to b-BC, 5-ring PAHs showed high increases in contribution, ranging from 164% to 1274%. For 6-ring PAHs, no clear trend in changes was observed with increasing doses of WL-derived n-BC. In contrast to the results after 1 hour of incubation, after 60 days of incubation, the 2.5% and 5% doses led to an increase in the contribution of 2-ring PAHs and a decrease in the contribution of 3-ring PAHs. The remaining doses showed opposite trends in these changes. For 4- and 6-ring PAHs, a decrease in contribution was noted across all doses, with no clear dose-dependent trend. Similarly, 5-ring PAHs continued to show substantial increases in contribution after 60 days, ranging from 311% to 369%. In the case of RH-derived n-BC, no distinct trend was observed in the changes for 2-, 3-, and 6-ring PAHs. However, it is notable that the 2.5% dose showed the highest increase in the contribution of 2-ring PAHs and the highest decrease in the contribution of 6-ring PAHs. For 4-ring PAHs, the 2.5% dose caused the highest decrease in contribution, while at higher doses (5% and 10%), this effect was reduced. For 5-ring PAHs, high increases in contribution were observed, ranging from 1370% to 1753%, except for the 1% dose, which showed a 42% decrease in contribution. After 60 days of incubation, an increase in the contribution of 2-ring PAHs was observed for all tested doses, with the highest increase recorded for the 2.5% dose. Conversely, for 3-, 4-, and 6-ring PAHs, a decrease in contribution was noted, with the largest reductions also observed for the 2.5% dose. Notably, 5-ring PAHs continued to show substantial increases in contribution after 60 days, ranging from 431% to 1683%.

Table S5. The content of PAHs groups C_{free} PAHs [ng/L] in sewage sludge-biochar/nano-biochar mixtures after 1 hour and 60 days of incubation.

PAHs	Sewage sludge (SSL)	1 hour of incubation										60 days of incubation									
		SSL+1% b-BC-WL-550	SSL+2.5% b-BC-WL-550	SSL+5% b-BC-WL-550	SSL+10% b-BC-WL-550	SSL+1% n-BC-WL-550	SSL+5% n-BC-WL-550	SSL+10% n-BC-WL-550	SSL+1% b-BC-RH-550	SSL+2.5% b-BC-RH-550	SSL+5% b-BC-RH-550	SSL+10% b-BC-RH-550	SSL+1% n-BC-RH-550	SSL+2.5% n-BC-RH-550	SSL+5% n-BC-RH-550	SSL+10% n-BC-RH-550					
2 rings	146.8 ± 1.262	84.70 ± 0.3031	83.53 ± 0.3056	65.31 ± 0.2650	91.24 ± 0.2279	78.79 ± 0.3201	54.60 ± 0.1544	58.23 ± 0.1325	58.38 ± 0.2170	35.86 ± 0.1296	31.10 ± 0.1302	37.00 ± 0.1049	29.92 ± 0.0872	26.91 ± 0.2691	26.29 ± 0.1053	3.87 ± 0.0800					
3 rings	52.76±1.235	52.49±0.1765	24.48±0.0757	40.55±0.1471	49.81±0.1828	40.71±0.1356	33.27±0.1334	30.87±0.0887	16.08±0.0526	20.31±0.0826	26.49±0.0933	20.96±0.0787	12.94±0.0407	10.36±0.8111	10.11±0.0376	12.39±0.0503					
4 rings	50.09±0.6456	33.32±0.1309	16.06±0.0850	27.20±0.1100	34.68±0.1210	29.39±0.1130	23.42±0.0998	20.27±0.0766	14.49±0.0642	12.22±0.0374	16.17±0.0649	15.71±0.0628	4.563±0.0175	1.822±0.0207	2.775±0.0107	6.443±0.0259					
5 rings	0.8010±0.0316	8.785±0.324	11.53±0.0652	2.904±0.0124	14.61±0.0595	6.848±0.0202	3.764±0.0158	2.241±0.0101	4.428±0.0115	2.974±0.0108	0.4612±0.0021	3.122±0.0109	0.0879±0.0007	1.928±0.0309	2.468±0.0077	2.261±0.0105					
6 rings	0.1167±0.0041	0.1122±0.0004	0.0370±0.006	0.0985±0.002	0.1250±0.003	0.1081±0.000	0.0814±0.000	0.0665±0.000	0.0796±0.000	0.0327±0.000	0.0639±0.000	0.0483±0.000	0.0094±0.000	0.0039±0.002	0.0080±0.000	0.0187±0.000					
		4	6	1	2	4	2	2	3	2	2	2	4	9	3	7					
2 rings	237.3 ± 1.084	222.4 ± 0.7004	128.3 ± 0.5624	131.0 ± 0.3201	252.5 ± 0.8376	138.0 ± 0.2986	128.5 ± 0.5432	151.8 ± 0.6414	123.0 ± 0.3878	109.3 ± 0.3994	102.2 ± 0.2719	109.3 ± 0.3839	121.4 ± 0.5137	87.29 ± 0.2929	84.90 ± 0.3006	92.05 ± 0.3786					
3 rings	77.550±0.2645	55.42±0.1979	78.25±0.3245	83.70±0.3332	47.65±0.1658	61.51±0.2140	58.23±0.2121	38.83±0.1154	67.14±0.2148	24.76±0.0972	19.93±0.0665	40.01±0.1314	34.838±0.1279	4.496±0.0159	6.665±0.0286	15.47±0.0491					
4 rings	74.15±0.2673	37.17±0.1602	52.80±0.2121	57.82±0.2005	35.89±0.1407	47.63±0.1639	35.58±0.1221	34.64±0.1574	36.16±0.1126	10.95±0.0411	15.40±0.0609	35.73±0.1097	25.972±0.1049	2.570±0.0073	3.454±0.0148	7.753±0.0260					

5 rings	1.355±0.0052	6.293±0.019	4.141±0.0164	1.829±0.0068	11.39±0.0445	10.87±0.0404	1.056±0.0046	4.635±0.0183	6.380±0.0230	3.508±0.0137	3.939±0.0195	3.436±0.0144	2.680±0.104	3.422±0.0139	2.570±0.0076	6.264±0.0292	3.076±0.0103
6 rings	0.3354±0.023	0.1453±0.001	0.2189±0.001	0.1908±0.001	0.1287±0.001	0.1798±0.001	0.1476±0.000	0.1155±0.000	0.1862±0.000	0.1257±0.000	0.0427±0.000	0.0774±0.000	0.1552±0.000	0.0939±0.000	0.0128±0.000	0.0162±0.000	0.0297±0.001

Table S6. The content of individual C_{free} PAHs [ng/L] in sewage sludge and sewage sludge-biochar/nano-biochar mixtures after 1 hour of incubation.

PAHs	Sewage sludge (SSL)																
	SSL+1% b-BC-WL-550	SSL+2.5% b-BC-WL-550	SSL+5% b-BC-WL-550	SSL+10% b-BC-WL-550	SSL+1% n-BC-WL-550	SSL+2.5% n-BC-WL-550	SSL+5% n-BC-WL-550	SSL+10% n-BC-WL-550	SSL+1% b-BC-RH-550	SSL+2.5% b-BC-RH-550	SSL+5% b-BC-RH-550	SSL+10% b-BC-RH-550	SSL+1% n-BC-RH-550	SSL+2.5% n-BC-RH-550	SSL+5% n-BC-RH-550	SSL+10% n-BC-RH-550	
NAP	146.8±1.262	84.70±0.3031	83.53±0.3056	65.31±0.2650	91.24±0.2279	78.79±0.3201	54.60±0.1544	58.23±0.1325	97.83±0.3355	58.38±0.2170	35.86±0.1296	31.10±0.1302	37.00±0.1049	29.92±0.0872	26.91±0.1053	26.29±0.1053	3.87±0.0800
ACY	2.006±0.0154	0.6909±0.0024	1.179±0.0049	1.034±0.0035	1.480±0.0054	1.205±0.0029	0.9102±0.0035	1.095±0.0042	1.347±0.0053	1.031±0.0034	0.8423±0.0029	1.703±0.0072	0.7060±0.0024	0.8745±0.0028	0.8111±0.0019	0.6916±0.0019	0.6471±0.0027
ACE	11.098±0.0729	12.12±0.0513	6.683±0.0184	9.382±0.0358	12.41±0.0497	5.523±0.0202	7.692±0.0347	7.499±0.0328	11.41±0.0466	7.554±0.0302	5.670±0.0199	8.151±0.0320	5.293±0.0174	3.639±0.0109	2.251±0.0055	1.806±0.0055	3.063±0.0109
FLO	9.377±0.1657	7.802±0.0228	2.865±0.0069	5.859±0.0228	6.767±0.0298	6.812±0.0285	4.884±0.0213	4.344±0.0089	6.366±0.0278	4.678±0.0119	2.730±0.0106	3.386±0.0146	2.740±0.0122	1.452±0.0058	1.129±0.0052	1.089±0.0052	1.388±0.0044
PHE	27.84±0.9392	28.81±0.0870	12.40±0.0392	21.99±0.0750	26.28±0.0846	24.79±0.0737	18.31±0.0683	15.91±0.0373	24.29±0.0990	1.192±0.0032	10.20±0.0464	12.03±0.0350	11.34±0.0436	6.319±0.0182	5.801±0.0243	6.346±0.0243	6.760±0.0301
ANT	2.438±0.0415	3.0660±0.0130	1.354±0.0062	2.286±0.0100	2.871±0.0133	2.380±0.0103	1.473±0.0056	2.025±0.0055	2.624±0.0097	1.628±0.0039	0.8677±0.0028	1.218±0.0045	0.8763±0.0031	0.6559±0.0030	0.3681±0.0007	0.1788±0.0007	0.5300±0.0022

FLA	34.56 ±	18.11 ±	8.183 ±	14.72 ±	18.51 ±	15.98 ±	12.39 ±	10.99 ±	17.16 ±	12.17 ±	6.457 ±	8.747 ±	8.308 ±	2.202 ±	0.8467 ±	1.283 ±	3.141 ±
PYR	0.1429 ±	0.0687 ±	0.0368 ±	0.0629 ±	0.0621 ±	0.0550 ±	0.0562 ±	0.0428 ±	0.0704 ±	0.0565 ±	0.0235 ±	0.0382 ±	0.0333 ±	0.0080 ±	0.8467 ±	0.0042 ±	0.0118 ±
	13.22 ±	12.92 ±	7.038 ±	10.58 ±	13.62 ±	11.42 ±	8.972 ±	7.931 ±	12.64 ±	0.9036 ±	5.088 ±	6.210 ±	6.546 ±	2.148 ±	0.8981 ±	1.366 ±	3.000 ±
	0.4361 ±	0.0528 ±	0.0205 ±	0.0395 ±	0.0507 ±	0.0500 ±	0.0363 ±	0.0286 ±	0.0324 ±	0.0032 ±	0.0112 ±	0.0219 ±	0.0262 ±	0.0088 ±	0.8981 ±	0.0060 ±	0.0128 ±
BaA	0.7268 ±	0.7298 6	0.2333 ±	0.5331 ±	0.8367 ±	0.5921 ±	1.031 ±	0.3845 ±	0.7752 ±	0.3322 ±	0.1602 ±	0.3894 ±	0.2080 ±	0.0489 ±	0.0170 ±	0.0245 ±	0.0650 ±
	0.0202 9	±	0.0036 ±	0.0023 ±	0.0035 ±	0.0021 ±	0.0044 ±	0.0015 ±	0.0024 ±	0.0016 ±	0.0005 ±	0.0017 ±	0.0008 ±	0.0002 ±	0.0170 ±	0.0001 ±	0.0003 ±
CHR	1.591 ±	1.572 ±	0.6007 ±	1.364 ±	1.717 ±	1.405 ±	1.027 ±	0.9662 ±	1.608 ±	1.083 ±	0.5113 ±	0.8225 ±	0.6506 ±	0.1644 ±	0.0597 ±	0.1013 ±	0.2360 ±
	0.0462 6	0.0062 ±	0.0241 ±	0.0053 ±	0.0047 ±	0.0059 ±	0.0029 ±	0.0037 ±	0.0055 ±	0.0029 ±	0.0022 ±	0.0031 ±	0.0025 ±	0.0005 ±	0.0597 ±	0.0004 ±	0.0010 ±
BbF	0.3623 4	0.3581 2	0.1235 ±	0.3030 ±	0.4036 ±	0.3377 ±	0.1956 ±	0.2145 ±	0.4029 ±	0.2221 ±	0.1170 ±	0.2034 ±	0.1431 ±	0.0355 7	0.0156 ±	0.0268 ±	0.0581 6
	±	±	0.0046 ±	0.0011 ±	0.0016 ±	0.0010 ±	0.0008 ±	0.0008 ±	0.0015 ±	0.0006 ±	0.0003 ±	0.0008 ±	0.0004 ±	0.0001 ±	0.0156 ±	0.0001 ±	0.0002 ±
BkF	0.2600 ±	0.2674 ±	0.0974 ±	0.1265 ±	0.3008 ±	0.2447 ±	0.1835 ±	0.1643 ±	0.2866 ±	0.1928 ±	0.0845 ±	0.1547 ±	0.1133 ±	0.0257 2	0.0043 ±	0.0172 ±	0.0442 6
	0.0083 65	0.0007 2	0.0045 ±	0.0005 ±	0.0009 ±	0.0010 ±	0.0007 ±	0.0008 ±	0.0014 ±	0.0008 ±	0.0004 ±	0.0005 ±	0.0005 ±	0.0001 ±	0.0042 ±	0.0005 ±	0.0002 ±
BaP	0.1664 ±	8.1478 ±	11.31 ±	2.463 ±	13.89 ±	6.255 ±	3.378 ±	1.859 ±	0.7995 ±	4.005 ±	2.769 ±	0.0967 ±	2.846 ±	0.0255 ±	1.908 ±	2.424 ±	2.157 ±
	0.0060 5	0.0295 ±	0.0504 ±	0.0083 ±	0.0563 ±	0.0175 ±	0.0140 ±	0.0083 ±	0.0018 ±	0.0097 ±	0.0099 ±	0.0004 ±	0.0098 ±	0.0001 ±	0.0090 8	0.0067 ±	0.0092 ±
IcdP	0.0554 01	0.0531 ±	0.0156 ±	0.0466 ±	0.0574 5	0.0507 5	0.0395 ±	0.0317 ±	0.0531 9	0.0377 ±	0.0149 3	0.0297 0	0.0212 ±	0.0041 ±	0.0017 ±	0.0034 ±	0.0082 ±
	±	0.0002 ±	0.0056 ±	0.002 ±	0.0002 ±	0.0002 ±	0.0001 ±	0.0001 ±	0.0002 ±	0.0001 ±	0.0001 ±	0.0001 ±	0.0001 ±	0.0002 ±	0.0017 ±	0.0001 ±	0.0003 ±
DahA	0.0121 3	0.0118 ±	0.0034 ±	0.0110 ±	0.0129 2	0.0109 3	0.0076 ±	0.0026 ±	0.0125 ±	0.0079 ±	0.0037 54	0.0064 ±	0.0195 ±	0.0011 ±	0.0003 ±	0.0006 ±	0.0013 4
	±	0.0004 ±	0.0001 ±	0.0005 ±	0.0005 ±	0.0005 ±	0.0002 ±	0.0001 ±	0.0005 ±	0.0003 ±	0.0001 ±	0.0003 ±	0.0001 ±	0.0002 ±	0.0003 ±	0.0003 ±	0.0006 ±
BghiP	0.0613 ±	0.0591 ±	0.0214 ±	0.0519 ±	0.0675 0	0.0573 8	0.0419 ±	0.0347 ±	0.0626 ±	0.0419 ±	0.0177 9	0.0342 ±	0.0271 ±	0.0053 ±	0.0022 ±	0.0047 ±	0.0106 ±
	0.0020 2	±	0.0001 ±	0.0001 ±	0.003 ±	0.0002 ±	0.0001 ±	0.0001 ±	0.0002 ±	0.0002 ±	0.0001 ±	0.0001 ±	0.0001 ±	0.0002 ±	0.0012 ±	0.0002 ±	0.0004 ±
Σ16 PAHs	250.3 ±	179.4 ±	135.6 ±	136.1 ±	190.5 ±	155.9 ±	115.1 ±	111.7 ±	177.7 ±	93.46 ±	71.40 ±	74.28 ±	76.84 ±	47.52 ±	41.03 ±	41.66 ±	44.98 ±
[ng/L]	2.394	5.133	3.077	4.705	4.879	6.561	3.695	0.9853	4.801	2.726	3.172	1.527	2.528	1.385	1.443	1.432	1.338

Values are mean of three repetitions. <LOD – below limit of detection. NAP - naphthalene; ACY - acenaphthylene; ACE - acenaphthene; FLO - fluorene; PHE - phenanthrene; ANT - anthracene; FLA - fluoranthene; PYR - pyrene; BaA - benz(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(g,h,i)perylene.

Table S7. The content of individual C_{free} PAHs [ng/L] in sewage sludge-biochar/nano-biochar mixtures after 60 days of incubation.

PAHs	Sewage sludge (SSL)																
	SSL+1% b-BC-WL-550	SSL+2.5% b-BC-WL-550	SSL+5% b-BC-WL-550	SSL+10% b-BC-WL-550	SSL+1% n-BC-WL-550	SSL+2.5% n-BC-WL-550	SSL+5% n-BC-WL-550	SSL+10% n-BC-WL-550	SSL+1% b-BC-RH-550	SSL+2.5% b-BC-RH-550	SSL+5% b-BC-RH-550	SSL+10% b-BC-RH-550	SSL+1% n-BC-RH-550	SSL+2.5% n-BC-RH-550	SSL+5% n-BC-RH-550	SSL+10% n-BC-RH-550	
NAP	237.3 ±	222.4 ±	128.3 ±	131.0 ±	252.5 ±	138.0 ±	128.5 ±	151.8 ±	183.3 ±	123.0 ±	109.3 ±	102.2 ±	109.3 ±	121.4 ±	87.29 ±	84.90 ±	92.05 ±
ACY	1.084 ±	0.7004 ±	0.5624 ±	0.3201 ±	0.8376 ±	0.2986 ±	0.5432 ±	0.6414 ±	0.5914 ±	0.3878 ±	0.3994 ±	0.2719 ±	0.3839 ±	0.5137 ±	0.2929 ±	0.3006 ±	0.3786 ±
ACE	3.328 ±	3.145 ±	2.739 ±	3.683 ±	5.711 ±	2.138 ±	1.912 ±	2.217 ±	3.128 ±	2.566 ±	0.2141 ±	2.537 ±	2.086 ±	1.681 ±	0.1566 ±	0.1298 ±	1.897 ±
	0.0097 ±	0.0135 ±	0.0126 ±	0.0147 ±	0.0231 ±	0.0082 ±	0.0081 ±	0.0072 ±	0.0127 ±	0.0071 ±	0.0008 ±	0.0062 ±	0.0070 ±	0.0067 ±	0.0007 ±	0.0005 ±	0.0073 ±
	18.59 ±	16.44 ±	20.82 ±	20.05 ±	14.69 ±	15.09 ±	15.44 ±	12.69 ±	19.54 ±	13.94 ±	5.864 ±	6.919 ±	12.21 ±	10.99 ±	1.203 ±	2.754 ±	6.442 ±
FLO	0.0767 ±	0.0655 ±	0.0797 ±	0.0854 ±	0.0517 ±	0.0454 ±	0.0515 ±	0.0442 ±	0.0775 ±	0.0400 ±	0.0168 ±	0.0256 ±	0.0343 ±	0.0391 ±	0.0045 ±	0.0134 ±	0.0152 ±
	15.67 ±	8.568 ±	12.31 ±	12.70 ±	6.064 ±	9.625 ±	5.508 ±	5.525 ±	10.81 ±	8.268 ±	3.140 ±	2.266 ±	5.438 ±	4.644 ±	0.9637 ±	0.9523 ±	1.744 ±
PHE	0.0497 ±	0.0319 ±	0.0508 ±	0.0527 ±	0.0260 ±	0.0345 ±	0.0304 ±	0.0216 ±	0.0260 ±	0.0312 ±	0.0085 ±	0.0089 ±	0.0194 ±	0.0181 ±	0.0033 ±	0.0040 ±	0.0077 ±
	36.01 ±	23.99 ±	37.29 ±	40.76 ±	18.09 ±	30.79 ±	27.84 ±	16.42 ±	29.90 ±	24.87 ±	14.15 ±	6.560 ±	17.84 ±	15.17 ±	1.595 ±	2.012 ±	4.212 ±
ANT	0.1176 ±	0.0745 ±	0.1615 ±	0.1587 ±	0.0516 ±	0.1088 ±	0.1090 ±	0.0343 ±	0.0871 ±	0.0793 ±	0.0662 ±	0.0202 ±	0.0604 ±	0.0542 ±	0.0060 ±	0.0083 ±	0.0152 ±
	3.952 ±	3.276 ±	5.095 ±	6.513 ±	3.099 ±	3.868 ±	3.535 ±	1.970 ±	3.763 ±	3.336 ±	1.393 ±	1.651 ±	2.438 ±	2.352 ±	0.5781 ±	0.8167 ±	1.176 ±
	0.0108 ±	0.0125 ±	0.0199 ±	0.0217 ±	0.0134 ±	0.0171 ±	0.0131 ±	0.0081 ±	0.0115 ±	0.0095 ±	0.0049 ±	0.0056 ±	0.0103 ±	0.0098 ±	0.0014 ±	0.0024 ±	0.0037 ±
FLA	47.15 ±	19.69 ±	27.89 ±	30.98 ±	18.32 ±	25.44 ±	19.16 ±	17.84 ±	23.95 ±	19.50 ±	5.076 ±	7.320 ±	18.73 ±	12.91 ±	1.054 ±	1.508 ±	3.589 ±
	0.1691 ±	0.0880 ±	0.1108 ±	0.1148 ±	0.0749 ±	0.0772 ±	0.0627 ±	0.0849 ±	0.0875 ±	0.0532 ±	0.0160 ±	0.0265 ±	0.0657 ±	0.0515 ±	0.0032 ±	0.0058 ±	0.0088 ±

PYR	23.12	14.75	21.10	22.99	15.39	18.70	13.83	14.78	18.63	14.04	5.347	7.304	14.46	11.65	1.399	1.795	3.812
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
BaA	0.0831	0.0599	0.0878	0.0705	0.0573	0.0729	0.0505	0.0666	0.0535	0.0505	0.0230	0.0313	0.0352	0.0491	0.0038	0.0083	0.0163
	1.216	0.8677	1.243	1.249	0.6236	1.159	0.8335	0.5069	0.9300	0.8502	0.1043	0.2078	0.7664	0.3676	0.0280	0.0357	0.0824
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
CHR	0.0040	0.0037	0.0027	0.0039	0.0026	0.0043	0.0041	0.0016	0.0024	0.0033	0.0004	0.0008	0.0024	0.0014	0.0001	0.0001	0.0002
	2.661	1.862	2.565	2.610	1.559	2.330	1.760	1.506	2.169	1.780	0.4193	0.5723	1.777	1.045	0.088	0.1152	0.2703
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	0.0111	0.0086	0.0108	0.0113	0.0059	0.0095	0.0048	0.0043	0.0094	0.0056	0.0017	0.0023	0.0064	0.0029	0.0002	0.0006	0.0007
BbF	0.6047	0.4407	0.5936	0.5243	3.108	0.5289	0.3833	0.3362	0.5027	0.4014	0.0953	0.1776	0.4216	0.3247	0.041	0.0412	0.0670
	±	±	±	±	±	±	±	±	±	±	1 ±	±	±	±	±	±	±
	0.0024	0.0014	0.0026	0.0021	0.0153	0.0023	0.0015	0.0015	0.0016	0.0014	0.0004	0.0008	0.0016	0.0011	0.0001	0.0002	0.0002
BkF	0.4353	0.2071	0.2858	0.2761	4.310	0.2551	0.1778	0.1853	0.2399	0.1809	0.0652	0.1331	0.2200	0.1555	0.0163	0.010	0.0377
	±	±	±	±	±	±	±	±	±	±	0 ±	±	±	±	±	±	±
	0.0015	0.0006	0.0011	0.0011	0.0125	0.0011	0.0008	0.0006	0.0011	0.0008	0.0002	0.0004	0.0008	0.0007	0.0005	0.0003	0.0002
BaP	0.2766	5.631	3.183	1.013	3.937	10.02	0.4868	4.104	5.546	2.917	3.778	3.081	2.030	2.940	2.499	6.211	2.935
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	0.0009	0.0159	0.0121	0.0026	0.0164	0.0365	0.0018	0.0158	0.0198	0.0110	0.0185	0.0129	0.0073	0.0118	0.0062	0.0281	0.0091
IcdP	0.0924	0.0494	0.0798	0.0693	0.0478	0.0631	0.0531	0.0422	0.0732	0.0379	0.0160	0.0357	0.0616	0.0364	0.0061	0.0047	0.0162
	±	±	±	±	±	±	9 ±	±	±	±	±	1 ±	±	±	±	±	±
	0.0003	0.0002	0.0003	0.0003	0.0002	0.0003	0.0002	0.0001	0.0002	0.0001	0.0001	0.0001	0.0003	0.0002	0.0003	0.0002	0.0007
DahA	0.0384	0.0141	0.0779	0.0154	0.0393	0.0682	0.0086	0.0097	0.0914	0.0092	0.0011	0.0444	0.0083	0.0026	0.014	0.0012	0.0361
	±	±	±	±	±	±	1 ±	4 ±	±	±	±	9 ±	±	±	±	±	±
	0.0001	0.0005	0.0003	0.0007	0.0001	0.0002	0.0003	0.0003	0.0003	0.0004	0.0003	0.0002	0.0004	0.0001	0.0005	0.0004	0.0001
BghiP	0.3354	0.0959	0.1391	0.1216	0.0809	0.1167	0.0944	0.0733	0.1130	0.0878	0.0267	0.0417	0.0936	0.0575	0.0067	0.0115	0.0137
	±	±	±	±	±	±	±	±	±	±	2 ±	±	±	±	±	±	±
	0.002	0.0003	0.0004	0.0005	0.0003	0.0004	0.0004	0.0003	0.0004	0.0004	0.0001	0.0001	0.0003	0.0002	0.0003	0.0005	0.0003
Σ16 PAHs	390.7±	321.4±	263.7±	274.6±	347.6±	258.2	223.5±	230.0±	302.7±	215.7±	148.9±	141.1±	187.9±	185.7±	96.94±	101.3±	118.4±
[ng/L]	2.818	5.413	4.031	3.904	5.702	±9.215	3.804	8.664	6.507	1.734	2.210	1.064	2.094	5.5112	0.972	3.197	5.725

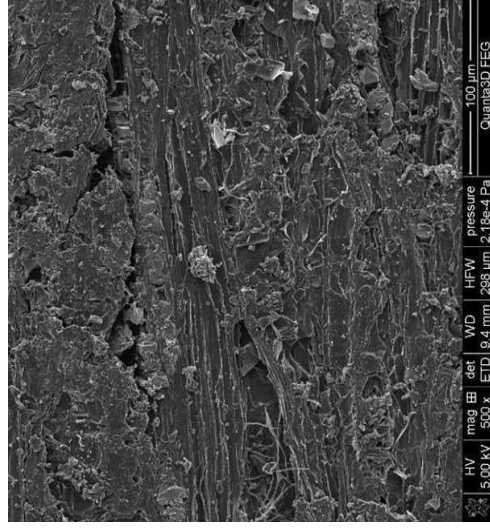
Values are mean of three repetitions. <LOD – below limit of detection. NAP - naphthalene; ACY - acenaphthylene; ACE - acenaphthene; FLO - fluorene; PHE - phenanthrene; ANT - anthracene; FLA - fluoranthene; PYR - pyrene; BaA - benz(a)anthracene; CHR - chrysene; BbF - benzo(b)fluoranthene; BkF - benzo(k)fluoranthene; BaP - benzo(a)pyrene; IcdP - indeno(1.2.3-cd)pyrene; DahA - dibenz(a,h)anthracene; BghiP - benzo(g,h,i)perylene.

Table S9. *Lepidum sativum* germination test results after 72H for elongation test.

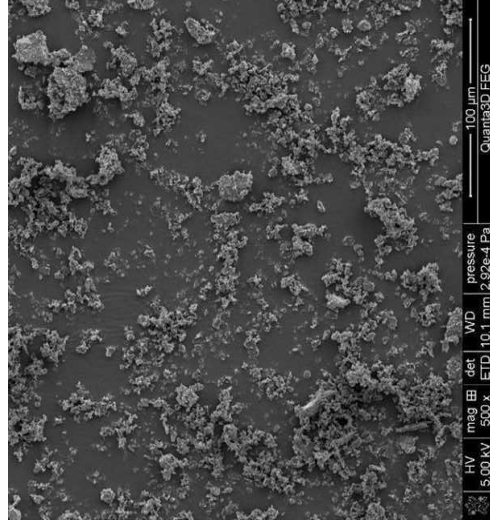
Sample	Replicates	The number of germinated seeds after 1 hour of incubation	The number of germinated seeds after 60 days of incubation
Control	K1	15	15
	K2	15	14
	K3	15	15
Sewage sludge (SSL)	I	8	7
	II	8	7
	III	7	6
SSL+1% b-BC-WL	I	14	13
	II	13	14
	III	14	15
SSL+2.5% b-BC-WL	I	15	13
	II	13	13
	III	13	13
SSL+5% b-BC-WL	I	14	14
	II	14	14
	III	14	14
SSL+10% b-BC-WL	I	13	13
	II	13	13
	III	14	14
SSL+1% b-BC-RH	I	14	13
	II	15	14
	III	13	15
SSL+2.5% b-BC-RH	I	15	13
	II	14	13
	III	13	13
SSL+5% b-BC-RH	I	14	14
	II	15	14

	III	13	14
SSL+10% b-BC-RH	I	13	13
	II	14	13
	III	14	14
SSL+1% n-BC-WL	I	14	14
	II	13	12
	III	14	13
SSL+2.5% n-BC-WL	I	15	15
	II	13	13
	III	13	14
SSL+5% n-BC-WL	I	14	15
	II	14	13
	III	14	13
SSL+10% n-BC-WL	I	13	13
	II	13	14
	III	14	14
SS+1% n-BC-RH	I	14	14
	II	15	13
	III	13	13
SSL+2.5% n-BC-RH	I	15	13
	II	14	14
	III	13	15
SSL+5% n-BC-RH	I	14	13
	II	15	13
	III	13	13
SSL+10% n-BC-RH	I	13	14
	II	14	14
	III	14	14

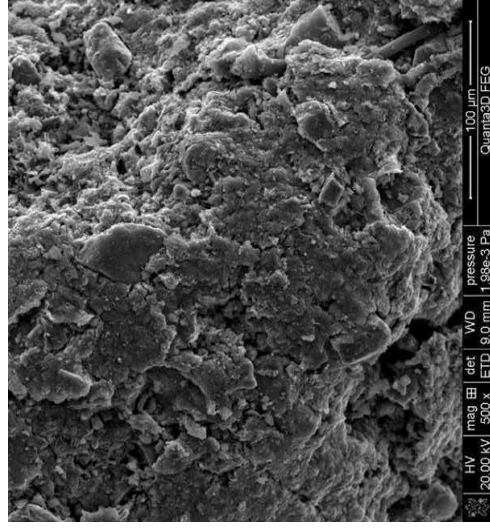
b-BC-WL



n-BC-WL



b-BC-RH



n-BC-RH

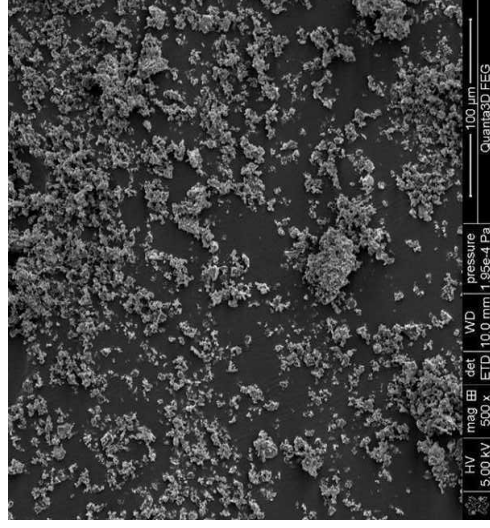


Figure S1. SEM images b-BC and n-BC samples.

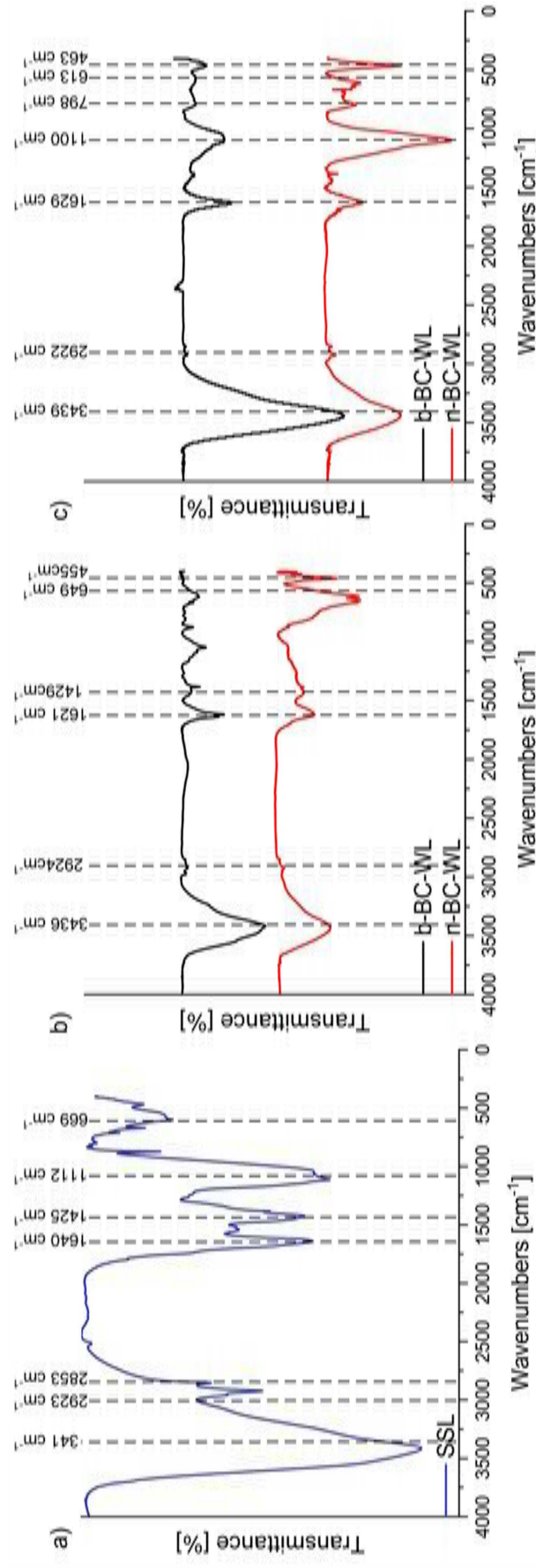


Figure S2. FT-IR spectra of SSL, b-BC and n-BC samples.

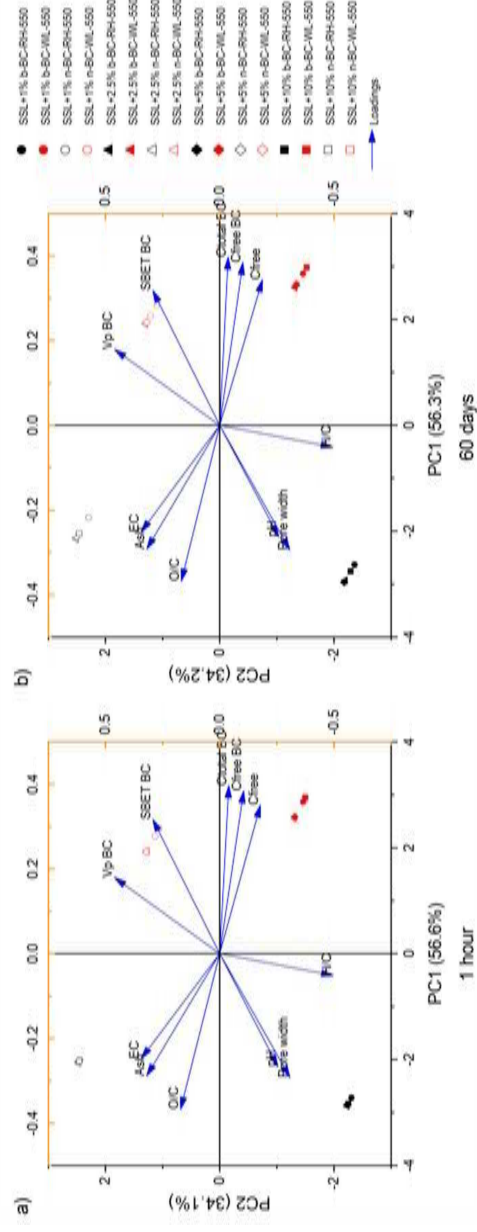


Figure S3. Principal component analysis (PCA) biplots illustrating the influence of BC type, dose, and contact time and physicochemical properties on Cfree $\Sigma 16$ PAHs immobilization in SSL-b-BC/n-BC mixtures leachate after 1 hour (a) and 60 days (b) of incubation.

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“Sustainable soil remediation using nano–biochar for improved food safety and resource recovery”

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Sustainable soil remediation using nano-biochar for improved food safety and resource recovery

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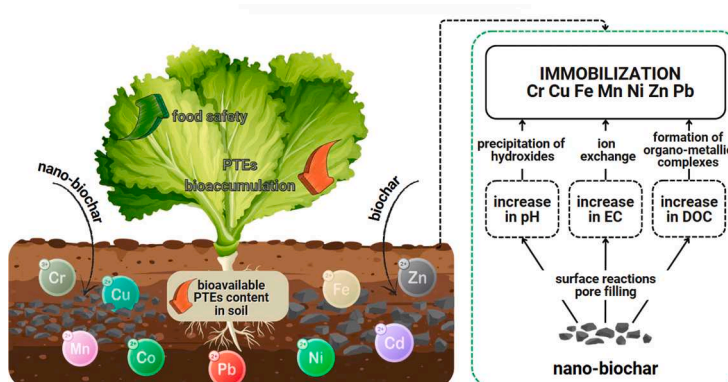
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HIGHLIGHTS

- Nano-biochar (n-BC) outperformed conventional biochar in immobilizing contaminants.
- N-BC led to a substantial decrease in PTEs bioaccumulation in lettuce leaves.
- Sludge-derived n-BC demonstrated the highest PTEs immobilization efficiency.

GRAPHICAL ABSTRACT



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ABSTRACT

The contamination of agricultural soils with potentially toxic elements (PTEs) poses serious environmental and health risks due to their persistence and adverse effects on crop productivity. The main objective of this study was to evaluate the potential of nano-biochar (n-BC) to immobilize PTEs in contaminated soil and its effect on PTEs bioaccumulation in lettuce (*Lactuca sativa* L.), with the hypothesis that n-BC—due to their unique and improved physicochemical properties—are more effective than bulk forms in reducing PTEs mobility and bioavailability. Biochars (BCs) were obtained from palm bunch (PB), rice husk (RH) and sewage sludge (SSL) at 550°C and subsequently processed into nanoscale forms. A six-week pot experiment demonstrated that n-BC amendments significantly reduced the bioavailable (extracted with H₂O and CaCl₂) fractions of Cr, Cu, Fe, Mn, Ni, Zn, and Pb in soil, with higher immobilization efficiencies by 4.2 % to even 305 % than corresponding bulk biochars (b-BC). According to NICA-Donnan modelling, the main immobilization mechanisms were precipitation and ion exchange. Application of n-BC also resulted in a notable decrease in PTEs concentrations in lettuce leaves (ranging from 29.7 % to 100 %), thereby reducing both the bioaccumulation factor and health risk index. Among the different BCs, SSL-derived n-BC demonstrated the highest immobilization capacity and the most substantial reduction in PTEs uptake by plants. These findings highlight the potential of n-BC as a highly effective and low-

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cost amendment for rapid mitigation PTEs contamination in agricultural soils, enhancing food safety, and supporting circular economy principles by utilizing organic waste materials.

1. Introduction

The increasing contamination of soils poses a significant threat to environment, agricultural productivity, and human health [1]. Potentially toxic elements (PTEs), such as Cr, Cu, Fe, Ni, Zn, Pb and As [2], persist in soils due to their non-degradable nature, accumulation potential which may disrupt soil microbial activity, reduce crop yields, and pose toxic risks to consumers of contaminated produce [3]. PTEs are introduced into the environment through industrial activities, agricultural inputs (e.g., fertilizers, pesticides), and improper waste disposal, leading to widespread environmental concerns [4].

Agricultural cultivation on contaminated soils further exacerbates the issue, as PTEs can accumulate in crops through root uptake, directly entering the food chain and threatening human health [5]. Crops grown in such soils often exhibit reduced growth, nutrient deficiencies, and physiological stress due to PTEs toxicity, resulting in lower yields and compromised quality [6–8]. This scenario is particularly concerning for staple crops and vegetables consumed raw, such as lettuce, which are more susceptible to PTEs accumulation. This issue is particularly severe in developing countries, including many regions of Africa, where rapid industrialization, increasing environmental pollution, and often insufficient enforcement of environmental protection regulations contribute to soil and water contamination [9]. In local conditions, where agriculture is often the backbone of the economy and a primary source of food for communities, PTEs pollution can lead to serious health and economic consequences. Food produced in contaminated soils, especially in uncontrolled urban and peri-urban environments, may contain high concentrations of PTEs, posing a threat to public health, particularly for children and individuals of lower socio-economic status who have limited access to safe food. Despite increasing awareness of the risks associated with PTEs contamination, there is still a lack of effective, affordable, easy-to-use, and locally applicable remediation strategies aimed at reducing PTEs bioavailability in soils and their accumulation in edible plants. There is a need to address this gap by evaluating the effectiveness of nano-biochar (n-BC) amendments, thereby contributing to the development of sustainable, practical, and safe agricultural practices for use in contaminated areas.

Biochars (BCs), derived from the pyrolysis of organic waste materials, have emerged as a promising solution for soil remediation [10–12]. Their unique properties, including a porous structure and high cation exchange capacity (CEC), make them effective in immobilizing PTEs, reducing their bioavailability in soils [13,14]. BC contributes to the stabilization of PTEs in soil through various pathways, such as binding with surface functional groups (complexation), forming low-solubility compounds (precipitation), and retaining PTEs via its porous matrix through adsorption processes. These mechanisms work synergistically to limit the mobility and environmental availability of PTEs ions [15]. Recent advances have highlighted that BCs can also enhance soil properties, such as water retention and microbial activity, thereby promoting plant growth [16,17]. Additionally, BCs can be easily produced locally from residues (e.g. agriculture residues), making them a cost-effective and sustainable solution for soil remediation in developing regions [18]. In many African countries, where soil degradation and contamination pose significant challenges to food security, BC application has been shown to improve soil fertility, enhance nutrient availability, and increase crop yields [19,20]. Given its ability to retain moisture and improve soil structure, BC is particularly beneficial for African soils, which are often prone to nutrient depletion and water scarcity [21]. Furthermore, BC production contributes to waste management by utilizing agricultural and organic waste materials that would otherwise contribute to pollution. This process aligns with the

principles of the circular economy and sustainability by converting waste into a valuable resource, reducing environmental impact, and promoting a closed-loop agricultural system [22]. By integrating BC into soil management practices, communities can enhance food security, mitigate PTEs contamination, and support a more sustainable and resilient agricultural sector.

While bulk biochars' (b-BCs) potential in soil improvement and remediation is well-established, nanoscale biochars - n-BC - demonstrate even more impressive properties and greater potential compared to traditional b-BC. The n-BC offers an exciting frontier for soil remediation research. These nano-sized particles, typically less than 100 nm in diameter, leads to more efficient soil enhancement capabilities. The n-BCs possess enhanced physicochemical properties compared to traditional b-BCs, including greater surface reactivity, higher adsorption capacities, and improved interactions with soil particles and contaminants [23,24]. Their smaller size allows them to penetrate soil structures more effectively, creating a more uniform distribution of beneficial properties throughout the soil matrix [25]. To date, the majority of studies have focused on the remediation potential of b-BCs, primarily examining their capacity to adsorb or immobilize PTEs in soils [26–29]. While these findings have demonstrated the efficacy of b-BCs, the n-BCs use is underexplored, despite existing evidence of their base remediation capabilities, suggesting untapped potential for optimizing both contaminant sequestration and soil ecosystem services. The n-BCs can be produced via various methods, mainly hydrothermal carbonization, sonication, and ball milling [30]. Among these, ball milling stands out as a top-down mechanical technique that reduces particle size to the nanoscale through the application of physical force without damaging the crystal structure. This approach has gained increasing interest due to its eco-friendly character, cost-effectiveness, scalability, and reliability in generating different types of nano-powders [23].

Conventional remediation techniques, including soil washing, chemical stabilization, and phytoremediation, often face limitations such as high costs, secondary pollution, and the inability to effectively address long-term contamination in agricultural soils [31,32]. Therefore, there is an urgent need for sustainable, cost-effective, and scalable solutions to mitigate PTEs pollution and ensure agricultural and food security. Immobilization-based technology is undoubtedly the leading remedial approach for soils contaminated with PTEs [33], and the use of carbon materials, like BCs in this context is gaining increasing recognition. The n-BC stands out as an exceptionally effective and versatile approach to environmental remediation, outperforming previous mentioned traditional methods. Due to its nanoscale structure, n-BC exhibits an extremely high surface area, enhanced porosity, and abundant functional groups, which greatly increase its adsorption capacity for PTEs [23,34]. Unlike phytoremediation, which is slow and limited in scope [35], n-BC works quickly and remains effective under diverse environmental conditions [36]. It also offers a safer alternative to chemical stabilization, without the risk of secondary pollution or negative impacts on soil chemistry [37]. Importantly, n-BC and b-BC not only immobilizes contaminants but also provides fertilizing and plant growth-promoting properties [38,39]. It contains readily available nutrients (such as potassium and calcium) and improves key soil properties, such as water retention, nutrient availability, and microbial activity [17]. This dual functionality—remediation and soil enhancement—makes n-BC a particularly attractive solution for the sustainable management of degraded and contaminated soils.

Our research seeks to bridge these gaps by evaluating the remediation potential of n-BC from palm bunch, rice husk and sewage sludge in PTEs-polluted soils. Utilizing these residues for n-BC production not only valorizes waste but also aligns with circular economy principles,

contributing to sustainable waste management practices [40]. Despite the growing interest in n-BC, most existing studies have concentrated on their applications in water treatment, with limited research addressing their role in soil remediation [41]. Moreover, the mechanisms underlying the interactions between n-BCs, PTEs, and plant growth remain insufficiently explored, particularly in the context of field-relevant scenarios involving edible crops. BCs can be produced from various materials. However, an essential direction is the utilization of waste-based feedstocks, particularly those generated locally. In many African countries, agricultural waste, such as palm bunches and rice husks, is abundant but often poorly managed [42]. Palm bunch waste is a significant issue in Africa, particularly in countries like Nigeria, where palm oil production is rapidly expanding. The growth of oil palm plantations contributes to deforestation, threatening biodiversity. Additionally, when left to decompose, these residues emit greenhouse gases (GHG) such as methane (CH_4) and carbon dioxide (CO_2), contributing to climate change. Additionally, open burning of agricultural waste, a common practice in many regions, releases toxic pollutants, further degrading air quality and harming both human health and the environment. Converting these locally available residues into BC and subsequently to more effective n-BC provides a sustainable waste management solution, simultaneously reducing emissions, mitigating pollution, and improving soil health.

This research examines how n-BCs interact with soil systems to enhance both soil properties and plant growth by reducing the accumulation of PTEs, while conducting thorough risk-benefit analyses of n-BCs applications in agriculture to evaluate potential impacts on food safety and human health. To further investigate the indirect effects of contaminant immobilization, we assessed the bioaccumulation of PTEs in lettuce (*Lactuca sativa* L.). Additionally, the health risk index (HRI) was used to evaluate potential health risks associated with lettuce consumption by adults and children, providing a comprehensive understanding of the implications for food safety and human health after use n-BC as an immobilization agent. Integrating of advanced nanomaterial science with agricultural and environmental research, this study offers a novel and holistic approach to managing PTEs-contaminated soils. By utilizing locally available waste materials for the production of advanced remediation materials, this approach not only enhances soil decontamination efficiency but also promotes circular economy principles and sustainability, transforming waste into valuable resources for environmental restoration and agricultural improvement.

2. Materials and method

2.1. Bulk biochar and nano-biochar preparation and characterization

Palm bunch (PB) rice husk (RH), and sewage sludge (SSL) were used to produce bulk biochars (b-BCs) and subsequently nano-biochars (n-BCs). These materials were selected due to their status as abundant agricultural and municipal waste products, aligning with the principles of waste valorisation and circular economy. PB and RH are widely available in many farming regions, where their utilization poses environmental and management challenges, attracting increasing attention for sustainable reuse. In contrast, SSL is a major byproduct of wastewater treatment plants worldwide, where its safe disposal and potential for resource recovery remain key environmental concerns. The PB and RH samples originated from Umudike, Nigeria (5.4956° N, 7.5296° E), while SSL was sourced from a municipal wastewater treatment facility in Lublin, Poland (51°14' N, 22°35' E). The b-BCs production process followed an optimized pyrolysis protocol [43]. Briefly, the feedstocks were dried at 100°C for 24 hours in a laboratory dryer (POL-EKO, Poland) and were subsequently ground to < 2 mm particle using a laboratory grinder (TESTCHEM, Poland). The pyrolysis was conducted in a horizontal tubular retort furnace (Czyłok, Poland) at 550°C for 3 hours under nitrogen (N_2) atmosphere, with a controlled heating rate of 10°C·min⁻¹ and gas flow 1 L·min⁻¹. To produce n-BCs, the b-BCs were first frozen at

Table 1

Pseudo-total content of potentially toxic elements (PTEs) in soil ($\text{mg}\cdot\text{kg}^{-1}$) and aqueous (H_2O) and CaCl_2 extract ($\mu\text{g}\cdot\text{L}^{-1}$) from the soil used in the experiment.

PTEs	Pseudo-total content [$\text{mg}\cdot\text{kg}^{-1}$]	Aqueous extract [$\mu\text{g}\cdot\text{L}^{-1}$]	CaCl_2 extract [$\mu\text{g}\cdot\text{L}^{-1}$]
Cr	54.47 ± 2.305	15.32 ± 0.7924	19.28 ± 0.6841
Cu	28.04 ± 2.305	25.50 ± 1.645	34.06 ± 2.228
Fe	14193 ± 627.9	160.9 ± 11.28	242.9 ± 5.487
Mn	750.1 ± 37.07	31.36 ± 1.3711	45.78 ± 2.804
Ni	17.37 ± 1.715	11.13 ± 0.3881	15.38 ± 0.8023
Zn	604.7 ± 22.43	21.19 ± 1.302	32.10 ± 1.364
Co	4.764 ± 0.134	-	-
Cd	1.985 ± 0.066	-	-
Pb	83.75 ± 4.156	6.286 ± 0.2591	7.844 ± 0.3236

−80°C for 24 hours to facilitate effective milling, then processed through ball milling using a planetary ball mill machine (PM100, Retsch Corporation, Germany), successfully reducing particle size to < 100 nm [24]. The comprehensive physicochemical characterization methodology, including elemental analysis (CHNO), ash content, pH, electrical conductivity (EC), size and zeta potential measurements, total organic carbon (TOC) content in solid b-BC/n-BC samples, dissolved organic carbon (DOC) content in b-BC/n-BC's leachates and porous structure analysis via N_2 adsorption-desorption is detailed in the [Supplementary Information \(SI\)](#).

2.2. Pot experiment

The PTEs contaminated soil used in the pot experiment was collected from industrial zone of the Silesian region in Poland (Dąbrowa Górnicza, 50°19' N, 19°12' E). The soil was slightly neutral (pH=6.75) with low salinity ($\text{EC}=151.9\ \mu\text{S}\cdot\text{cm}^{-1}$), indicating optimal conditions for plant cultivation without pH or salt-induced stress factors. All PTEs included in legal regulations [44] were determined in the soil (Table 1). No exceedance of permissible limits for most elements was found, with only Zn slightly exceeding regulatory thresholds for agricultural applications (orchards, meadows, and pastures). Bioavailability assessment using the aqueous (H_2O) and CaCl_2 extraction detected measurable concentrations of Cr, Cu, Fe, Mn, Ni, Zn, and Pb (Table 1). The pot experiment was conducted in 500 mL containers in triplicate. The b-BC/n-BC were added to the soil at a dose of 2 % w/w (dry weight), thoroughly mixed to create a homogeneous mixture and transferred to experimental pots. The soil-amendment matrices were hydrated with Milli-Q® water to maintain 65 % water-holding capacity. Lettuce (*Lactuca sativa* L.), being a globally significant leafy vegetable, is highly sensitive to contamination by PTEs [45]. Since it is often consumed raw, it poses a direct risk to human health when grown in contaminated soils. Its high susceptibility to PTEs uptake makes it an ideal model crop for studying soil remediation strategies and assessing food safety implications [46]. *L. sativa* L. seeds underwent sequential pre-treatment: hydration (2 hours in Milli-Q® water), sterilization (15 minutes in 2 % calcium hypochlorite followed by 1 minute in 70 % ethanol), triple rinsing with Milli-Q® water [47]. Seeds germination occurred on hydrated filter paper in Petri dishes under controlled conditions (Conviron GEN100, Canada, 22°C, dark environment). After 4 days, five seedlings were selected and transplanted into each pot including unamended control units. Environmental parameters were precisely regulated (Conviron GEN100, Canada, photoperiod – 12:12 hour light:dark cycle, temperature gradient −22°C day/18°C night, and constant humidity – 65 %). Bi-weekly irrigation with Milli-Q® water maintained consistent soil moisture levels throughout the experiment. Soil samples (approximately 50–60 g) were collected at the beginning of the experiment (T_0) and after six weeks after harvest (T_1). The soil samples were air-dried for 24 hours and stored at 4°C before further analysis. The six-week cultivation period concluded with harvesting and systematic separation of roots and leaves for PTEs translocation analysis.

Table 2

Physicochemical properties of bulk (b-BC) and nano-biochars (n-BC) used in the experiment.

Sample	S _{BET} [m ² /g]	V _p [cm ³ /g]	Pore width [nm]	TOC [mg/g]	DOC [mg/L]	C [%]	H [%]	O [%]	H/C [molar ratio]	O/C [molar ratio]	Ash [%]
b-BC-PB	1.14	0.004	13.08	464.9	460.6	67.46	1.54	9.1	0.274	0.101	20.66
	±	±	±	±	±	±	±	± 0.4888	±	± 0.0054	± 1
n-BC-PB	0.0616	0.0002	0.6974	23.71	24.394	3.471	0.0817		0.0149		.074
	11.54	0.020	6.8	308.8	299.8	59.66	1.7	15.1	0.342	0.190	22.40
b-BC-RH	±	±	±	±	±	±	±	± 0.8284	±	±	±
	0.529	0.0008	0.3832	16.68	11.95	2.894	0.0645		0.0178	0.0067	1.178
n-BC-RH	175.8	0.110	2.2	12.50	1.8	43.77	1.18	5.65	0.324	0.097	48.50
	±	±	±	±	±	±	± 0.05921	± 0.2518	±	± 0.0047	±
b-BC-SSL	7.877	0.005	0.0967	0.5597	0.0959	2.097			0.0138		2.320
	195.6	0.141	3.12	96.00	0.10	35.81	1.36	6.53	0.455	0.137	55.37
n-BC-SSL	±	±	±	±	±	±	± 0.07156	± 0.3497	±	± 0.00707	±
	10.09	0.007	0.1585	4.999	0.0053	1.837			0.0239		2.806
b-BC-SSL	41.61	0.055	5.27	5.27	6.95	20.53	0.74	3.69	0.433	0.135	72.31
	±	±	±	±	±	±	±	± 0.2145	± 0.02523	±	±
n-BC-SSL	2.459	0.003	0.303	0.3035	0.4056	1.188	0.0428			0.0076	4.285
	51.23	0.090	7.03	4.36	15.30	23.06	0.53	7.83	0.276	0.255	65.54
b-BC-SSL	±	±	±	±	±	±	±	± 0.4131	±	± 0.01332	±
	2.674	0.005	0.3584	0.2294	0.7997	1.197	0.0278		0.0145		3.392

Where: b-BC- bulk biochar, n-BC-nano-biochar, PB-palm bunch, RH-rice husk. SSL-sewage sludge, S_{BET}-specific surface area, V_p-pore volume, TOC- total organic carbon, DOC-dissolved organic carbon, C, H, O and Ash [%] total content, O/C and H/C- molar ratios. The values are mean of three repetitions.

2.3. Determination of PTEs content

The pseudo-total PTEs content in the samples was analyzed using an inductively coupled plasma optical emission spectrometer (ICP-OES). For this analysis, 200 mg of b-BC/n-BC or soil or 500 mg of *Lactuca sativa* L. leaves were digested in a microwave system with a mixture of HNO₃ and HCl in a 1:3 (v/v) ratio for 1.5 hours at 200°C. After digestion, the samples were allowed to cool to room temperature, then filtered through a 22 µm pore filter to remove any solid residues. The resulting filtrate was transferred quantitatively to a 50 mL volumetric flask.

To assess the bioavailable PTEs fraction in b-BC/n-BC or soil amended with 2 % b-BC/n-BC, 2 g of the sample was dispersed in 20 mL of Milli-Q water (H₂O) or a 0.01 mol·L⁻¹ calcium chloride (CaCl₂) solution at a solid-to-liquid ratio of 1:10 (w/v) according to Wang et al. [48]. The suspensions were shaken on a horizontal shaker for 24 hours, after which the solutions were filtered through a 0.45 µm syringe filter, acidified with 1 % HNO₃ (v/v), and analyzed for trace PTEs using ICP-OES. High-purity argon (99.9999 %, AirLiquide, Poland) was used as the plasma generation gas and for sample nebulization, with respective flow rates of 12 L·min⁻¹ and 0.5 L·min⁻¹. PTEs quantification was performed using a six-point calibration curve ranging from 0.001 to 2 mg·L⁻¹. Detailed calibration parameters are provided in the [Supplementary Information](#) (SI).

2.4. Bioaccumulation factor (BAF)

The bioaccumulation factor (BAF) is a key parameter used to evaluate the mobility and uptake of PTEs in plants [49]. It is defined as the ratio of the concentration of a PTEs in plant tissues, such as leaves or roots, to its concentration in the soil. This factor provides insight into the ability of a plant to accumulate metals from its growing medium and is expressed as:

$$BAF = \frac{C_{\text{leaf}}}{C_{\text{soil}}} \quad (5)$$

where C_{plant} – the concentration of the PTEs in the plant tissue (mg/kg), C_{soil} – the concentration of the PTEs in the soil (mg/kg).

A higher BAF value indicates greater PTEs mobility and accumulation in plant tissues, reflecting the plant's capacity to uptake PTEs from the soil [50]. This parameter is widely used in environmental and agricultural studies to assess the risk of PTEs contamination in crops and to determine the efficiency of remediation techniques such as BC

amendments in reducing PTEs bioavailability [51].

2.5. Health risk index (HRI)

The health risk index (HRI) was used to assess potential health risks linked to lettuce consumption. HRI considers the daily PTEs intake (DMI), which is determined based on the vegetable consumption rates, and compares it to the oral reference dose (RFD) established for each specific PTEs. This approach provides a quantitative measure of the potential health hazards associated with dietary exposure to PTEs.

$$DMI = \frac{C_{\text{PTE}} \cdot C_f \cdot IR}{BW} \quad (6)$$

where C_{PTE} – the concentration of the PTEs in the lettuce (mg/kg), C_f – the conversion factor is 0.085 for the conversion of fresh to dry vegetable mass, IR – the ingestion rate of lettuce by person (0.345 kg/day/person), BW- body weight of the individual (kg/person) and is 60 kg for adults and 32.7 kg for children.

$$HRI = \frac{DMI}{RFD} \quad (7)$$

where DMI – the daily PTEs intake (mg/kg), The RFD for Cr, Cu, Fe, Mn, Ni, Zn and Pb is 1.5, 0.04, 0.003, 0.14, 0.02, 0.3 and 0.003 mg/kg/day, respectively [52]. Our study aimed to assess PTEs exposure through lettuce consumption without age limits or categorization into carcinogenic or non-carcinogenic impacts.

2.6. Statistical analysis and modeling

The data were analyzed statistically using OriginPro 2024 (Origin-Lab Corporation, USA) and Statistica 13.0 (TIBCO Software Inc., USA) software. Mean values were calculated based on triplicate measurements. Relationships between the physicochemical properties of b-BC/n-BC and the immobilization efficiency of PTEs were assessed using Pearson's correlation coefficient tests. Statistical significance was determined at *p ≥ 0.05. Cluster analysis was performed to identify similarities across different b-BC/n-BC amendment treatments using Ward's method. To determine the dominant mechanism governing PTEs ion binding, geochemical speciation modeling was conducted using Visual MINTEQ 4.0 (USA). The NICA-Donnan model was applied, as it enables the simulation of PTE complexation by constituents characterized by significant heterogeneity in binding site affinities.

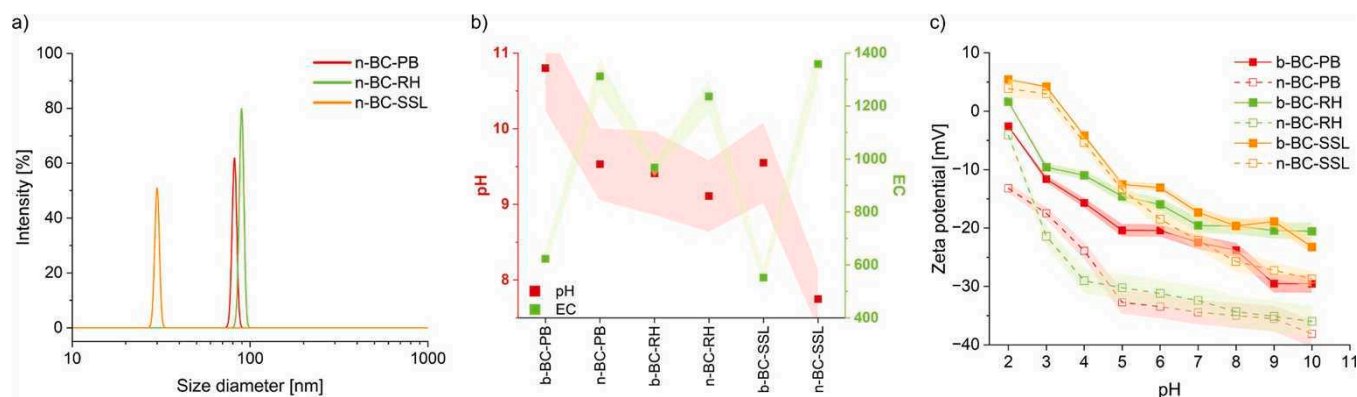


Fig. 1. Size distribution of n-BC derived from different feedstocks (a), showing particle size in the nanometer range, pH and EC values for b-BC and n-BC (b) and zeta potential as a function of pH for b-BC and n-BC solution (c), where the shaded areas represent standard deviation.

3. Results and discussion

3.1. Physicochemical properties of tested bulk biochar and nano-biochar

Analysis of the physicochemical properties of the b-BCs and n-BCs showed distinct variations correlated with both feedstock used and particle size (Table 2). The size distribution of n-BC indicates that the analyzed n-BCs ranged from 30.0 nm to 89.6 nm (Fig. 1a), whereas the size of b-BC was larger than 1 μm . Generally, the b-BC were characterized by an alkaline pH ranging from 9.41 to 10.8 and EC from 552 to 968 $\mu\text{S}\cdot\text{cm}^{-1}$ (Fig. 1b). Particle size reduction to nanoscale dimensions decreased pH by 3.2–22 %, and an increased EC from 27.7 % to 378 %, with variations dependent on the feedstock. SSL-derived n-BCs exhibited the most pronounced modifications in both pH and EC after size reduction. Both b-BCs and n-BCs demonstrated mostly relatively low porosity (Table 2), which was in general, higher for n-BC than b-BC. Among the tested feedstocks, RH-derived b-BC and n-BC exhibited the highest specific surface area (S_{BET}) (175.8 $\text{m}^2\cdot\text{g}^{-1}$ for b-BC-RH and 195.6 $\text{m}^2\cdot\text{g}^{-1}$ for n-BC-RH), which was 2.3- to 154-fold higher than for BCs produced from other feedstocks. This enhanced S_{BET} corresponded with elevated pore volume (V_p), which was 1.2- to 27.5-fold higher, with RH-derived b-BCs and n-BCs consistently displaying superior porosity metrics across all evaluated BCs (Table 2).

The PB- and RH-derived b-BCs and n-BCs were predominantly composed of C (from 35.8 % to 67.5 %), while SSL-derived b-BCs and n-BCs showed a higher ash content (from 65.5 % to 72.3 %), which is attributed to the mineral-rich composition of the SSL as a feedstock [53]. The effect of ball milling on elemental (CHNO) composition and ash content varied depending on the type of feedstock (Table 2). Consistent with our previous studies [24], a decrease in C content and an increase in ash content were observed after ball milling for PB- and RH-derived b-BCs, likely due to the breaking of bonds within the BC's structure and subsequent oxidation (confirmed by increase of O) occurring during the milling [24]. However, for SSL-derived b-BC, ball milling had the opposite effect. The mechanical forces applied during milling may have caused the mineral-rich feedstock, such as SSL, to disintegrate into finer particles, ultimately leading to a decrease in ash content, while simultaneously increasing C content. The reduction in ash content—primarily composed of inorganic minerals—results in a relative enrichment of the carbon fraction within the material. These changes in the molecular composition affected the molecular ratios (Table 2). In general, an increase in both H/C and O/C ratios was noted, suggesting that n-BCs possess more polar functional groups on their surface and exhibit lower aromaticity compared to their bulk counterparts [54,55]. In the case of SSL-derived b-BCs and n-BC, when particle size was reduced, the opposite pattern was observed for H/C ratio compared to other types of b-BCs and n-BCs. As ash-rich materials, SSL-derived b-BC and n-BC may lose release of volatile H-containing compounds during ball milling,

further reducing H content and consequently lowering the H/C ratio. Additionally, ball milling can promote structural reorganization toward a more ordered, graphite-like form, where H/C naturally decreases due to H loss and the development of more condensed carbon structures [56].

The total organic carbon (TOC) and dissolved organic carbon (DOC) contents were much higher in PB-derived b-BC and n-BC (Table 2) than in other tested materials, which can be attributed to the organic richness of the PB feedstock, lower ash and mineral content, and the chemical composition of the resulting BC [57]. For all tested b-BC, a reduction in particle size to the nanoscale resulted in a decrease in TOC depending on the feedstock from 14.9 % to 94.4 %. However, the DOC measurements exhibited feedstock-dependent variability. A decrease in DOC values was observed for PB- and RH-derived n-BCs, whereas SSL-derived n-BCs demonstrated an inverse correlation, showing elevated DOC levels following particle size reduction. It may suggest the release of additional dissolved organic components during the ball milling process. In highly mineralized BCs, some organic compounds may be physically trapped within the matrix. Mechanical milling can break down this structure, exposing and releasing previously inaccessible dissolved organic components into solution, leading to increased DOC levels. Additionally, the milling process generates soluble organic matter by detaching structures that were originally bound to the insoluble solid fraction, allowing them to dissolve into the aqueous phase [58].

The zeta potential (ζ) analysis (Fig. 1c) showed significant differences in surface charge among BC depending on their feedstock and size. The n-BC exhibited more ζ than corresponding b-BC, what may suggest a higher density of surface functional groups (Fig. S1 and S2) on the n-BC's surface capable of ionization and enhanced colloidal stability. Among the different feedstocks, PB-derived b-BC and n-BC demonstrated the highest negative ζ , particularly in the pH range of 5–10 (Fig. 1c), indicating a high concentration of oxygen-containing surface groups. Conversely, RH-based b-BC and n-BC exhibited less negative ζ , likely due to the higher ash content, which may reduce the availability of ionizable surface groups [59]. SSL-derived b-BC and n-BC had the least negative ζ , implying a lower density of dissociable functional groups on their surface. Regardless of the feedstock type, all BCs showed a decreasing trend in ζ with increasing pH, consistent with the progressive deprotonation of surface functional groups (Fig. 1c). The observed differences in ζ between BC derived from different feedstocks and of varying sizes highlight the influence of BC structure and composition on surface charge characteristics, which may play a crucial role in determining their stability and adsorption performance in environment.

3.2. Effect of bulk biochar/nano-biochar amendment on soil properties

The addition of b-BC or n-BC to the contaminated soil increased both

Table 3
Physicochemical properties of soil amendment with 2 % of bulk biochar (b-BC) or nano-biochar (n-BC) at T₀ and T₁ term.

Sample	Term	TOC [mg/g]	DOC [mg/L]	pH	EC [μS/cm]
Soil (S)	T ₀	8.602 ± 0.2043	13.48 ± 0.1480	6.75 ± 0.2524	151.9 ± 7.931
	T ₁	7.952 ± 0.3360	12.52 ± 0.2228	6.76 ± 0.2501	150.9 ± 6.426
S+b-BC-PB	T ₀	12.74 ± 0.1209	22.01 ± 0.1220	8.65 ± 0.2071	330.7 ± 13.34
	T ₁	13.60 ± 0.3130	25.42 ± 0.1254	9.24 ± 0.1411	352.6 ± 18.52
S+n-BC-PB	T ₀	13.35 ± 0.1130	19.33 ± 0.1933	9.33 ± 0.2032	423.3 ± 24.01
	T ₁	14.60 ± 0.2230	22.62 ± 0.3226	9.87 ± 0.1904	454.2 ± 23.22
S+b-BC-RH	T ₀	11.78 ± 0.2090	14.55 ± 0.3455	7.58 ± 0.1509	146.5 ± 4.431
	T ₁	13.20 ± 0.1816	16.58 ± 0.2165	8.12 ± 0.2669	145.7 ± 7.706
S+n-BC-RH	T ₀	10.36 ± 0.2346	14.00 ± 0.2440	7.49 ± 0.1152	149.7 ± 5.214
	T ₁	11.28 ± 0.2465	13.53 ± 0.1653	8.03 ± 0.2895	151.7 ± 5.613
S+b-BC-SSL	T ₀	10.65 ± 0.1166	9.906 ± 0.1991	7.49 ± 0.1023	152.0 ± 7.206
	T ₁	11.59 ± 0.1704	11.34 ± 0.1340	8.04 ± 0.1028	163.0 ± 7.466
S+n-BC-SSL	T ₀	12.02 ± 0.1015	16.44 ± 0.1644	7.72 ± 0.1221	196.4 ± 11.91
	T ₁	13.24 ± 0.2993	18.37 ± 0.1837	8.34 ± 0.1383	210.9 ± 10.98

Where: S-soil-BC- bulk biochar, n-BC-nano-biochar, PB-palm bunch, RH-rice husk. SSL-sewage sludge, TOC- total organic carbon, DOC-dissolved organic carbon, pH- in H₂O, EC- electrical conductivity. The values are mean of three repetitions.

pH and EC immediately after application (T₀) and these values continued to increase during the experiment duration (T₁) (Table 3). Notably, the gradual increase in soil pH over time indicates the sustained influence of b-BC and n-BC on soil chemistry. This effect may enhance soil buffering capacity and long-term fertility [60], making b-BC and n-BC valuable amendments for remediating acidic soils and improving soil quality in agricultural and environmental contexts. The addition of b-BC caused a pH increase compared to control of 10.9–36.6 %, whereas n-BC resulted in much higher increase of 11.0–46.0 %, depending on the incubation time (Table 3). Generally, n-BC induced a greater increase in soil pH compared to b-BC by 23.4–35.8 % depending on the contact time. It was observed that over the time, the difference between b-BC

and n-BC became more pronounced. An exception was RH-derived n-BC, for which no statistically significant changes ($p > 0.05$) in pH were observed compared to RH-derived b-BC, regardless of the contact time. The greatest pH increase (by 28.1–46.0 %) relative to the control soil was observed for b-BC-PB and n-BC-PB, which also exhibited the most pronounced differences between b-BC and n-BC. This elevated values can be attributed to PB-derived b-BC and n-BC demonstrating superior alkaline pH levels relative to BCs produced from other feedstocks (Table 2). An increase in soil pH is a critical factor influencing the bioavailability of PTEs [61]. Higher pH levels can reduce the solubility and mobility of many PTEs, by promoting their adsorption onto BC surfaces [62] and inducing precipitation as less soluble hydroxides or carbonates [63].

The EC analysis demonstrated patterns analogous to those observed in the pH (Table 3). After the EC increased following most BC application, this upward trend continued until the end of the experiment. Compared to control soil, the amendment with n-BC resulted in a greater increase (by 50.4 % to 44,433 %, depending on incubation time) of EC than after b-BCs application, attributed to the higher inherent EC values of n-BC than their bulk counterparts. An exception was observed for RH-derived b-BC and n-BC, where no statistically significant changes ($p > 0.05$) in EC values were observed compared to the control soil. This phenomenon can be explained by the fact that RH-derived b-BC and n-BC exhibited the highest specific surface area (S_{BET}) (Table 2), which may have led to the adsorption of ions from the soil, thereby balancing the EC changes and reducing the number of available ions in the soil solution. In the case of feedstock, the highest increase in EC was observed after application of b-BC-PB and n-BC-PB, which can be attributed to the composition of PB feedstock rich in water-soluble minerals such as potassium (K), calcium (Ca), and magnesium (Mg). Increasing of EC values with time may suggest the gradual mineralization and release of soluble minerals [64] from b-BC and n-BC. Over time, an increase in EC values was observed for both b-BC and n-BC. It is worth noting that for n-BC (except n-BC-RH), the changes between time points were 7.96–99.7 % lower compared to those observed for b-BC. This observation suggests that n-BC contributes to greater stability in EC values in soil conditions over time compared to b-BC, likely due to the gradual release of ions as a result of better dispersion of n-BC particles in the soil.

TOC and DOC in the soil significantly ($p \leq 0.05$) increased after both b-BC and n-BC application (Table 3). The addition of b-BC and n-BC, depending on feedstock, resulted in an increase in TOC by 23.8–66.0 % and by 20.4–83.6 %, respectively compared to the control soil. The n-BC generally caused a greater increase in TOC (by 14.8–66.7 %) compared to b-BC after their addition to the soil, with the exception of n-BC-RH, for which a 36.5–44.8 % lower increase was recorded. In the case of DOC,

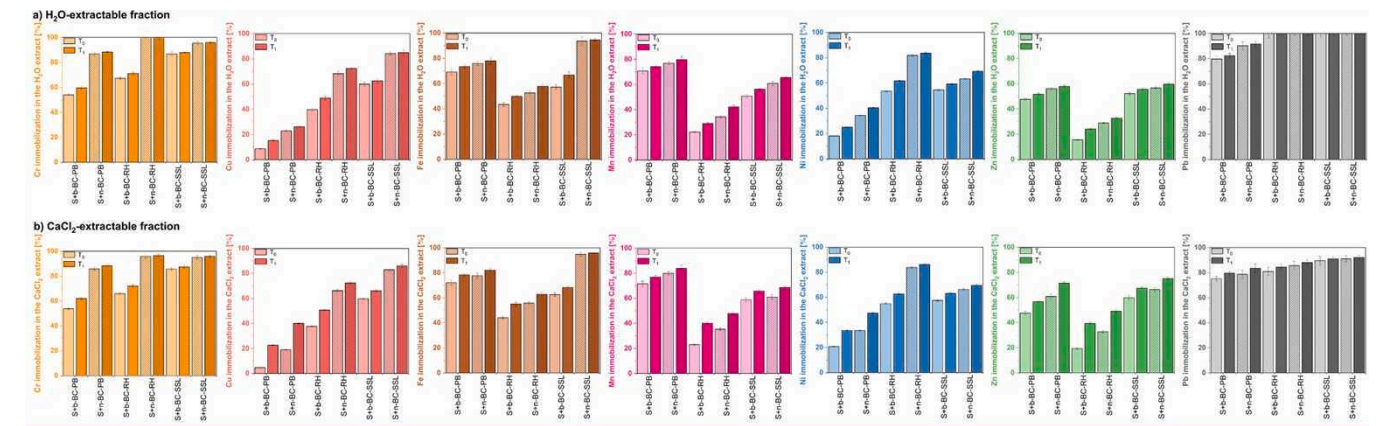


Fig. 2. Immobilization percentage (calculated compared to control) of individual PTEs depending on the experimental variant T₀ term (lighter shades) and T₁ term (darker shades), measured the a) H₂O-extractable fraction and b) CaCl₂-extractable fraction. The x-axis represents different b-BC/n-BC amendments. Dashed bars represents n-BC-amendments. Error bars represent the standard deviation of the mean (n = 3).

increase was observed for most b-BCs and n-BCs and ranged from 7.95 % to 103 % and from 3.86 % to 80.6 %, respectively, except for b-BC-SSL (but not for n-BC-SSL) where decrease of DOC (from 9.45 % to 26.5 %, depending on incubation time) was observed (Table 3). A lower increase in DOC content (by 21.7–75.3 %) was observed for n-BC compared to b-BC after soil application. However, an exception was noted for n-BC-SSL, which led to an increase in DOC values, unlike b-BC-SSL, where a decrease was recorded. This trend is linked to the fact that n-BC-PB and n-BC-RH had lower DOC contents, whereas n-BC-SSL exhibited a higher DOC content compared to their bulk counterparts (Table 2). After a 6-week incubation period of b-BC/n-BC-amended soil, the differences in TOC and DOC content became even more pronounced compared to both the initial measurement in b-BC/n-BC-amended soil (T_0) and the control soil at T_1 . A trend was observed in which the highest increase in TOC and DOC content was recorded for b-BC-PB and n-BC-PB. PB-derived b-BC and n-BC contained more TOC and DOC than others (Table 2), which may explain higher TOC and DOC increase after addition to soil than for other BCs. After particle size reduction, DOC increased for SSL-derived n-BC, making this material a greater source of this component. An increase in TOC and DOC contents are typical for most studies where b-BC is used as an amendment [65,66]. However, these studies focus only on b-BC, and data on the effects of n-BC on TOC and DOC dynamics remain limited.

3.3. Effect of bulk biochar/nano-biochar amendments on immobilization of PTEs

Fig. 2 illustrates the reduction in potentially bioavailable PTEs content extracted with water (H_2O) and $CaCl_2$. The addition of b-BCs or n-BCs to the soil resulted in a significant decrease ($p \leq 0.05$) in the extractable levels of Cr, Cu, Fe, Mn, Ni, Zn, and Pb compared to their content in contaminated control soil. This reduction was consistent across both (H_2O and $CaCl_2$) solvents tested. The Co and Cd was not detected in the bioavailable fraction (extracted with H_2O and $CaCl_2$) either in the control or in any of treatments, even presence of these PTEs were confirmed in pseudo-total content of control soil (Table 1). The absence of Co and Cd can be attributed to these PTEs' strong binding to more stable soil fractions as was previously suggested by Tessier et al. [67].

At the initial stage of the experiment (T_0), the addition of b-BC resulted in a significant ($p \leq 0.05$) immobilization of PTEs and ranged from 8.69 % to 100 % in H_2O -extract and from 4.69 % to 89.5 % for $CaCl_2$ -extract. Application of n-BC caused higher reduction of bioavailability of specific PTEs ranging from 22.9 % to 100 % for H_2O -extract and from 18.9 % to 95.9 % for $CaCl_2$ -extract (Fig. 2). These variations were dependent on the feedstock used and the specific PTEs. The n-BC exhibited a superior immobilization effect, highlighting its enhanced efficacy in reducing the mobility of PTEs in the soil. In the H_2O extract, immobilization was 8.38–163 % higher for n-BC than b-BC at T_0 , while in the $CaCl_2$ extract, these values were from 5.78–305 % higher compared to b-BC at T_0 , depending on the feedstock and specific PTEs.

During the experiment (T_1), PTEs bioavailability in control soil also significantly decreased depending on specific PTEs from 9.21 % to 14.9 % in H_2O -extract and from 7.34 % to 13.9 % for $CaCl_2$ -extract relative to the beginning of the experiment (T_0), which is a typical process connected with sequestration mechanisms in plants tissues [68]. Beyond the natural decrease observed in the control, the application of b-BC and n-BC led to a substantially greater reduction in PTEs bioavailability, particularly as the experiment progressed (T_1). In H_2O -fraction and $CaCl_2$ -fraction, depending on the specific PTEs, extending the contact time to 6 weeks enhanced the effectiveness of immobilization (Fig. 2). The reduction of specific PTEs in the H_2O fraction ranged from 15.3 % to 100 % for b-BC and from 26.2 % to 100 % for n-BC and in the $CaCl_2$ fraction ranged from 22.6 % to 90.9 % for b-BC and from 40.1 % to 96.5 % for n-BC, indicating a time-dependent stabilization effect. Notably, for Pb- H_2O extractable

forms, an increase was recorded only for b-BC-PB and n-BC-PB, whereas for all other b-BC and n-BC treatments, a complete (100 %) reduction was observed regardless of time. Although extending the contact time improved PTEs immobilization more noticeably for b-BC than n-BC, n-BC remained the more effective amendment at T_1 also due to its higher initial effectiveness (Fig. 2). Moreover, n-BC's ability to act more rapidly and maintain superior performance over time further highlights its potential as a more effective and sustainable solution for PTEs rapid stabilization in soil. The n-BC achieves significant immobilization at an earlier stage due to its superior distribution, resulting from its smaller particle size and the higher likelihood of rapid interaction between soil particles and n-BC. Its faster action in binding PTEs makes it a more efficient solution for rapid remediation, effectively limiting the mobility and spread of contaminants immediately after application. The b-BC, with its larger particle size, undergoes gradual surface aging, progressively developing oxygen-containing functional groups that aid in PTEs complexation and enhance its ability to retain PTEs over time. This demonstrates that b-BC is also effective in immobilizing PTEs, but it requires a longer time to reach its maximum efficiency, making it suitable for areas where immediate results are not a priority.

The observed difference in long-term trends (T_1) for b-BC or n-BC amendments can be attributed to multiple synergistic mechanisms. The extended interaction time between b-BC or n-BC and soil components facilitated enhanced PTEs immobilization. This phenomenon can be attributed to the progressive saturation of adsorption sites on BC, the formation of stable precipitates [69], and the development of stronger PTEs-BC complexes over time [70]. Additionally, surface chemistry transformations of b-BC/n-BC in the soil environment, including the gradual oxidation of BC's surfaces and shifts in pH and redox potential, further augmented PTEs sequestration capacity. The cumulative effect of these physicochemical processes resulted in quantifiable reductions in PTEs extractability in both the H_2O and $CaCl_2$ extracts during the experiment.

The increased levels of PTEs immobilization observed following the application of b-BC and n-BC can be primarily attributed to the increase in soil pH and EC induced by these amendments, which have been previously identified as critical environmental factors influencing PTEs solubility [71–73]. Additionally, the higher levels of DOC in soils amended with b-BC and n-BC further contribute to the reduction of PTEs bioavailability [74,75], because it provides functional groups capable of forming stable organo-metallic complexes. The increase in soil pH induces the deprotonation of soil surfaces, increasing their negative charge, which enhances the adsorption of PTEs ions. Furthermore, under alkaline conditions, hydroxylated PTEs cation species, which exhibit higher affinities for soil surfaces, are generated [76]. Simultaneously, elevated EC enhances ion exchange processes, effectively sequestering PTEs in less bioavailable forms [77]. Based on the modelling data for heterogeneous soil matrix with elevated DOC content presented in Table S7, the immobilization mechanisms of PTEs following b-BC/n-BC application can be broadly categorized into two main processes: (1) ion exchange and (2) precipitation into low-permeability waste forms. These mechanisms often occur simultaneously, although their relative contributions vary depending on the specific PTEs.

Ion exchange was identified as the dominant mechanism for Cr, Fe, Mn, and Ni. These PTEs are typically present as hydrated cations or cations with relatively high mobility and low tendencies to form insoluble compounds. Their immobilization primarily occurs through electrostatic interactions and exchange with native cations (e.g., Ca^{2+} , Mg^{2+} , H^+) on negatively charged surfaces of BC and soil particles. This process is enhanced by the higher specific surface area (S_{BET}) and surface charge density of n-BC (Fig. 1c), which increases the number of active sorption sites. Moreover, surface functional groups such as carboxylic ($-COOH$), hydroxyl ($-OH$), carbonyl ($C=O$), and phenolic groups play a crucial role in facilitating cation exchange. FT-IR analysis (Fig.S1) confirmed the presence of these groups across all b-BCs and n-BCs, with differences in intensity depending on the feedstock. Notably, ball milling intensified

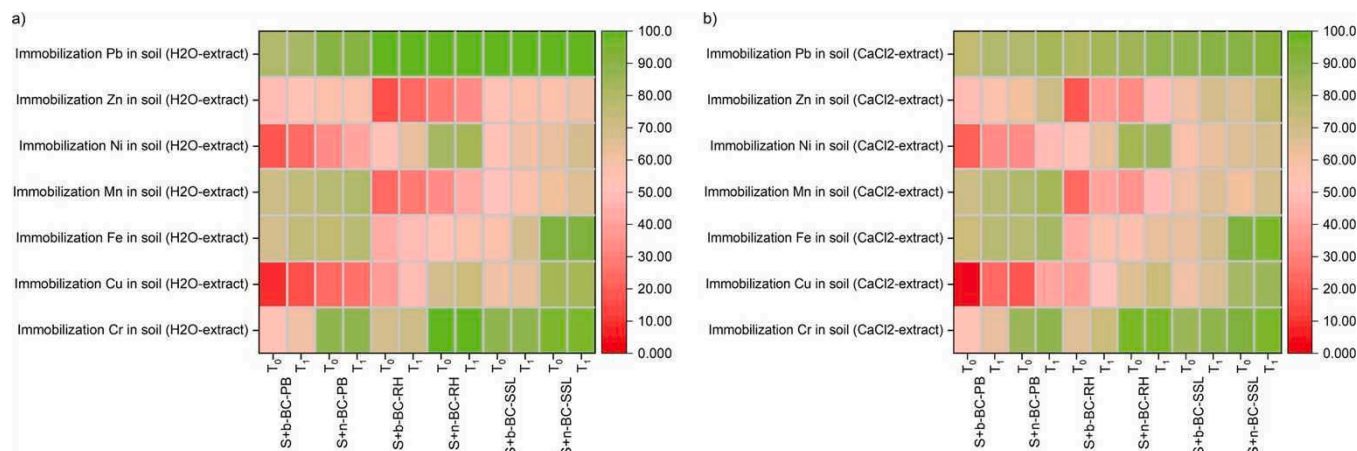


Fig. 3. Heatmap comparison of the immobilization efficiency of different b-BC and n-BC for PTEs extracted with (a) H₂O and (b) CaCl₂. The color scale represents the degree of immobilization, with red indicating the lowest and green the highest immobilization efficiency. Data are shown for two time points: T₀ (initial stage) and T₁ (after 6 weeks).

certain bands (e.g., C=O stretching in aromatic rings), indicating increased exposure of functional sites. Additionally, XPS data (Fig. S2) revealed a higher abundance of oxygen-containing groups on n-BC surfaces, particularly for SSL-derived materials. The presence of C–OH, C=O, COOR, and Me–O functionalities contributes to enhanced surface polarity and reactivity, promoting stronger interactions with metal cations through complexation and ion exchange.

In contrast, Cu, Zn, and Pb were predominantly immobilized through

precipitation mechanisms. The increase in soil pH following b-BC/n-BC amendment facilitates the formation of insoluble hydroxides (e.g., Cu(OH)₂, Zn(OH)₂, Pb(OH)₂), effectively reducing the mobility and bioavailability of these PTEs. These precipitated forms are typically stable under alkaline conditions and may be further integrated into the soil matrix or BC's surface. Additionally, the higher ash content may contribute to PTEs immobilization by providing mineral components—such as carbonates, phosphates, and silicates—that can

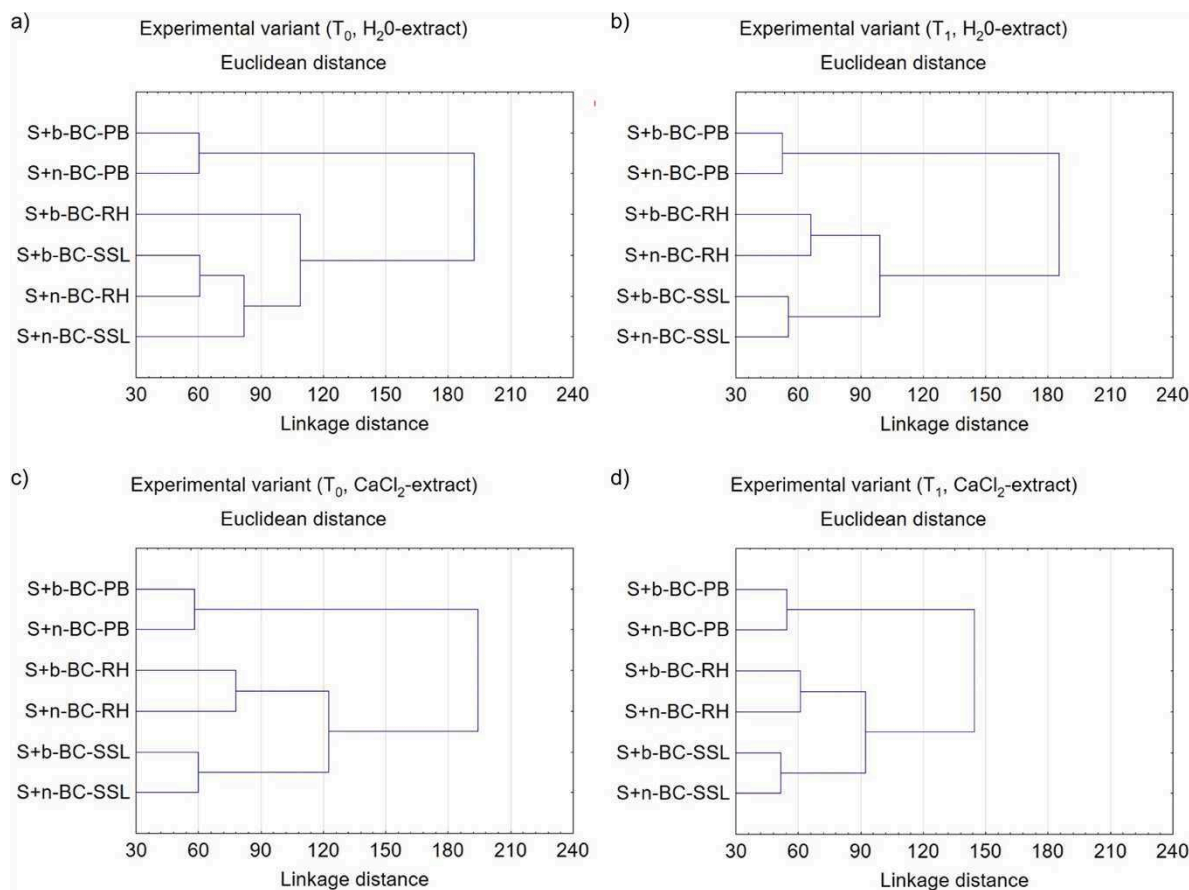


Fig. 4. Hierarchical clustering dendrograms illustrating the similarities in PTEs immobilization among different b-BC and n-BC amendments in soil, based on the percentage immobilization of Cr, Cu, Fe, Mn, Ni, Zn, and Pb. Panels represent different extraction methods and time points: (a) H₂O extract at T₀, (b) H₂O extract at T₁, (c) CaCl₂ extract at T₀, and (d) CaCl₂ extract at T₁.

participate in co-precipitation or direct chemical binding with PTEs ions, further reducing their mobility and enhancing long-term stability in the soil system. Notably, the modelling data clearly show that both ion exchange and precipitation mechanisms were more pronounced in soils amended with n-BC than with b-BC, indicating the enhanced efficiency of n-BC in immobilizing PTEs. This can be attributed to its greater surface reactivity, finer particle size, and higher density of functional groups, all of which promote stronger and more stable interactions with PTEs ions.

Previous studies have consistently reported that a range of BC [78–80], increased soil pH and subsequently immobilized PTEs when incorporated into soils. For example, Beesley et al. [81] reported an increase in soil pH and a decrease in pore water concentrations of Cd and Zn after applying hardwood-derived BC to multi-element-contaminated soil. Similarly, Jiang et al. [82] demonstrated a reduction in acid-extractable Cu and Pb concentrations following the application of rice straw-derived BC to artificially contaminated soil, coinciding with an increase in soil pH. Kim et al. [83] observed that the addition of rice hull-derived BC into PTEs contaminated soil significantly reduced the phytoavailable PTEs content, primarily due to increased PTEs adsorption onto the BC and a concomitant increase in soil pH.

Fig. 3 presents a heatmap visualization of the immobilization efficiency of different b-BC and n-BC amendments for PTEs in both H₂O- and CaCl₂-extractable fractions at two experimental time points (T₀ and T₁). The colour gradient indicates the degree of immobilization, with red representing the lowest and green the highest efficiency. This visualization allows for a comparative assessment of how different BC types (feedstock) and modification scales (bulk vs. nano) influence the stabilization of specific PTEs over time. Grouping the b-BC and n-BC based on their ability to immobilize specific PTEs, we observed b-BC-SSL and n-BC-SSL were the most often the most effective in immobilization of most of PTEs in both solutions (H₂O- and CaCl₂-extractable fractions) regardless of the experimental time point (Fig. 3). In the case of n-BC-SSL superior performance can likely be attributed to the significantly smaller particle size of n-BC-SSL (Fig. 1a) compared to other n-BC, which enhances its distribution throughout the soil matrix, increasing the likelihood of direct interaction with contaminants. The finer particle size provides a greater reactive surface area and also facilitates improved penetration into soil micropores, allowing for more effective adsorption and complexation of PTEs. Moreover, both SSL-derived b-BC and n-BC were characterized by the highest ash content among all tested materials (Table 2), which may contribute to PTEs immobilization through

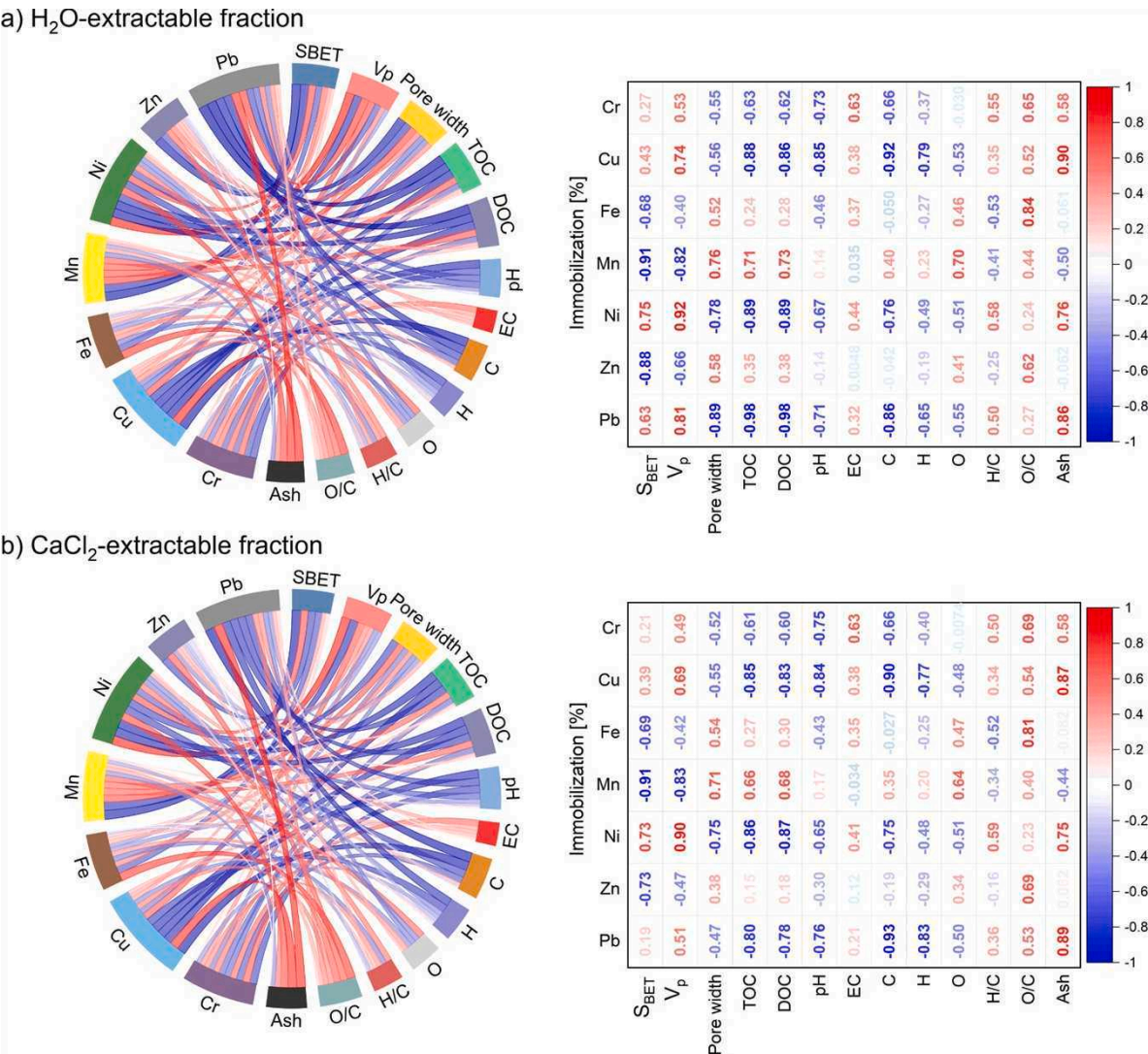


Fig. 5. Correlation analysis between the reduction efficiencies of PTEs in two extractable fractions and the physicochemical properties of b-BC and n-BC. Panel (a) represents the H₂O-extractable fraction, and panel (b) represents the CaCl₂-extractable fraction. The network relationships diagrams illustrate the network of Pearson's correlations, with red and blue lines indicating positive and negative correlations, respectively. The correlation values on the right depict the strength and direction of the Pearson's correlations between physicochemical properties of BC and n-BC and the reduction efficiencies of PTEs in two extractable fractions.

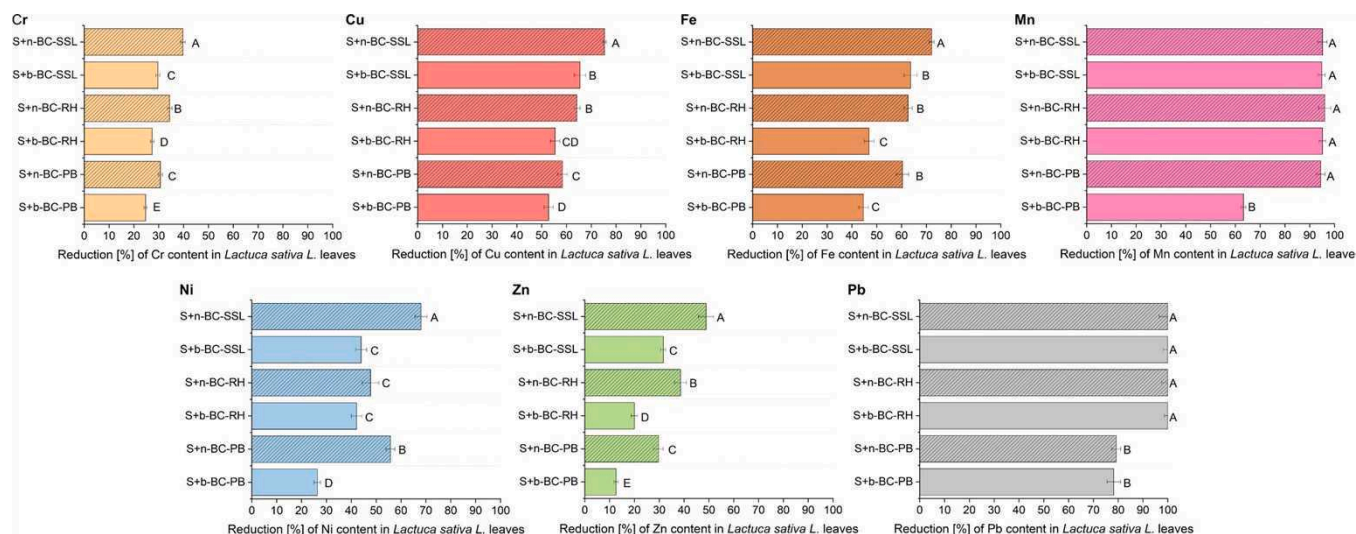


Fig. 6. Reduction in the content of individual PTEs in *Lactuca sativa L.* leaves depending on the experimental variant. Error bars represent the standard deviation of the mean ($n = 3$). The dashed pattern represents n-BC, while the solid pattern represents b-BC.

precipitation mechanisms involving mineral components. Additionally, these BCs possessed a rich abundance of surface functional groups, which further promote binding through complexation and ion exchange mechanisms, enhancing their overall immobilization capacity. Depending on the specific PTEs there were some exceptions, showing that particular PTEs were the best immobilized by particular BC. For H_2O -extracted PTEs, exceptions were observed for b-BC, where the highest immobilization of Fe and Mn was noted for b-BC-PB. For n-BC, the highest immobilization of Cr and Ni was observed for n-BC-RH, while for Mn, the highest immobilization was demonstrated by n-BC-PB. Similarly, for $CaCl_2$ -extracted PTEs, for b-BC, the highest immobilization of Fe and Mn was observed for b-BC-PB (Fig. 3). For n-BC, the greatest immobilization efficiency for Mn and Ni extracted with $CaCl_2$ was noted for n-BC-PB and n-BC-RH, respectively.

The dendrograms analysis (Fig. 4) illustrate the similarities in PTEs immobilization among different b-BC or n-BC amendments added to the soil, based on the percentage immobilization of Cr, Cu, Fe, Mn, Ni, Zn, and Pb. The hierarchical clustering analysis using Euclidean distance metrics demonstrated that feedstock type predominantly determines immobilization patterns, with three distinct clusters emerging from PB-, RH-, and SSL-derived b-BC/n-BC. At the initial (T_0) and final (T_1) time points, b-BC and n-BC derived from identical feedstocks exhibit strong clustering tendencies in both H_2O (Fig. 4a-b) and $CaCl_2$ (Figs. 4a and 4c-d) extracts in most cases. This clustering pattern suggests that that chemical properties of feedstock rather than particle size, serves as a dominant factor in PTEs immobilization efficiency in amended soil. The data particularly highlights that PB-derived b-BC and n-BC demonstrate significantly different immobilization characteristics compared to SSL- and RH-derived b-BC and n-BC, indicating distinct mechanistic pathways for PTEs immobilization between these groups. The n-BC consistently exhibits superior immobilization efficiency, as indicated by the stronger clustering of soil amended with n-BC within each feedstock group. The increased differentiation over time, particularly in $CaCl_2$ -extractable PTEs, suggests that n-BC enhances the long-term stability of immobilized PTEs more effectively than BC. The observed similarities in the immobilization abilities of different materials were further confirmed by dendrogram analysis (Fig. 4), which indicated comparable PTEs immobilization properties depending on the type of BC.

3.4. Effect of size reduction on immobilization of specific PTEs

It is also interesting to examine which PTEs were most significantly affected by size reduction. The reduction of particle size to the nanoscale

significantly enhanced Cu immobilization in both H_2O - and $CaCl_2$ -extractable fractions, particularly for n-BC-PB and n-BC-RH, and for Fe, the greatest enhancement in immobilization in H_2O - and $CaCl_2$ -extractable fractions was observed for n-BC-SSL compared to corresponding b-BC. However, this enhancements did not correspond to the highest overall immobilization efficiency, as the highest immobilization was noted for Pb in both H_2O - and $CaCl_2$ -extractable fractions across all b-BC/n-BC amendments (Fig. 2). The enhanced immobilization efficiency of Cu and Fe following the reduction of b-BC to nanoscale can be attributed to their unique physicochemical properties and optimized interactions with the n-BC matrix. Their relatively small ionic radii (Table S3) facilitate effective penetration into n-BC's nanoporous structure of n-BC, maximizing surface contact and strengthening binding potential. Additionally, the high hydration energy of Cu and Fe promotes efficient dehydration [84], enabling robust adsorption and the formation of stable complexes or precipitates. Furthermore, Cu exhibits particularly effective immobilization due to its characteristically low solubility product and ability to undergo redox transformations, leading to the formation of stable mineral complexes on the n-BC surface [85]. Fe demonstrates analogous redox behavior, with precipitation occurring predominantly through interactions with mineral ash components in n-BC-SSL, explaining its preferential immobilization in both extractable fractions. While other PTEs also showed improvement in immobilization, the enhancement was less pronounced compared to Cu and Fe. This diminished effect can be attributed to their larger ionic radii (Table S3), which may limit their penetration into the nanoporous structure of n-BC and reduce overall adsorption efficiency. In competitive multi-PTEs systems, the combination of ionic dimensions and binding affinities enables Cu and Fe to optimally utilize the physicochemical properties of n-BC, resulting in markedly improved immobilization efficiencies compared to their bulk counterparts.

3.5. Effect of bulk biochar/nano-biochar properties on immobilization efficiency of PTEs

The varying effectiveness of PTEs immobilization-which differed based on both BC type and PTEs species-suggests that multiple physicochemical parameters influence the immobilization process. While specific surface area (S_{BET}) was highest in b-BC-RH and n-BC-RH samples (Table 2), this factor alone does not fully explain the observed differences, indicating that other chemical and physical properties play crucial roles in determining immobilization efficiency. Fig. 5 presents the Pearson's correlation coefficients and network relationships

Table 4

The bioaccumulation factor (BAF) for each specific PTEs calculated for different experimental variants in soil amended with 2 % bulk biochar (b-BC) or nano-biochar (n-BC).

Experimental variant	Cr	Cu	Fe	Mn	Ni	Zn	Co	Cd	Pb
Soil (S)	0.0463 ± 0.00135	3.298	0.04448	0.2510	0.5466	0.3558	-	-	0.8822
S+b-BC-PB	0.03486	1.558	0.02462	0.09212	0.4029	0.3109	-	-	0.1922
	± 0.001425	± 0.05929	± 0.0009795	± 0.004394	± 0.01439	± 0.01215			± 0.005005
S+n-BC-PB	0.03212	1.373	0.01763	0.01392	0.242	0.2502	-	-	0.1836
	± 0.001198	± 0.05296	± 0.000622	± 0.0003618	± 0.008735	± 0.009813			± 0.007216
S+b-BC-RH	0.03363	1.470	0.02361	0.01221	0.3163	0.2849	-	-	-
	± 0.001448	± 0.06245	± 0.001008	± 0.0005181	± 0.01362	± 0.01212			
S+n-BC-RH	0.03038	1.180	0.01661	0.009926	0.2858	0.2187	-	-	-
	± 0.001169	± 0.02966	± 0.0004835	± 0.0003343	± 0.01231	± 0.009274			
S+b-BC-SSL	0.03256	1.139	0.01618	0.013	0.3063	0.2434	-	-	-
	± 0.0009588	± 0.03715	± 0.0005124	± 0.0005303	± 0.01313	± 0.0098			
S+n-BC-SSL	0.02789	0.8125	0.01241	0.01215	0.1749	0.1821	-	-	-
	± 0.001077	± 0.03211	± 0.000498	± 0.0004473	± 0.004447	± 0.005468			

Where: S-soil, b-BC- bulk biochar, n-BC-nano-biochar, PB-palm bunch, RH-rice husk. SSL-sewage sludge, '-' not calculated, because metal concentrations in lettuce leaves were below the detection limits (<LOD).

between the reduction efficiencies of individual PTEs and the physicochemical properties of b-BC and n-BC. The study elucidates the critical correlations between b-BC/n-BC physicochemical characteristics and their respective PTEs reduction efficacies in soil. The findings demonstrate that specific properties - notably ash content, S_{BET} , and pore width - play instrumental roles in enhancing PTEs immobilization mechanisms across extractable fractions. Ash components, contribute to PTEs immobilization through two mechanisms. Firstly, they may directly immobilize PTEs through sequestration via precipitation reactions, cation exchange processes, and adsorption phenomena [86]. Secondly, they indirectly stabilize PTEs by elevating pH [87], which promotes the formation of insoluble PTEs species, thereby reducing the metal's mobility in the system. These mechanisms were further supported by NICA-Donnan modelling, which revealed the dominant PTEs species present in the studied systems and confirmed their reduced availability under the conditions created by ash-rich BCs. Additionally, high S_{BET} and V_p further enhance immobilization efficiency by increasing the number of available reactive sites for PTEs adsorption and complexation, improving the entrapment of PTEs within the BC matrix. Distinct variations were observed in the immobilization behaviour between H_2O - and $CaCl_2$ - extractable phases, suggesting different binding mechanisms.

3.6. Bioaccumulation of PTEs in *Lactuca sativa* L in bulk biochar/nano-biochar-amended soil

The concentration of investigated PTEs in *Lactuca sativa* L. leaf tissue decreased by 12.6–100 % for b-BC amendment and by 29.7–100 % for n-BC amendment, depending on specific PTEs and BC type (Fig. 6). Notably, n-BC demonstrated higher efficacy in immobilizing PTEs in soil, leading to a greater reduction in their accumulation in lettuce leaves. Specifically, the addition of n-BC resulted in higher reduction of PTEs concentrations in lettuce leaves by 13.3–135 % compared to the addition of their bulk counterparts, depending on the specific PTEs. An exception was only observed for n-BC-PB, n-BC-RH, and n-BC-SSL for Pb, as well as n-BC-RH and n-BC-SSL for Mn, where no statistically significant ($p > 0.05$) difference in the reduction of Pb and Mn accumulation in *Lactuca sativa* L leaves was noted compared to their respective b-BC amendments. The observed differences in the ability of b-BC and n-BC amendments to reduce PTEs accumulation in lettuce leaves can be primarily attributed to phytoextraction, where *Lactuca sativa* L. absorbed bioavailable PTEs from the soil. This process was more pronounced in soil amended with b-BC, as it retained a higher concentration of PTEs within the mobile fraction, making them more accessible for plant uptake.

In terms of feedstock, the highest reductions in PTEs concentrations

in lettuce leaves were observed for b-BC and n-BC derived from SSL. The reductions were 4.33–150 % higher for b-BC and 15.1–64.5 % higher for n-BC, depending on the specific PTEs, compared to those achieved using other feedstocks. This trend correlates with the superior immobilization capacity of SSL-derived b-BC and n-BC for PTEs in the soil (Fig. 3), highlighting their effectiveness in reducing PTEs bioavailability and subsequent uptake by plants. However, for Mn and Pb, no significant differences were observed between RH-derived b-BC and n-BC and SSL-derived b-BC and n-BC.

Analyzing individual PTEs, it was demonstrated that Pb and Mn showed the strongest reduction in bioaccumulation, regardless of feedstock, followed by $Cu > Fe > Ni > Cr > Zn$ after application of b-BC or n-BC to soil (Fig. 6). Pb reduction efficiency was between 78.2 % and 100 % using b-BC and 79.2–100 % using n-BC. For Mn, b-BC achieved removal rates of 63.3–95.1 %, while n-BC demonstrated removal rates of 94.55–96.1 %. Reduction in bioaccumulation in the case of other PTEs depended on the b-BC/n-BC used and ranged from 12.6 % to 75.4 %, with n-BC to be more effective by 10.6–135 %. The pronounced reduction in Pb accumulation in lettuce leaves can be linked to its preferential immobilization in the soil, as was previously confirmed by the results of H_2O - and $CaCl_2$ extraction (Fig. 2). This significant immobilization of Pb results from Pb's low hydration energy and strong affinity to oxygen-containing functional groups and mineral components of b-BC/n-BC, which facilitate its adsorption, precipitation, and stabilization in less bioavailable forms. The correlation analysis (Fig. S3) revealed that the reduction in bioaccumulation in *Lactuca sativa* L. leaves is strongly positively correlated with specific physicochemical properties of b-BC and n-BC, particularly the O/C ratio, EC and ash content. Furthermore, the strong positive correlations observed between bioaccumulation reduction and immobilization efficiency of individual PTEs in both the H_2O -extractable and $CaCl_2$ -extractable fractions indicate that higher PTEs immobilization in soil is directly linked to lower PTEs uptake in lettuce leaves (Fig. S3). This finding suggests that these properties enhance the immobilization of PTEs in soil, thereby reducing their bioavailability and subsequent uptake by plants and highlight the crucial role of BC's physicochemical characteristics in mitigating PTEs contamination and reducing PTEs accumulation in edible crops.

To the best of our knowledge, there are no published studies investigating n-BC for reducing PTEs accumulation in plants. The majority of existing research has focused on b-BC applications for PTEs immobilization and bioavailability reduction. Our findings demonstrate that reducing BC particle size to the nanoscale substantially enhances its remediation efficiency. Previous studies, such as Kim et al. [83], examined that application of b-BC derived from rice husks effectively reduced the concentrations of Cd, Pb, and Zn in lettuce by 88 %, 60 %, and 66 %, respectively, compared to the control. Similarly, Zheng et al.

Table 5
The health risk index (HRI) for each specific PTEs calculated for different experimental variants in soil amended with 2 % bulk biochar (b-BC) or nano-biochar (n-BC) for adults and child.

Experimental variant	Cr		Cu		Fe		Mn		Ni		Zn		Co		Cd		Pb	
	adult	child	adult	child	adult	child	adult	child	adult	child	adult	child	adult	child	adult	child	adult	child
Soil (S)	0.0008 ± 2.40•10 ⁻⁵	0.0015 ± 4.41•10 ⁻⁵	1.129 ± 0.0287	2.0731 ± 0.0526	102.9 ± 2.741	188.7 ± 5.031	0.6574 ± 0.0237	1.206 ± 0.0435	0.2320 ± 0.0060	0.4257 ± 0.0110	0.3506 ± 0.0126	0.6433 ± 0.0231	-	-	-	-	0.6848 ± 0.026	1.2564 ± 0.0476
S+b-BC-PB	0.0006 ± 2.53•10 ⁻⁵	0.0011 ± 4.64•10 ⁻⁵	0.5337 ± 0.0203	0.9792 ± 0.0373	56.92 ± 2.265	104.5 ± 4.156	0.2412 ± 0.0115	0.4427 ± 0.0211	0.1710 ± 0.0061	0.3138 ± 0.0112	0.3063 ± 0.0120	0.5620 ± 0.0220	-	-	-	-	0.1492 ± 0.004	0.2737 ± 0.0071
S+n-BC-PB	0.0006 ± 2.13•10 ⁻⁵	0.0011 ± 3.90•10 ⁻⁵	0.4703 ± 0.0181	0.8629 ± 0.0333	40.76 ± 1.438	74.79 ± 2.639	0.0365 ± 0.0009	0.0669 ± 0.0017	0.1027 ± 0.0037	0.1885 ± 0.0068	0.2465 ± 0.0097	0.4523 ± 0.0177	-	-	-	-	0.1425 ± 0.006	0.2615 ± 0.0103
S+b-BC-RH	0.0006 ± 2.57•10 ⁻⁵	0.0011 ± 4.72•10 ⁻⁵	0.5035 ± 0.0214	0.9239 ± 0.0393	54.61 ± 2.331	100.2 ± 4.277	0.0320 ± 0.0014	0.0587 ± 0.0025	0.1343 ± 0.0058	0.2464 ± 0.0106	0.2807 ± 0.0119	0.5150 ± 0.0219	-	-	-	-	-	-
S+n-BC-RH	0.0005 ± 2.07 •10 ⁻⁵	0.0009 ± 3.81•10 ⁻⁵	0.4043 ± 0.0102	0.7419 ± 0.0186	38.41 ± 1.118	70.4789 ± 2.052	0.0260 ± 0.0009	0.0477 ± 0.0016	0.1213 ± 0.0052	0.2226 ± 0.0096	0.2155 ± 0.0091	0.3954 ± 0.0168	-	-	-	-	-	-
S+b-BC-SSL	0.0006 ± 1.70•10 ⁻⁵	0.0011 ± 3.12•10 ⁻⁵	0.3901 ± 0.0127	0.7158 ± 0.0234	37.42 ± 1.185	68.6616 ± 2.174	0.0341 ± 0.0014	0.0625 ± 0.0025	0.1300 ± 0.0056	0.2386 ± 0.0102	0.2398 ± 0.0097	0.4401 ± 0.0177	-	-	-	-	-	-
S+n-BC-SSL	0.0005 ± 1.91•10 ⁻⁵	0.0009 ± 3.51•10 ⁻⁵	0.2784 ± 0.0110	0.5108 ± 0.0202	28.70 ± 1.152	52.6668 ± 2.113	0.0318 ± 0.0012	0.0584 ± 0.0021	0.0742 ± 0.0019	0.1362 ± 0.0035	0.1794 ± 0.0054	0.3292 ± 0.0099	-	-	-	-	-	-

Where: S-soil, b-BC- bulk biochar, n-BC-nano-biochar, PB-palm bunch, RH-rice husk, SSL-sewage sludge, ‘-’ not calculated, because PTEs concentrations in lettuce leaves were below the detection limits (<LOD).

[88] demonstrated that the use of rice-derived BC enhanced the immobilization of PTEs during rice cultivation, as evidenced by reductions in Cd, Pb, and Zn concentrations in rice grains by 98 %, 72 %, and 83 %, respectively. In comparison, our research observed relatively lower Zn immobilization rates, which can likely be attributed to soil-specific properties known to significantly influence PTEs mobility in the soil matrix. The observed PTEs values align with previous research findings [89,90], suggesting, which may confirm, that n-BC could be in general more effective in reduction of the risk related to PTEs compared to b-BC.

Table 4 presents the bioaccumulation factor (BAF) for specific PTEs in lettuce leaves, calculated for various experimental treatments. Adding b-BC and n-BC significantly reduced BAF values compared to the control. For b-BC, the reduction in BAF ranged from 12.6 % to 100 %, while for n-BC, it ranged from 29.7 % to 100 %, depending on the specific PTEs. Analysis of feedstock showed no clear trends between specific feedstock types and their effectiveness in reducing BAF for PTEs. However, n-BC consistently demonstrated greater efficacy in reducing BAF compared to b-BC, with reductions being 10.6–134 % higher than those observed for BC, depending on the specific PTEs. Among the analyzed PTEs, the BAF values increased in the following order: Fe < Cr < Mn < Pb < Zn < Ni < Cu. Notably, the addition of b-BC or n-BC was most effective in reducing BAF for Pb and Mn, with average reductions of 94.7 % and 91.4 %, respectively. The enhanced performance of n-BC highlights its potential as a superior amendment for managing PTEs contamination in agricultural soils.

3.7. Health risk index (HRI)

Table 5 presents the health risk index (HRI) values linked to lettuce consumption for each PTEs and experimental variant calculated for adults or children. An HRI < 1 is considered likely safe, while an HRI values ≥ 1 may pose a potential health risk [91]. In control soil, HRI values for adults were > 1 only for Cu and Fe, whereas for children for Cu, Fe, Mn, and Pb. Notably, the addition of b-BC and n-BC significantly reduced HRI values. For b-BC, the reduction in HRI ranged from 12.6 % to 100 %. The n-BC demonstrated greater efficacy, with reductions being 1.1- to 2.5-fold higher than for b-BC and ranged from 29.7 % to 100 %. After application of b-BC and n-BC into the soil the HRI values were < 1 for all PTEs (except for Fe) and for both adults and children. These values ranged from 0.0006 to 0.9792 and from 0.0005 to 0.8629 for b-BC and n-BC amendments, respectively. For Fe, the HRI index ranged from 37.4 to 105 for b-BC amendments and from 28.7 to 74.8 for n-BC amendments. However, the HRI for Fe after the addition of b-BC or n-BC decreased by 44.6–63.6 % and 60.4–72.1 %, respectively, compared to the untreated soil.

The risk mitigation effects of b-BC and n-BC are especially critical for raw-consumed vegetables such as lettuce, which undergo no thermal or chemical processing and could potentially decrease PTEs concentrations post-harvest. The observed reduction in HRI values through b-BC and n-BC application has significant implications for public health safety, particularly among children populations who exhibit heightened susceptibility due to their lower body mass indices and proportionally higher dietary intake rates.

4. Conclusion

The results demonstrated that b-BC and n-BC effectively immobilize PTEs in the contaminated soil, significantly reducing their bioavailability and uptake by lettuce. Notably, n-BC exhibited superior performance compared to b-BC, with higher immobilization efficiencies and more excellent and rapid reductions in PTEs accumulation in lettuce. The study also revealed the critical role of feedstock in determining the efficacy of b-BC and n-BC. The SSL-derived b-BC and n-BC exhibited the highest PTEs immobilization and reduction in bioaccumulation PTEs in lettuce leaves. It make this problematic and inconvenient biowaste

valuable feedstock for BC production. Notably, n-BC, regardless feedstock used to production, consistently outperformed b-BC in reducing both BAF and HRI, emphasizing its superior capacity for PTEs immobilization and uptake mitigation. These improvements are strongly linked to the enhanced physicochemical properties of n-BC, including increased surface area, higher density of oxygen-containing functional groups, greater surface reactivity, and smaller particle size that promotes better dispersion in soil and more efficient interaction with PTEs ions.

Mechanistic analysis using NICA-Donnan modelling confirmed that the dominant immobilization processes were ion exchange (for Cr, Fe, Mn, and Ni) and precipitation (for Cu, Zn, and Pb), and both were more pronounced in n-BC treatments. Spectroscopic analyses (FT-IR and XPS) further supported this, revealing an increased abundance of surface functional groups and oxygenated species in n-BC, particularly those derived from SSL, which enhanced its sorption capacity.

By improving soil health, reducing PTEs bioavailability, and enhancing crop safety, b-BC and especially n-BC serve as practical tools for reducing the risk of PTEs-contaminated soil and safeguarding human health. By effectively reducing the bioavailability and dietary transfer of PTEs from contaminated soils to edible plants, b-BC and n-BC play a crucial role in safeguarding human health, particularly for raw vegetables that are consumed directly without any further treatment.

Although the results are promising, some limitations should be considered when evaluating the broader applicability of n-BC for PTEs immobilization. Site-specific factors such as soil type, pH, texture, organic matter content, and background contamination can influence its effectiveness. Moreover, the inherent heterogeneity of n-BC—resulting from differences in feedstock, pyrolysis conditions (e.g. temperature, atmosphere), and post-processing (e.g. milling time)—may lead to variable performance.

Future research should validate these findings across diverse environmental contexts and focus on standardizing or tailoring n-BC production. Long-term field studies and mechanistic insights are essential to optimize and ensure the reliability of n-BC-based remediation strategies.

Environmental implications

This research has significant environmental benefits, including soil remediation, waste valorization, and greenhouse gas reduction. Nano-biochar (n-BC) immobilizes potentially toxic elements (PTEs), preventing contamination of crops and water sources while enhancing soil health and microbial diversity. By converting waste materials into biochar, it promotes a circular economy and reduces landfill pollution. Additionally, n-BC aids in carbon sequestration, lowering CO₂ emissions and improving climate resilience. Its application in agriculture enhances water retention, boosts biodiversity, and supports sustainable food production. This approach aligns with global sustainability goals, offering a cost-effective, eco-friendly solution for mitigating soil contamination and protecting ecosystems.

CRediT authorship contribution statement

Akachukwu Doris: Methodology, Investigation, Data curation. **Oleszczuk Patryk:** Writing – review & editing, Supervision, Resources, Formal analysis, Conceptualization. **Raczkiewicz Monika:** Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Data curation, Conceptualization.

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. Supporting information

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Data availability

Data will be made available on request.

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Supplementary material for:

Sustainable soil remediation using nano-biochar for improved food safety and resource recovery

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This file includes:

Supplementary text

Tables S1-S4

Figure S1-S3

1. Physicochemical analysis

The porous structure of BC and n-BC was analyzed using low-temperature nitrogen adsorption-desorption isotherms at 77,3 K with an ASAP 2420 analyzer (Micromeritics, USA), Key parameters, including specific surface area (SBET), micropore surface area (Smicro), total pore volume, micropore volume, and average pore diameter, were derived from nitrogen desorption data, as described by (Michot and Villieras, 2013). The pH and electrical conductivity (EC) of the biochar samples were assessed following the procedure reported by Kończak et al. (2020), Suspensions were prepared by mixing 1 g of biochar with 10 mL of deionized water, achieving a solid-to-liquid ratio of 1:10 (w/v), Measurements of pH and EC were taken using a digital meter (HQ430d Benchtop Single Input, HACH, USA or CP-401, ELMETRON, Poland), Total organic carbon (TOC) in the solid biochar samples and dissolved organic carbon (DOC) in water extracts (prepared as described above) were quantified using a TOC analyzer (TOC-VCSH, SHIMADZU, Kyoto, Japan) equipped with a Solid Sample Module (SSM-5000), Elemental composition, including carbon, hydrogen, and nitrogen, was determined using a Perkin–Elmer 2400 CHN analyzer (Akron, OH, USA), The ash content was measured according to the ASTM D3176 standard by combusting dried samples at 760°C for 6 hours (Abdel-Fattah et al., 2015). The ash content was calculated using the following equation:

$$\% Ash = \frac{\text{weight after ashing} - \text{weight of crucible}}{\text{dry weight of BC sample}} \cdot 100\% \quad (1)$$

The total content of O was determined by the following equation:

$$\% O = 100\% - \% Ash - \% C - \% H - \% N \quad (2)$$

The functional groups of BCs and n-BC were determined by FTIR-ATR spectra in the region of 400–4000 cm⁻¹ with a resolution of 1 cm⁻¹ (Nicolet 8700A, Thermo Scientific, USA) and X-ray photoelectron spectroscopy (XPS) (analytical system UHV Prevac, Poland). The hydrodynamic diameter of n-BCs was determined by dynamic light scattering (DLS) using a Zetasizer Nano ZS90 analyzer (Malvern Instrument Inc., UK) equipped with a universal dip cell. The intensity-weighted hydrodynamic particle size was determined assuming the Stokes-Einstein equation for spherical particles (Chen and Elimelech, 2008). The zeta potential of the b-BC n-BC suspensions was calculated from the

electrophoretic mobility measurements and the Smoluchowski equation (Zetasizer Nano ZS, Malvern Instruments) (Yang et al., 2019). Briefly, 100 mg/L b-BC n-BC suspensions were prepared from the background electrolyte solution (10^{-3} M NaCl) and treated with ultrasound for 1 min. Then, the zeta potential was measured in the pH range from 2 to 10, adjusted by adding 0.1 M HCl or NaOH solution. All measurements were performed 10 times, and the average values are reported.

2. FT-IR analysis

The FT-IR spectra of the analyzed biochars (BCs) reveal complexity due to the presence of multiple bands associated with various functional groups on the biochar surface, as noted by Ilić et al. (2022). However, there is no significant distinction between the spectra of BCs and n-BCs other than differences in peak intensities. More pronounced variations in FT-IR spectra are observed between BCs and n-BCs derived from different feedstocks. Across all BCs and n-BCs, several characteristic peaks are consistently identified: a broad peak near 3400 cm^{-1} (corresponding to -OH stretching vibrations from carboxylic groups or adsorbed water in pores), a weak peak at 2920 cm^{-1} (associated with aliphatic -C-H bands and =C-H stretching in aromatic compounds) and strong peaks in the range of $1430\text{--}1621\text{ cm}^{-1}$ (attributed to -C=C and -C=O stretching vibrations in aromatic rings, indicating the presence of carboxylic and phenol groups) (Ternero-Hidalgo et al., 2016; Gromov et al., 2017; Cordero-Lanzac et al., 2018). Notably, the -C=C and -C=O stretching vibrations in aromatic rings exhibit a slight shift to higher wavenumbers after ball milling, likely due to a reduced conjugation effect. Peaks within the $1200\text{--}1000\text{ cm}^{-1}$ range correspond to -C-H deformation vibrations in aromatic structures, while those in the $1060\text{--}783\text{ cm}^{-1}$ range are attributed to -Si-O vibrations from inorganic materials in BCs. Additionally, the bands between $950\text{--}600\text{ cm}^{-1}$ represent -C-H bending in aromatic compounds (Barroso-Bogeat et al., 2015). These findings indicate that n-BCs retain diverse functional groups and aliphatic chains similar to BCs, highlighting their high environmental reactivity.

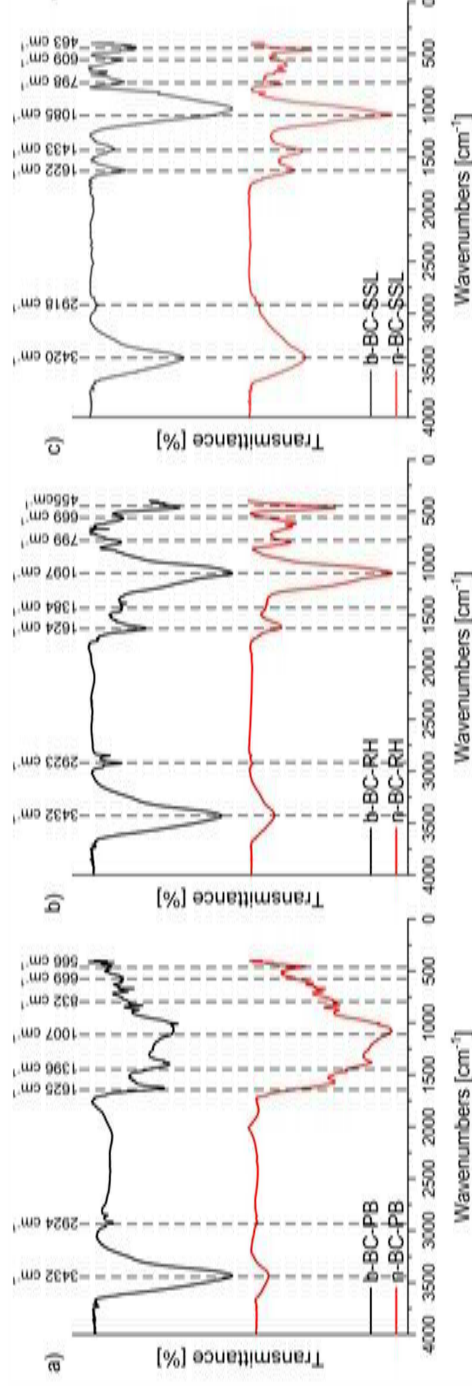


Figure S1. FT-IR spectra of obtained bulk-(BC) and nano-biochars (n-BC).

3. XPS analysis

The X-ray Photoelectron Spectroscopy (XPS) analysis of BC and n-BC derived from different feedstock- PB, RH and SSL-revealed notable differences in surface chemistry, particularly in the distribution of carbon- and oxygen-containing functional groups. In all cases, BC and n-BC exhibited a dominant C-C/C-H peak (~ 285 eV), characteristic of graphitic structures, with n-BC showing an increased presence of oxygenated functional groups (C-OH, C=O, COOR), indicating higher surface oxidation. Among the feedstocks, PB-derived BC had the highest degree of carbonization, as evidenced by the most intense C-C/C-H signal and minimal oxygen-containing groups, whereas RH-derived BC exhibited a more pronounced presence of oxygenated species, suggesting higher surface reactivity. SSL-derived BC displayed the highest intensities of C-OH, C=O, and COOR peaks, along with a distinct carbonate signal (~ 290 – 292 eV), likely due to inorganic residues from sewage sludge. These trends were further supported by the XPS O1s spectra, which showed significant variations in oxygen-containing surface functionalities. The transition from BC to n-BC led to a substantial increase in surface oxygenation across all feedstocks, with SSL-derived BC and n-BC exhibiting the highest

intensity of oxygen-related peaks, particularly in the Me-O/carbonates/O=C (~530–531 eV) and Me-OH/O=C/O=C-O-R (~532–533 eV) regions, suggesting an abundance of inorganic residues. RH-derived BC showed a prominent C-OH/Si-O peak (~533–534 eV), reflecting its naturally high silica content, while PB-derived BC had a more balanced distribution of oxygen functionalities, indicating moderate oxidation. The transition to n-BC amplified oxidation across all feedstocks, with SSL-derived n-BC displaying the most pronounced increase in oxygenated groups, including hydroxyls, esters, and carbonates. The enhanced presence of these functionalities suggests that n-BC, particularly from SSL, possesses improved surface reactivity and a higher potential for adsorption interactions. These findings indicate that SSL-derived n-BC may exhibit superior sorption properties, making it a promising material for environmental applications, especially in pollutant removal.

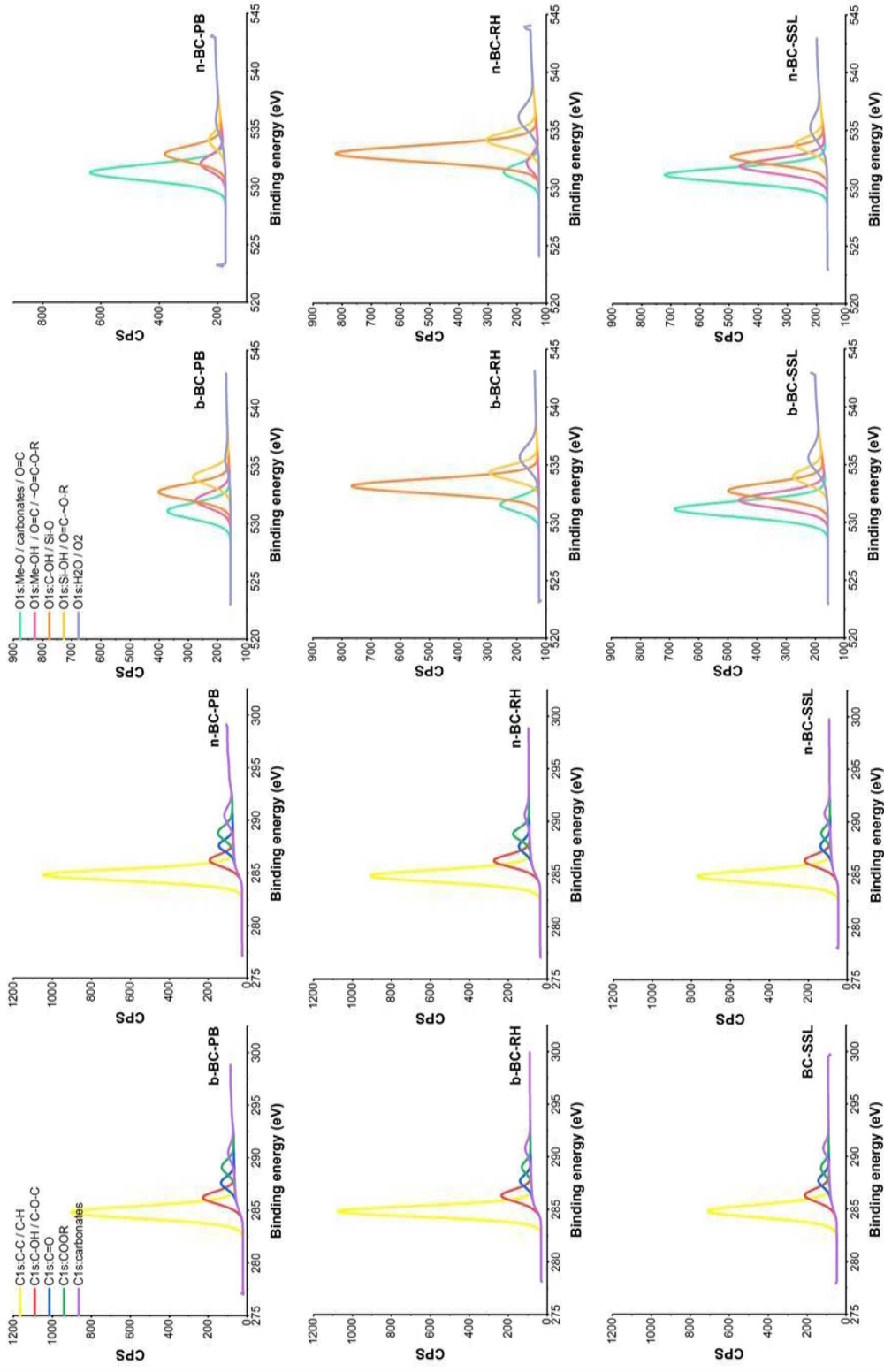


Figure S2. XPS spectra of obtained bulk-(b-BC) and nano-biochars (n-BC).

4. ICP-OES analysis

The quantification of metals was performed based on the six-point calibration curve prepared for selected metals in the range from 0.001 to 2 mg/L. Solutions for the calibration curve were prepared by diluting the multi-element standard (concentration of metals 1 g/L) with MiliQ- water and acidified using HNO₃ (1 %, v/v). Metal quantification on ICP-OES was carried out at wavelength on radial or axial system,

Table. S1. Parameters of the calibration curves for the metals analyzed.

Element	Emission wavelength (nm)	Calibration curve equation	Correlation coefficient (r)	Limit of detection (µg/L)
Cr	283.563	y = 3113.0x + 31.82	0.9999	0.0004
Cu	324.754	y = 3490.9x + 64.03	0.9999	0.0008
Fe	259.940	y = 2036.7x + 29.73	0.9999	0.0028
Mn	257.610	y = 15145.7x + 152.5	0.9999	0.0003
Ni	231.604	y = 644.54 + 7.434	0.9999	0.0005
Zn	213.856	y = 3402.97x + 41.53	0.9999	0.0526
Cd	228.802	y = 2189.9x + 14.21	0.9999	0.0007
Co	228.616	y = 1130.9x + 11.01	0.9999	0.00599
Pb	220.353	y = 245.48x + 1.295	0.9999	0.0047

Table S2. Person correlation between the immobilization efficiencies in H₂O- and CaCl₂-extracts of individual metals and the physicochemical properties of b-BC and n-BC. The significant correlations (* p ≤ 0.05) were indicated by red color.

H ₂ O-extract										
Soil (S)	T ₀	15.32 ± 0.7924	25.5 ± 1.645	160.9 ± 11.28	31.36 ± 1.371	11.13 ± 0.3881	21.19 ± 1.302	6.287 ± 0.2591	<LOD	<LOD
	T ₁	13.17 ± 0.6811	21.74 ± 1.403	143.4 ± 10.06	28.03 ± 1.226	9.669 ± 0.337	18.03 ± 1.108	5.708 ± 0.2352	<LOD	<LOD
S-b-BC-PB	T ₀	7.046 ± 0.3644	23.28 ± 1.502	49.62 ± 3.48	9.179 ± 0.4013	9.127 ± 0.3181	11.1 ± 0.6816	1.276 ± 0.05257	<LOD	<LOD
	T ₁	5.331 ± 0.2757	18.42 ± 1.188	37.95 ± 2.662	7.254 ± 0.3172	7.254 ± 0.2528	8.702 ± 0.5345	1.003 ± 0.04134	<LOD	<LOD
S+n-BC-PB	T ₀	2.022 ± 0.1046	19.66 ± 1.269	38.7 ± 2.714	7.251 ± 0.317	7.31 ± 0.2548	9.312 ± 0.572	0.6177 ± 0.02545	<LOD	<LOD
	T ₁	1.539 ± 0.07961	16.04 ± 1.035	31.64 ± 2.219	5.658 ± 0.2474	5.752 ± 0.2005	7.593 ± 0.4664	0.4784 ± 0.01971	<LOD	<LOD
S+b-BC-RH	T ₀	5.029 ± 0.2601	15.37 ± 0.9919	91.01 ± 6.383	24.37 ± 1.065	5.174 ± 0.1803	17.92 ± 1.100	<LOD	<LOD	<LOD
	T ₁	3.799 ± 0.1965	11.12 ± 0.7172	71.95 ± 5.046	19.92 ± 0.8708	3.705 ± 0.1291	13.7 ± 0.8416	<LOD	<LOD	<LOD
S+n-BC-RH	T ₀	<LOD	8.075 ± 0.521	76.07 ± 5.335	20.61 ± 0.9012	2.019 ± 0.07036	15.06 ± 0.9249	<LOD	<LOD	<LOD
	T ₁	<LOD	5.973 ± 0.3854	60.51 ± 4.244	16.19 ± 0.7078	1.59 ± 0.05541	12.15 ± 0.7463	<LOD	<LOD	<LOD
S+b-BC-SSL	T ₀	2.037 ± 0.1054	10.21 ± 0.6588	68.8 ± 4.825	15.42 ± 0.674	5.071 ± 0.1768	10.13 ± 0.6224	<LOD	<LOD	<LOD
	T ₁	1.584 ± 0.08194	8.147 ± 0.5257	47.55 ± 3.335	12.27 ± 0.5365	3.947 ± 0.1376	8.017 ± 0.4924	<LOD	<LOD	<LOD
S+n-BC-SSL	T ₀	0.7125 ± 0.03685	4.018 ± 0.2592	10.08 ± 0.7072	12.3 ± 0.5378	4.091 ± 0.1426	9.205 ± 0.5654	<LOD	<LOD	<LOD
	T ₁	0.5452 ± 0.0282	3.268 ± 0.2109	7.943 ± 0.5571	9.659 ± 0.4223	2.977 ± 0.1038	7.287 ± 0.4476	<LOD	<LOD	<LOD
CaCl ₂ -extract										
Soil (S)	T ₀	19.28 ± 0.6841	34.05 ± 2.228	242.9 ± 5.487	45.78 ± 2.804	15.38 ± 0.8023	32.1 ± 1.364	7.844 ± 0.3236	<LOD	<LOD
	T ₁	16.6 ± 0.5891	30.35 ± 1.985	214.6 ± 9.267	41.43 ± 2.538	13.28 ± 0.6927	29.74 ± 1.264	6.836 ± 0.282	<LOD	<LOD

S-b-BC-PB	T ₀	8.909 ± 0.3161	32.46 ± 2.124	67.84 ± 2.929	13.06 ± 0.7999	12.19 ± 0.636	16.8 ± 0.714	1.956 ± 0.08066	<LOD	<LOD
	T ₁	6.31 ± 0.2239	23.49 ± 1.537	46.57 ± 2.011	9.554 ± 0.5852	8.83 ± 0.4606	12.87 ± 0.5471	1.397 ± 0.05763	<LOD	<LOD
S+n-BC-PB	T ₀	2.726 ± 0.09672	27.6 ± 1.805	54.25 ± 2.342	9.183 ± 0.5625	10.22 ± 0.5328	12.57 ± 0.5342	1.679 ± 0.06924	<LOD	<LOD
	T ₁	1.93 ± 0.06848	18.19 ± 1.19	38.39 ± 1.658	6.635 ± 0.4064	6.989 ± 0.3645	8.491 ± 0.3608	1.127 ± 0.04648	<LOD	<LOD
S+b-BC-RH	T ₀	6.565 ± 0.233	21.22 ± 1.389	135.8 ± 5.864	35.26 ± 2.16	6.961 ± 0.3631	25.91 ± 1.101	1.496 ± 0.06173	<LOD	<LOD
	T ₁	4.635 ± 0.1645	14.95 ± 0.9782	96.46 ± 4.165	24.8 ± 1.519	4.958 ± 0.2586	18.03 ± 0.7663	1.063 ± 0.04385	<LOD	<LOD
S+n-BC-RH	T ₀	0.8006 ± 0.02841	11.92 ± 0.7795	107.3 ± 4.635	29.6 ± 1.813	2.499 ± 0.1303	21.56 ± 0.916	1.129 ± 0.04659	<LOD	<LOD
	T ₁	0.5777 ± 0.0205	8.383 ± 0.5484	79.8 ± 3.446	21.6 ± 1.323	1.829 ± 0.09538	15.12 ± 0.6426	0.8212 ± 0.03387	<LOD	<LOD
S+b-BC-SSL	T ₀	2.778 ± 0.09856	13.73 ± 0.8983	90.84 ± 3.922	18.89 ± 1.157	6.548 ± 0.3416	12.9 ± 0.5483	0.8259 ± 0.03407	<LOD	<LOD
	T ₁	2.083 ± 0.07392	10.3 ± 0.6738	68.13 ± 2.942	14.17 ± 0.8679	4.911 ± 0.2562	9.678 ± 0.4113	0.6194 ± 0.02555	<LOD	<LOD
S+n-BC-SSL	T ₀	0.9686 ± 0.03437	5.805 ± 0.3798	12.63 ± 0.5456	17.88 ± 1.095	5.176 ± 0.27	10.82 ± 0.4597	0.7091 ± 0.02925	<LOD	<LOD
	T ₁	0.6801 ± 0.02413	4.277 ± 0.2798	8.951 ± 0.3865	12.99 ± 0.7956	4.049 ± 0.2112	7.393 ± 0.3141	0.5415 ± 0.02234	<LOD	<LOD

Table S5. Concentrations ($\mu\text{g} \cdot \text{L}^{-1}$) of individual PTEs depending on the experimental variant, measured in the H₂O-extractable fraction and the CaCl₂-extractable fraction.

T ₀	99.603	CrOH ⁺	47.97 2.735 0.186	CuOH ⁺ Cu(OH) ₂ Cu ₂ (OH) ₂ ²⁺	1.215	FeOH ⁺	0.078	MnOH ⁺	0.386	NiOH ⁺	2.962 1.157	ZnOH ⁺ Zn(OH) ₂	43.505 0.427	PbOH ⁺ Pb(OH) ₂
	0.115	Cr ³⁺	19.702 66.771	Cu ²⁺ CuOH ⁺	95.905	Fe ²⁺	99.731	Mn ²⁺	98.556	Ni ²⁺	79.843	Zn ²⁺	26.429	Pb ²⁺
T ₁	99.885	CrOH ⁺	0.055 13.201	Cu(OH) ₂ Cu ₂ (OH) ₂ ²⁺	4.092	FeOH ⁺	0.268	MnOH ⁺	1.33	NiOH ⁺	8.557 11.597	ZnOH ⁺ Zn(OH) ₂	71.146 2.422	PbOH ⁺ Pb(OH) ₂
	0.397	Cr ³⁺	49.072	Cu ²⁺	98.784	Fe ²⁺	99.922	Mn ²⁺	99.603	Ni ²⁺	95.879	Zn ²⁺	56.058	Pb ²⁺

S+n-BC-RH

T ₀	99.603	CrOH ⁺	2.734 0.235	Cu(OH) ₂ Cu ₂ (OH) ₂ ²⁺	1.215	FeOH ⁺	0.078	MnOH ⁺	0.387	NiOH ⁺	2.963 1.158	ZnOH ⁺ Zn(OH) ₂	43.515 0.427	PbOH ⁺ Pb(OH) ₂
	0.112	Cr ³⁺	19.252 66.802	Cu ²⁺ CuOH ⁺	95.811	Fe ²⁺	99.725	Mn ²⁺	98.52	Ni ²⁺	79.241	Zn ²⁺	25.958	Pb ²⁺
T ₁	99.888	CrOH ⁺	0.058 13.517	Cu(OH) ₂ Cu ₂ (OH) ₂ ²⁺	4.185	FeOH ⁺	0.275	MnOH ⁺	1.361	NiOH ⁺	8.695 12.06	ZnOH ⁺ Zn(OH) ₂	71.546 2.493	PbOH ⁺ Pb(OH) ₂
	0.233	Cr ³⁺	35.345 58.806	Cu ²⁺ CuOH ⁺	97.948	Fe ²⁺	99.868	Mn ²⁺	99.315	Ni ²⁺	91.948	Zn ²⁺	42.669	Pb ²⁺

S+b-BC-SSL

T ₀	99.767	CrOH ⁺	0.012 5.699	Cu(OH) ₂ Cu ₂ (OH) ₂ ²⁺	2.052	FeOH ⁺	0.132	MnOH ⁺	0.658 0.028	NiOH ⁺ Ni(OH) ₂	4.838 3.214	ZnOH ⁺ Zn(OH) ₂	56.39 0.941	PbOH ⁺ Pb(OH) ₂
	0.056	Cr ³⁺	9.285 64.36	Cu ²⁺ CuOH ⁺	91.961	Fe ²⁺	99.452	Mn ²⁺	96.859	Ni ²⁺	54.748	Zn ²⁺	14.514	Pb ²⁺
T ₁	99.944	CrOH ⁺	0.222 25.996	Cu(OH) ₂ Cu ₂ (OH) ₂ ²⁺	8.025	FeOH ⁺	0.548	MnOH ⁺	2.673 0.467	NiOH ⁺ Ni(OH) ₂	12.001 33.227	ZnOH ⁺ Zn(OH) ₂	79.916 5.558	PbOH ⁺ Pb(OH) ₂
	CaCl₂-extract													

S+n-BC-SSL

T ₀	0.028	Cr ³⁺	3.673 51.799 0.744 42.668	Cu ²⁺ CuOH ⁺ Cu(OH) ³⁻ Cu(OH) ₂	84.875	Fe ²⁺	98.887	Mn ²⁺	92.916	Ni ²⁺	25.201	Zn ²⁺	7.247	Pb ²⁺
	99.972	CrOH ⁺	0.871 0.246	Cu ₂ (OH) ₂ ²⁺ Cu ₃ (OH) ₄ ²⁺	15.071 0.054	FeOH ⁺ Fe(OH) ₂	1.108	MnOH ⁺	1.859	Ni(OH) ₂	25.201	Zn(OH) ₂	11.515	Pb(OH) ₂
T ₁	99.993	CrOH ⁺	0.411 22.502 4.889 72.037	Cu ²⁺ CuOH ⁺ Cu(OH) ₃ - Cu(OH) ₂	58.866 40.541	Fe ²⁺ FeOH ⁺	95.635	Mn ²⁺	65.589	Ni ²⁺	2.445	Zn ²⁺	1.453	Pb ²⁺
			0.119 0.041	Cu ₂ (OH) ₂ ²⁺ Cu ₃ (OH) ₄ ²⁺	0.561	Fe(OH) ₂	4.156	MnOH ⁺	14.284	NiOH ⁺	4.229	ZnOH ⁺	63.134	PbOH ⁺

S-b-BC-PB

T ₀	99.994	CrOH ⁺	0.281 18.909 6.218 74.449	Cu ²⁺ CuOH ⁺ Cu(OH) ³⁻ Cu(OH) ₂	53.712	Fe ²⁺	94.584	Mn ²⁺	57.695	Ni ²⁺	1.642	Zn ²⁺	1.094	Pb ²⁺
	99.998	CrOH ⁺	0.099 0.041	Cu ₂ (OH) ₂ ²⁺ Cu ₃ (OH) ₄ ²⁺	0.774	Fe(OH) ₂	0.365	Mn ₂ OH ³⁺	26.295	Ni(OH) ₂	94.222	Zn(OH) ₂	39.615	Pb(OH) ₂
T ₁			0.023 5.385	Cu ²⁺ CuOH ⁺	24.274	Fe ²⁺	78.269	Mn ²⁺	12.921	Ni ²⁺	0.142	Zn ²⁺	0.155	Pb ²⁺

S+n-BC-PB

S+b-BC-RH	T ₀	0,013	Cu(OH) ₄ ²⁻	70,584	FeOH ⁺	14,36	MnOH ⁺	11,881	NiOH ⁺	1,035	ZnOH ⁺	28,368	PbOH ⁺
		21,289	Cu(OH) ₃ ⁻										
		73,283	Cu(OH) ₂	4,155	Fe(OH) ₂	7,371	Mn ₂ OH ³⁺	69,939	Ni(OH) ₂	96,526	Zn(OH) ₂	66,479	Pb(OH) ₂
	T ₁	43,545	Cu ²⁺	98,513	Fe ²⁺	99,905	Mn ²⁺	99,511	Ni ²⁺	94,686	Zn ²⁺	50,923	Pb ²⁺
		52,202	CuOH ⁺	1,487	FeOH ⁺	0,095	MnOH ⁺	0,475	NiOH ⁺	3,59	ZnOH ⁺	48,491	PbOH ⁺
		0,579	Cu ₂ (OH) ₂ ²⁺					0,014	Ni(OH) ₂	1,724	Zn(OH) ₂	0,585	Pb(OH) ₂
	T ₁	16,069	Cu ²⁺	95,016	Fe ²⁺	99,67	Mn ²⁺	98,201	Ni ²⁺	74,015	Zn ²⁺	22,497	Pb ²⁺
		66,885	CuOH ⁺	4,979	FeOH ⁺	0,33	MnOH ⁺	1,627	NiOH ⁺	9,743	ZnOH ⁺	74,385	PbOH ⁺
		0,084	Cu(OH) ₂					0,171	Ni(OH) ₂	16,235	Zn(OH) ₂	3,114	Pb(OH) ₂
	T ₀	16,26	Cu ₂ (OH) ₂ ²⁺										
49,101		Cu ²⁺	98,786	Fe ²⁺	99,923	Mn ²⁺	99,604	Ni ²⁺	95,886	Zn ²⁺	56,1	Pb ²⁺	
47,9		CuOH ⁺	1,213	FeOH ⁺	0,077	MnOH ⁺	0,387	NiOH ⁺	2,958	ZnOH ⁺	43,473	PbOH ⁺	
S+n-BC-RH	T ₀	0,264	Cu ₂ (OH) ₂ ²⁺										
		19,694	Cu ²⁺	95,908	Fe ²⁺	99,732	Mn ²⁺	98,558	Ni ²⁺	79,859	Zn ²⁺	26,446	Pb ²⁺
		66,688	CuOH ⁺	4,088	FeOH ⁺	0,268	MnOH ⁺	1,329	NiOH ⁺	8,551	ZnOH ⁺	71,131	PbOH ⁺
	T ₁	0,055	Cu(OH) ₂										
		13,181	Cu ₂ (OH) ₂ ²⁺							11,586	Zn(OH) ₂	2,421	Pb(OH) ₂
		49,056	Cu ²⁺	98,786	Fe ²⁺	99,923	Mn ²⁺	99,603	Ni ²⁺	95,883	Zn ²⁺	56,081	Pb ²⁺
	T ₀	47,894	CuOH ⁺	1,214	FeOH ⁺	0,077	MnOH ⁺	0,387	NiOH ⁺	2,96	ZnOH ⁺	43,492	PbOH ⁺
		2,73	Cu(OH) ₂							1,157	Zn(OH) ₂	0,427	Pb(OH) ₂
		0,315	Cu ₂ (OH) ₂ ²⁺										
	T ₁	19,246	Cu ²⁺	95,814	Fe ²⁺	99,725	Mn ²⁺	98,521	Ni ²⁺	79,257	Zn ²⁺	25,974	Pb ²⁺
66,726		CuOH ⁺	4,182	FeOH ⁺	0,275	MnOH ⁺	1,36	NiOH ⁺	8,689	ZnOH ⁺	71,531	PbOH ⁺	
0,058		Cu(OH) ₂							12,049	Zn(OH) ₂	2,492	Pb(OH) ₂	
S+b-BC-SSL	T ₀	13,498	Cu ₂ (OH) ₂ ²⁺										
		35,336	Cu ²⁺	97,949	Fe ²⁺	99,868	Mn ²⁺	99,315	Ni ²⁺	91,953	Zn ²⁺	42,682	Pb ²⁺
		58,758	CuOH ⁺										
	T ₁	0,012	Cu(OH) ₂										
		99,767	CuOH ⁺	2,05	FeOH ⁺	0,132	MnOH ⁺	0,657	NiOH ⁺	4,835	ZnOH ⁺	56,377	PbOH ⁺
		5,693	Cu ₂ (OH) ₂ ²⁺					0,028	Ni(OH) ₂	3,211	Zn(OH) ₂	0,941	Pb(OH) ₂
	T ₀	9,283	Cu ²⁺	91,963	Fe ²⁺	99,452	Mn ²⁺	96,86	Ni ²⁺	54,756	Zn ²⁺	14,517	Pb ²⁺
		64,332	CuOH ⁺										
		0,222	Cu(OH) ₂										
	T ₁	25,982	Cu ₂ (OH) ₂ ²⁺	8,027	FeOH ⁺	0,547	MnOH ⁺	2,672	NiOH ⁺	12	ZnOH ⁺	79,913	PbOH ⁺
99,944		CuOH ⁺					0,467	Ni(OH) ₂	33,221	Zn(OH) ₂	5,557	Pb(OH) ₂	

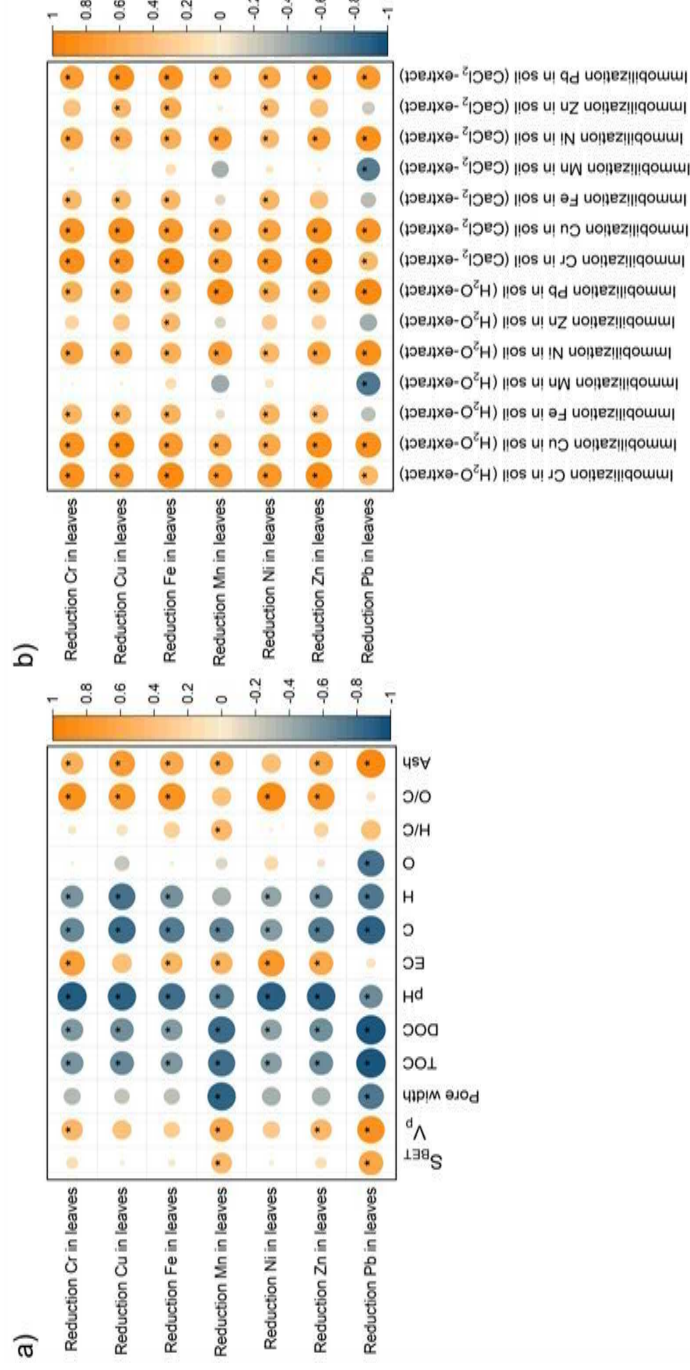


Figure S3. Pearson's correlation analysis between the reduction in bioaccumulation in *Lactuca sativa L.* leaves and a) the physicochemical properties of b-BC and n-BC, b) the immobilization efficiency of individual PTEs in the H₂O-extractable fraction and CaCl₂-extractable fraction. Statistically significant correlations are marked with * ($p \leq 0.05$).

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Publikacja D7

M. Raczkiewicz, D. Akachukwu, P. Oleszczuk

“Nanoscale biochar outperforms conventional biochar in enhancing soil health and reducing risks from polycyclic aromatic hydrocarbon-contaminated soil”

Environmental Research (po pierwszym etapie recenzji, w trakcie decyzji)

September 7, 2025

Professor Dear Prof. Apostolos Giannis, PhD,
Associate Editor
Environmental Research

Dear Dear Prof. Apostolos Giannis, PhD,

Enclosed is our revised manuscript (ER-25-12801R1) entitled *Nanoscale biochar outperforms conventional biochar in enhancing soil health and reducing risks from polycyclic aromatic hydrocarbon-contaminated soil*, by Monika Raczekiewicz, Doris Akachukwu and Patryk Oleszczuk (corresponding author). We really appreciate your revision suggestions. We thank the Reviewers for his/her comments. Based on the comments of the Reviewers, we made significant revisions to improve the manuscript.

Thank you very much for your help and time.

Sincerely yours,

Patryk Oleszczuk

Nanoscale biochar outperforms conventional biochar in enhancing soil health and reducing risks from polycyclic aromatic hydrocarbon-contaminated soil

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Abstract

Soil contamination with polycyclic aromatic hydrocarbons (PAHs) poses significant environmental and health risks due to their persistence, toxicity, and bioaccumulation. While conventional bulk biochar (b-BC) has been used for PAHs immobilization, limited studies have evaluated nano-biochar (n-BC) for this purpose. This study investigated the potential of n-BC to immobilize PAHs in industrially contaminated soil, focusing on its impact on PAHs bioavailability. B-BC were produced from palm bunch (PB), rice husk (RH), and sewage sludge (SSL) at 550°C under N₂ atmosphere and processed using a ball-milling method into nanoscale particles. Compared to b-BC, n-BC exhibited higher specific surface area, porosity, and abundance of oxygen-containing functional groups, resulting in higher immobilization efficiency immediately after soil application. Notably, n-BC exhibited high immobilization efficiency immediately after mixing with soil, underscoring its rapid remediation potential. A 6-week pot experiment showed that n-BC amendments reduced both total (C_{total} Σ 16 PAHs) and freely dissolved (C_{free} Σ 16 PAHs) PAHs contents. In n-BC-amended soils, C_{total} Σ 16 PAHs and C_{free} Σ 16 PAHs were 50.7–55.0% and 76.1–88.2% lower, respectively, compared to control soil, and significantly exceeding the values observed for b-BC-amendments. PB- and RH-derived b-BC and n-BC were most effective in PAHs immobilization. Additionally, n-BC amendments showed lower ecological risk based on the Nemerow Pollution Index (NIPI). This work represents the first comparative study directly evaluating n-BC and b-BC for PAHs immobilization, demonstrating that the rapid sorption and enhanced remediation efficiency of n-BC are strongly linked to its nanoscale properties. These findings highlight the superior remediation potential of n-BC for PAHs-contaminated soils, supporting its application as an effective and sustainable amendment. The enhanced quick and long-term immobilization effectiveness of n-BC compared to b-BC suggests its promising role in mitigating PAHs pollution and reducing related environmental risks.

Keywords: soil; amendment; contamination; environmental pollution; remediation; nanocarbons

1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a group of persistent organic pollutants (POPs) widely recognized for their toxic, mutagenic, and carcinogenic properties (Wang et al., 2021). Thus, PAHs contamination may be a global issue, requiring international attention and regulation. This structural stability of PAHs renders them highly resistant to environmental degradation, leading to their long-term persistence in ecosystems (Premnath et al., 2021). PAHs are primarily generated through incomplete combustion of organic materials, with major anthropogenic sources including fossil fuel combustion, industrial emissions (e.g., petroleum refining, coke production), vehicular exhaust, and biomass burning (Dat and Chang, 2017). While natural processes like volcanic eruptions contribute marginally to their release, anthropogenic activities dominate their global distribution (Abdel-Shafy and Mansour, 2016). PAHs are designated as priority pollutants by major environmental regulatory agencies including United States Environmental Protection Agency (US EPA), European Union Environmental Protection Agency (UE EPA) and Chinese Ministry of Ecology and Environment (MEE) (European Parliament and the Council of the European Union, 2013; Ministry of Ecology and Environment, 2018; United States Environmental Protection Agency, 2008), due to their documented adverse environmental impacts (Teixeira et al., 2024). Soils act as a significant reservoir for PAHs (Ferraro et al., 2021). Industrialized regions, particularly near refineries and urban centres, often exhibit PAHs concentrations exceeding regulatory thresholds (Wang et al., 2020). Once deposited, PAHs, persisting for decades (Garagnon et al., 2023) and threatening groundwater through leaching or atmospheric pathways via volatilization. This multi-matrix contamination underscores the urgent need for targeted

remediation strategies to mitigate environmental and health risks. Beyond their persistence in soils and mobility across environmental matrices, PAHs also pose serious health risks. Human exposure occurs via inhalation, dermal contact, and ingestion of contaminated food or water. They are linked not only to cancer but also to respiratory, cardiovascular, and neurodevelopmental disorders, underscoring the urgency of addressing PAH contamination to protect both ecosystems and public health (World Health Organization, 2021).

In situ immobilization has emerged as a rational strategy to reduce PAHs bioavailability and mobility through adsorption or sequestration, minimizing ecological disturbance while preventing contaminant transfer to groundwater or crops (Kuppusamy et al., 2017). Various immobilization agents have been employed to reduce the mobility and bioavailability of PAHs in contaminated soils, with activated carbon (AC) being a traditional choice (Eldos et al., 2022). In recent years, biochar (BC) has occurred as a promising alternative sorbent for PAHs remediation (Bianco et al., 2021; Ni et al., 2018; Shyam et al., 2025), drawing increased attention from the scientific community due to its cost-effectiveness and sustainable production methods. BC, a carbon-rich material produced via pyrolysis of biomass under oxygen-limited conditions (Amalina et al., 2022). Its high surface area, porous structure, and oxygen-containing functional groups (e.g., carboxyl, hydroxyl) enable robust interactions with hydrophobic pollutants via π - π stacking, hydrophobic partitioning, and pore-filling mechanisms (Ahmed et al., 2018; Barquilha and Braga, 2021). Furthermore, BC offers an advantage over AC by enhancing soil fertility through improved water retention, nutrient cycling, and microbial activity (Alkharabsheh et al., 2021; Waheed et al., 2025), while AC may actually limit nutrient availability (Oleszczuk et al., 2019).

Conventional BC's efficacy is constrained by variability in feedstock, pyrolysis conditions, and particle size, which influence its adsorption capacity and long-term stability (Crombie et al., 2013). To address these limitations, nano-biochar (n-BC) -engineered at the nanoscale (1–

100 nm) offers enhanced physicochemical properties compared to conventional BC (Racziewicz et al., 2024; Ramanayaka et al., 2020a, 2020b). Reduced particle size of n-BC amplifies surface area-to-volume ratios, exposes reactive sites, and improves colloidal mobility in soil, enabling deeper penetration and efficient PAHs interaction (Song et al., 2019). Functionalized surfaces on n-BC facilitate stronger adsorption via hydrogen bonding and electrostatic interactions, while its microporous structure acts as a molecular sieve, selectively trapping PAHs (Jiang et al., 2023). Furthermore, n-BC forms stable complexes with soil organic matter and minerals (Kianfar et al., 2022), which may reduce PAH remobilization risks under environmental stressors like pH fluctuations after the application of BC to soil. Despite the promising potential of n-BC, there is a significant research gap concerning its application for the immobilization of PAHs in contaminated soil. While b-BC or AC role for PAHs immobilization has been documented (Hale et al., 2012; Kołtowski et al., 2016), critical questions regarding the kinetics, molecular interactions, and long-term stability of n-BC in contaminated soils remain still unexplored.

This study examines the effectiveness of n-BC in immobilizing PAHs within contaminated soil, focussing on soil-particle interactions and long-term environmental stability. Through systematic optimization of n-BC parameters, including particle sizes distribution, and feedstock types, the study aims to develop an implementation protocol for cost-effective remediation of PAHs in industrial contaminated zones. The research advances nanotechnology applications in soil restoration, introducing enhanced remediation approaches that surpass conventional treatment methods while maintaining environmental sustainability. We hypothesize that n-BC, owing to its higher surface area, greater porosity, and improved accessibility of surface functional groups after particle size reduction, will exhibit stronger PAHs sorption capacity than conventional b-BC, while differences in immobilization efficiency among n-BC types will be primarily determined by the feedstock origin and the properties of the corresponding b-BC

used for their production. Importantly, this work represents the first comparative study directly evaluating the immobilization performance of n-BC and b-BC for PAHs in contaminated soils, thereby highlighting the novelty of applying nanoscale carbon materials for remediation purposes.

2. MATERIALS AND METHODS

2.1. Bulk biochar and nano-biochar

All BC, including bulk biochars (b-BCs) and nano-biochars (n-BCs) were obtained using palm bunch (PB) and rice husk (RH) sourced from African areas (Umudike, Nigeria) and sewage sludge (SSL) obtained from a local municipal wastewater treatment facility (Lublin, Poland). The b-BC production process was adapted from our previous research (Siatecka et al., 2021). Feedstocks were dried in an oven at 100°C for 24 hours (POL-EKO, Poland) and ground < 2 mm using a laboratory grinder (TESTCHEM, Poland). Pyrolysis was conducted in a horizontal tubular retort furnace (Czylok, Poland) at 550°C for 3 hours under an inert atmosphere of N₂ with a controlled gas flow 1L·min⁻¹ and heating rate of 10°C·min⁻¹. The production of n-BCs followed established protocol. In brief, the b-BCs particles underwent size reduction via mechanical processing in a planetary ball mill (1.5 hours, 440 rpm, biochar-to-ball mass ratio 1:100, 5-minute intervals with a change of rotation direction). (PM100, Retsch, Germany). The b-BC samples were subjected to cryogenic pretreatment at -80°C for 24 hours to optimize milling efficiency (Rackiewicz et al., 2024). The comprehensive physicochemical characterization of both b-BC and n-BC is detailed in the SI (Table S1). Additionally, the contents of C_{total} and C_{free} Σ16 PAHs are presented in Figure 1, with a detailed description available in the SI (Table S2-S3). Details on the methodologies used for the physicochemical characterization of b-BC and n-BC, including elemental analysis (CHNO), ash content, pH and electrical conductivity (EC) measurements, total and dissolved organic carbon (TOC and DOC)

content, porous structure analysis via N₂ adsorption-desorption, and FT-IR and XPS analysis are provided in the SI.

2.2. Pot experiment

Soil utilized in this study originated from an industrial region (Dąbrowa Górnicza, Poland) and was not sterilized prior to the pot experiment. The experimental setup was carried out in 500 mL pots, with triplicate treatments. To prepare the amendments, b-BC/n-BC were incorporated into the soil at a 2% (w/w, dry weight) application rate, ensuring thorough homogenization of the mixture. Although BC is typically applied at rates below 1% for fertilization (Joseph et al., 2021), a higher rate of 2% was selected in this study, as previous remediation studies indicate that such levels (~2%) lead to significantly improved overall remediation performance (Godlewska and Oleszczuk, 2022a; Kończak et al., 2023). Following homogenization, the amended soils were transferred into pots and moistened with Milli-Q® water to reach 65% of the soil's water holding capacity (WHC). Unamended soil was prepared in parallel as a control. The pots were maintained under controlled conditions (12-hour light/dark cycle, 22°C Day/18°C night, and constant humidity) in a growth chamber (Conviron GEN100, Canada). To maintain consistent moisture, Milli-Q® water was added twice weekly. Soil samples (50 g) were collected at two stages: immediately before the experiment commenced (T₀) and after six weeks of incubation (T₁). The samples were air-dried for 24 hours and then stored at 4°C for subsequent physicochemical analysis. Detailed physicochemical properties of both the unamended and b-BC/n-BC amended soils are provided in the SI (Table S4).

2.3. Organic solvent extractable (C_{total}) and freely dissolved (C_{free}) PAHs content

Organic-solvent extractable PAHs (C_{total} Σ16 PAHs) were extracted using an accelerated solvent extraction system (Dionex ASE 350, ThermoFisher Scientific, USA) according to

(Hilber et al., 2012). In this procedure, a 22 mL stainless-steel extraction cell was prepared by layering a cellulose filter at the bottom, followed by 6 g of powdered activated silica gel mixed with 0.1 g of powdered Cu and 0.1 g of EDTA, and 4 g of soil samples or 0.5g of b-BC/n-BC in the presence of an internal standard (16 deuterated PAHs at a concentration of 100 ng· μ L⁻¹). PAHs were extracted using n-hexane at a temperature of 150°C and a pressure of 14 MPa.

The concentration of freely dissolved PAHs (C_{free} Σ 16 PAHs) was assessed using the polyoxymethylene (POM) passive samplers (Cornelissen and Gustafsson, 2004). Specifically, 1 g of sample was placed in a 50 mL flask with two POM strips (40 x 40 mm). Untreated soil was used as a control. To inhibit microbial activity, each flask received 40 mL of sodium azide solution (200 mg·L⁻¹). The flasks were placed on a rotary shaker (Rotax 6.8, VELP, Usmate Velate, Italy) for 30 days until equilibrium was reached. After this period, the POM strips were carefully removed, cleaned with Milli-Q water to eliminate any solid residues, and dried to obtain the passive samplers mass measurements needed for subsequent analysis. PAHs were extracted from the POMs using the mixture of acetone and n-heptane (ratio 1:4, v/v) over a 48-hour period in the presence of an internal standard (16 deuterated PAHs at a concentration of 100 ng· μ L⁻¹).

2.4. The resulting extracts for determination C_{total} and C_{free} PAHs were concentrated to a final volume of 1 mL using a rotary vacuum concentrator (RVC 2–25CD plus, Martin Christ, Germany) and analyzed by gas chromatography-mass spectrometry (Trace 1300, ThermoFisher Scientific, USA), utilizing a Restek Rxi-5 ms capillary column (30 m length, 0.25 mm internal diameter, and 0.25 μ m film thickness. Mass spectra were obtained via electron ionization (EI), with SIM mode targeting molecular ions of individual PAHs. To ensure quality assurance and quality control (QA/QC), each batch of samples was analyzed with a blank sample and triplicates ($n = 3$) containing an internal standard. No PAHs were detected in the blank samples. Details regarding the detection limits and recovery rate of PAHs are provided in the SI (Table S5 and S6). Nemerow multifactor pollution index (NIPI)

The Nemerow Multifactor Pollution Index (NIPI) was used to assess the ecological risk associated with soil pollutant exposure before and after amendments. NIPI provides a comprehensive evaluation of the impact of soil contaminants, particularly in heavily polluted environments. The NIPI is calculated using the following method (Sawut et al., 2018):

$$P_i = \frac{C_i}{C_s} \quad (1)$$

$$NIPI = \sqrt{\frac{P_{iavg}^2 + P_{imax}^2}{2}} \quad (2)$$

where C_i is the measured concentration of PAHs, C_s is the soil environmental quality standard value of PAHs, and P_i is the pollution index of PAHs in the soil. P_{iavg} is the arithmetic mean of PAHs pollution index of each monomer, P_{imax} is the maximum value of the PAHs single factor pollution index at the sampling point, and NIPI is the multi-factor pollution index at the sampling point.

2.5. Statistical analysis

Statistical analyses were performed using OriginPro 2025 (OriginLab Corporation, USA). Mean values were derived from triplicate measurements. To evaluate the relationships between the physicochemical parameters of b-BC/n-BC and the efficiency of PAHs immobilization, Pearson's correlation analyses were conducted. Additionally, one-way ANOVA followed by Tukey's post hoc test was applied to determine the variability and significance of differences among the tested groups. The level of statistical significance was set at $p \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1. C_{total} and C_{free} $\Sigma 16$ PAHs content in control soil

The C_{total} $\Sigma 16$ PAHs content in control soil was $15,258 \pm 1033 \mu\text{g} \cdot \text{kg}^{-1}$ at the beginning of experiment (T_0) and was reduced by 17.8% after six weeks (T_1) (Fig. 2a). While the overall change was not statistically significant ($p > 0.05$), several individual compounds showed significant ($p = 0.0013$ - 0.0387) variations (Table S7), primarily among the light-weight compounds. These decreases could be attributed to natural degradation processes, such as microbial activity or volatilization (Ghosal et al., 2016). In control soil, 3- and 4-rings PAHs were predominant, accounting 59.2% and 21.9% (T_0), respectively (Fig. 2d). After six weeks (T_1), the contribution slightly changed and reached values of 53.6% and 25.0%, respectively. This change suggests that 3-rings PAHs underwent faster biodegradation due to their higher water solubility and greater availability to microorganisms (Wen et al., 2021). In contrast, the relative increase in 4-rings PAHs may be attributed to their lower biodegradability and stronger affinity for soil organic matter, which slows down their degradation and mobility (Ukalska-Jaruga et al., 2019). Acenaphthylene (ACY), phenanthrene (PHE) and fluoranthene (FLA) were the most abundant in C_{total} $\Sigma 16$ PAHs of the control soil, both at the beginning of the experiment

(T₀) and after six weeks (T₁) (Table S7). However, their absolute concentrations decreased by 6.55-37.2% compared to the initial values at T₀.

The C_{free} Σ16 PAHs content in control soil was 681±52.7 ng·L⁻¹ at the beginning of experiment (T₀) and was reduced by 14.1% after six weeks (T₁) reaching an absolute value of 585±46.0 ng·L⁻¹ (Fig. 2e). The contribution of C_{free} Σ16 PAHs was dominated by 2-rings PAHs, accounting 73.6% at the beginning of the experiment (T₀). Over time (T₁), the proportion of 2-ring PAHs increased by 12.4% (reached absolute value of 82.7%), while the contribution of 3- and 4-ring PAHs decreased by 37.0% and 32.6%, respectively (Fig. 2h). In contrast, no significant (p>0.05) changes were observed for 5- and 6-ring PAHs. The distribution of C_{free} Σ16 PAHs was primarily composed of naphthalene (NAP) and phenanthrene (PHE).

According to the European Union (EU) regulation regards to C_{total} PAHs in soils from industrial areas (Ministry of Climate and Environment, 2024), the permissible levels should not exceed 20,000 µg·kg⁻¹. Although these limits were not exceeded in the control soil, the measured values were close to the upper threshold. There are no EU regulations regarding C_{free} PAHs in soil. However, when compared to the regulatory limit for C_{free} PAHs in groundwater (European Parliament and the Council of the European Union, 2013), which is set at 270 ng·L⁻¹, the observed values in soil suggest a potential risk of leaching and groundwater contamination. Based on above criteria, the studied soil can be classified as contaminated, despite the fact that the permissible concentrations for C_{total} PAHs were not exceeded. Nevertheless, the detected C_{free} PAHs level in the soil solution suggests potential risks of leaching into surrounding groundwater, where it exceeds the permissible limit by approximately 2.5 times. This degree of contamination indicates that the soil is unsuitable for agricultural use due to potential adverse effects on crop quality and risks to human and environmental health.

3.2. Changes in C_{total} $\Sigma 16$ PAHs content in soil amended with b-BC/n-BC

The addition of b-BC into contaminated soil showed no statistically significant ($p > 0.05$) effect on the C_{total} $\Sigma 16$ PAHs content across all experimental treatments with b-BC at the beginning of the experiment (T_0) (Fig. 2a). In contrast, the addition of n-BC resulted in C_{total} $\Sigma 16$ PAHs within decreases ranging from 23.4-28.4% compared to the control soil (Fig. 2b). This reduction can be attributed to a mass/volume-based dilution effect, as n-BC contained inherently lower PAHs concentrations than the control soil, with the effect being more pronounced for n-BC than b-BC due to its lower initial PAHs content (Table S2) and its superior particle distribution within the soil matrix. These characteristics of n-BC contributed to a more substantial relative decrease in PAHs concentrations upon soil mixing.

Comparing the contribution of C_{total} $\Sigma 16$ PAHs based on the number of aromatic rings, 3-rings PAHs dominated in both unamended soil and soil amended with b-BC/n-BC. The initial application of b-BC/n-BC (T_0) led to a significant ($p \leq 0.05$) increase in 3-rings PAHs contribution, increasing by 10.2-15.4%, compared to the untreated soil, ultimately comprising 55.2-68.4% of total PAHs content, with variation depending on the BC type. Analysis of individual C_{total} PAHs demonstrated significant ($p \leq 0.05$) contribution changes after BC application for selected compounds (Fig. S5). The effect varied by compound and BC type, with some PAHs showing increased concentrations while others decreased.

After a six-week period (T_1), a statistically significant ($p \leq 0.05$) decrease of the C_{total} $\Sigma 16$ PAHs content was noted (Fig. 2c) compared to initial values (T_0). Specifically, treatments with b-BC showed decreases ranging from 35.7-41.3%, while n-BC amendments demonstrated reduction between 46.9- 48.3% relative to T_0 (Fig. 2c). These reductions were more pronounced for the n-BC (higher by 17.0-32.3%) than for the b-BC amendments. Among the b-BC variants, RH- and SSL-derived b-BC demonstrated the most effective PAHs reduction capabilities (Fig. 2c). In contrast, all n-BC treatments showed comparable effectiveness in PAHs reduction, with

no statistically significant variations ($p>0.05$) between different n-BC sources (Fig. 2c). Comparing C_{total} $\Sigma 16$ PAHs concentrations in the control soil at T_1 , significantly ($p>0.01$) lower concentrations (from 35.0-55.0%) were observed in samples containing b-BC/ n-BC amendments (Fig. 2b). These findings suggest that b-BC and n-BC amendments create more favourable conditions for PAHs sequestration compared to raw soil conditions. In all cases, after six-week period (T_1) n-BC demonstrated final lower content of PAHs (from 21.4-45.3%) than corresponding b-BC.

After six weeks (T_1), a dominance of 3-rings PAHs was observed in the contribution of C_{total} $\Sigma 16$ PAHs, ranging from 63.0- 71.2%, depending on the type of b-BC/n-BC (Fig. 2d). Analysis of individual C_{total} $\Sigma 16$ PAHs, revealed several compounds with particularly significant reductions ($>26.7\%$). These compounds included acenaphthylene (ACY), anthracene (ANT), fluoranthene (FLA), chrysene (CHR), benzo[b]fluoranthene (BbF), and benzo[k]fluoranthene (BkF) (Fig. S3). This pattern remained consistent across all b-BC and n-BC treatments. While the overall C_{total} $\Sigma 16$ PAHs reduction varied by feedstock source, no specific BC type exhibited preferential sequestration of particular PAHs.

The correlation analysis showed strong positive relationships ($r=0.86-0.99$, $p\leq 0.05$) between the reductions of C_{total} $\Sigma 16$ PAHs content compared to the control soil after application of b-BC/n-BC at both T_0 or T_1 and specific physicochemical parameters of applied b-BC/n-BC (Fig. 3a). Strong correlations were found between C_{total} $\Sigma 16$ PAHs content reduction and specific surface area (S_{BET}) ($r=0.98-0.99$), total pore volume (V_p) ($r=0.95-0.99$), and electrical conductivity (EC) ($r=0.86-0.94$). These parameters were generally higher in n-BC than b-BC, suggesting they play a key role in n-BC's superior performance in reducing PAHs after soil amendment. It is well established that high surface area (S_{BET}) and porosity are crucial factors in binding and immobilizing PAHs in soil (Tan et al., 2021), so these correlations align with existing research. The approximately 4.1 times higher surface area (S_{BET}) of n-BC (Table S1)

facilitates rapid PAHs immobilization via π - π stacking and pore-filling (Shamloo et al., 2024). Higher EC values may enhance the interactions between PAHs and soil components, potentially stabilizing contaminants through electrostatic interactions or changes in soil chemistry (Peralta and Costa, 2013). The increase in EC can influence the surface charge of n-BC (Li et al., 2016), affecting the availability of active sites for adsorption. Although PAHs are non-ionic, they can interact with n-BCs via dipole and π - π interactions. Additionally, higher EC may induce the salting-out effect (Turner and Rawling, 2001), where highly hydrated ions may reduce PAHs solubility in water, enhancing their adsorption onto the hydrophobic surfaces of n-BC's and soil (Oh et al., 2013). While EC is often overlooked as a significant factor affecting PAHs fate in soil environment, our correlations suggest it plays a crucial role, as EC values were 1.3-2.5 times higher in n-BC compared to b-BC. The Principal Component Analysis (PCA) results (Fig. 3c) further support these findings, illustrating distinct separation of soil samples treated with n-BC from those treated with b-BC and confirms that the specific physicochemical properties of n-BC, contribute significantly to its effectiveness in reducing PAHs in soil. The FTIR and XPS spectra (Fig. S1 and S2) further confirm that n-BC exhibits a higher presence of oxygenated functional groups (C-OH, C=O, COOR), indicating enhanced surface functionality. Moreover, the correlation analysis (Fig. S7) indicates that reductions in $C_{\text{total}} \Sigma 16$ PAHs are strongly influenced by the presence of specific functional groups in b-BC/n-BC. Significant positive correlations ($r=0.71$ - 0.79) were observed between reduction in $C_{\text{total}} \Sigma 16$ PAHs and C-C/C-H, C-OH/C-O-C, and Si-OH/O=C-O-R, suggesting that PAHs immobilization is primarily driven by hydrophobic interactions and hydrogen bonding. Functional groups such as C=O, COOR, and carbonates exhibited weaker or no correlation, implying a limited role in PAHs retention. These findings highlight the importance of aromatic (C-C/C-H) and oxygen-containing (C-OH/C-O-C, Si-OH/O=C-O-R) functional groups in PAHs immobilization, with interactions likely governed by hydrophobic forces and hydrogen bonding rather than

electrostatic mechanisms. Consequently, n-BC has emerged as a promising material for environmental remediation, demonstrating potential for reducing C_{total} $\Sigma 16$ PAHs, thereby mitigating the mobility and bioavailability of hazardous organic contaminants, such as PAHs, in polluted environments. However, over time, the influence of certain groups such as Me–O/carbonates/O=C may enhance desorption, affecting the long-term stability of PAHs in soil.

To evaluate the effect of change in physicochemical properties of soil matrix on PAHs immobilization, the correlation between changes of selected soil properties (TOC, DOC, pH, EC) and C_{total} $\Sigma 16$ PAHs was also calculated (Fig. 3b). Statistically significant positive correlations were observed for all analysed parameters, suggesting that the extent of soil property alterations induced by b-BC/n-BC amendments plays a key role in determining the efficiency of PAHs immobilization (Fig. 3b). In particular, higher increases in TOC and DOC following n-BC addition may provide additional sorption domains and complexation capacity, thereby contributing to the reduction of PAHs content in soil. The simultaneous increase in DOC content, may also reflect the partial release of soluble organic fractions from n-BC, which can interact with PAHs and influence their overall distribution between soil phases (Cai et al., 2019). Importantly, higher TOC generally enhances the retention of hydrophobic compounds such as PAHs, reinforcing the immobilization effect observed in amended soils (Han et al., 2015).

Recent studies showed that adding organic materials like compost, b-BC and AC can really boost soil health (Marwein et al., 2023). Furthermore, BC and AC exhibit an affinity for organic pollutants, such as PAHs, reducing their bioavailability and mobility (Beljin et al., 2024). Our observations support this finding, as we observed a reduction in C_{total} $\Sigma 16$ PAHs content following the addition of b-BC/n-BC in the soil after a six-week incubation period. Furthermore, our results align with previous reports (Kończak et al., 2023; Shang et al., 2024; B. Tomczyk et al., 2020) regarding changes in C_{total} $\Sigma 16$ PAHs content in BC-amended soils,

both immediately after b-BC application and over extended incubation durations. However, they expand the existing knowledge by incorporating findings on n-BC and emphasizing the critical six-week period. Tomczyk et al. (2020) reported no statistically significant changes ($p>0.05$) in C_{total} $\Sigma 16$ PAHs content immediately following the addition of different SSL-derived b-BC to soil. However, after six months of incubation, a reduction of 18-56% was observed, depending on the specific type of b-BC used. Similarly, Kończak et al. (2023) found no statistically significant changes ($p>0.05$) in C_{total} $\Sigma 16$ PAHs content directly after adding SSL-derived b-BC to three distinct soil types. Nevertheless, after 30–180 days, reductions ranging from 7.5-32% were observed, depending on the soil type. Likewise, Shang et al. (2024) demonstrated that the addition of RH-derived b-BC to soil resulted in a 51.1% reduction in C_{total} $\Sigma 16$ PAHs content after 75 days, which is consistent with the reductions observed in our current investigation. To date, no research has investigated the effect of n-BC on C_{total} $\Sigma 16$ PAHs persistence in soils or other matrices. Previous research only confirmed n-BC remarkable adsorption capacity for a wide range of organic contaminants (Anupama and Khare, 2021; Cui et al., 2025; Pathak et al., 2024). This enhanced sorption capacity compared to conventional b-BC is attributed to n-BC higher surface area, porosity, and the presence of functional groups introduced during the nanoscale modification process (Raczkiewicz et al., 2024). In our research we also give a hypothesis that EC, which is high in n-BC, may have significant effect of the observed superior immobilization of PAHs in contaminated soil. On the basis of sorption capacity, it can be assumed that these properties not only facilitate the adsorption of contaminants but also increase the persistence and long-term retention of organic pollutants in soil and water systems by reduced bioavailability and mobility.

3.3. Changes in C_{free} $\Sigma 16$ PAHs content in soil amended with b-BC/n-BC

The addition of b-BC/n-BC primarily impacted C_{free} $\Sigma 16$ PAHs content (Fig. 2e). For all variants involving b-BC/n-BC amendments, a statistically significant ($p\leq 0.05$) reduction in C_{free}

361 $\Sigma 16$ PAHs was observed immediately after b-BC/n-BC application (T_0). Depending on the type
 362 of b-BC/n-BC treatment, reductions in C_{free} $\Sigma 16$ PAHs content ranged from 11.8-52.5% (for b-
 363 BC – from 11.8-28.4% and n-BC – from 25.0-52.5%) (T_0). Notably, n-BC showed a
 364 substantially higher reductions range in C_{free} $\Sigma 16$ PAHs (by 68.4-113%) compared to
 365 corresponding b-BC. This finding indicates that, the initial phase following the addition of b-
 366 BC and especially n-BC into the soil involves rapid adsorption and retention of available PAHs,
 367 as previously reported for contaminated agricultural BC-amended soil (Oleszczuk et al., 2016).
 368 This fast binding of PAHs, primarily for n-BC is associated with the high availability of active
 369 surfaces and changes in the physicochemical properties of the material upon contact with the
 370 aqueous solution (Wang et al., 2023). For individual compounds, no discernible pattern
 371 emerged to indicate a preferential binding affinity in the presence of b-BC/n-BC. All PAHs
 372 were immobilized at comparable level or- in some cases- slight increases in their concentrations
 373 were noted (Fig. S5). Among all b-BC and n-BC amendments, PB-derived b-BC and n-BC
 374 were the most effective in immobilizing C_{free} $\Sigma 16$ PAHs compared to other BC, exhibiting
 375 reductions in C_{free} $\Sigma 16$ PAHs content by 28.4% (PB-derived b-BC) and 52.5% (PB-derived n-
 376 BC), respectively. These values were higher by 11.7-142% compared to other b-BC and n-BC
 377 immediately after their application (T_0). It is generally accepted that surface area (S_{BET}) is the
 378 primary factor influencing the efficiency of PAHs immobilization in soils with carbonaceous
 379 materials (Yaashikaa et al., 2024). Therefore, it is challenging to explain why PB-derived b-BC
 380 and n-BC are the most effective at reducing C_{free} $\Sigma 16$ PAHs in this study, even though these
 381 materials have the lowest S_{BET} values (Table S1). This observation may suggest that other
 382 properties of b-BC/n-BC than S_{BET} are important in this stage experiment (T_0). Immediately
 383 after addition (T_0), the superior performance of PB-derived b-BC and n-BC, despite their low
 384 S_{BET} can be attributed to carbon-driven mechanisms rather than surface area effects. Elevated
 385 TOC provides abundant organic domains that act as partitioning phases for hydrophobic PAHs,

while DOC released from PB-derived b-BC/n-BC may form transient complexes with PAHs. These DOC–PAHs associations can facilitate the transfer of contaminants towards the BC’s surface, where they are subsequently stabilized by π – π interactions, hydrogen bonding, and electrostatic attraction with functional groups (Sarma et al., 2024). In this initial stage, the dominance of such organic carbon–mediated processes outweighs the role of surface area, explaining why PB-derived b-BC/n-BC immobilized the highest fraction of C_{free} $\Sigma 16$ PAHs at the beginning of the experiment (T_0). Over longer incubation periods, however, the contribution of structural parameters such as S_{BET} and pore architecture is likely to become more pronounced, gradually shifting the balance of mechanisms that govern PAH immobilization. This interpretation is consistent with the PCA analysis (Fig. 3c) which indicates that the superior performance of b-BC-PB and n-BC-PB in reducing C_{free} $\Sigma 16$ PAHs is driven by a synergy of large pore structures (enhancing mass transfer), high TOC/DOC content (increasing PAHs interactions), and optimal hydrophobicity (improving PAHs retention). Their positioning on the right side of the PCA plot, closely aligning with TOC and DOC and elemental composition, suggests a strong correlation between high organic carbon availability and surface chemistry, and PAHs immobilization. The separation of these BC along PC1 (48.02%) indicates that their distinct physicochemical properties (like pore width and pH) contribute significantly to their sorption efficiency. Additionally, C_{free} $\Sigma 16$ PAHs are located in the same quadrant as TOC and DOC, reinforcing the idea that higher organic carbon content enhances PAHs sequestration through stronger interactions between PAHs and the BC’s matrix. Although other physicochemical properties are present in the PCA plot, their weaker alignment with, C_{free} $\Sigma 16$ PAHs content reduction suggests they are not the dominant drivers of PAHs immobilization compared to TOC/DOC, pore width and surface chemistry. Moreover, PB-derived BC and n-BC exhibited the highest abundance of surface functional groups (Fig. S1 and S2) compared to other b-BC/n-BC. The functional groups plays a crucial role in binding PAHs by hydrogen

bonding, π - π interactions, and electrostatic attraction (Ahmed et al., 2018). Notably, PB-derived b-BC and n-BC contained the lowest C_{free} $\Sigma 16$ PAHs content among the tested BCs (Table S2), which may limit the release of C_{free} $\Sigma 16$ PAHs from the b-BC/n-BC matrix itself and, to some extent, influence the efficiency of immobilization.

Extended contact between b-BC/n-BC and soil (T_1) significantly ($p \leq 0.05$) improved immobilization of C_{free} $\Sigma 16$ PAHs compared to initial measurements (T_0) (Fig. 2g). The immobilization effectiveness increased from 58.9-83.5% depending on feedstock. Notably, PB- and RH-derived b-BC and n-BC exhibited the most substantial temporal increases in immobilization, increasing from 83.1-83.5% for b-BC and 78.7-81.9% for n-BC, respectively. Particle size reduction significantly effected only for SSL-derived n-BC, demonstrating a 23.3% greater reduction in C_{free} $\Sigma 16$ PAHs content relative to SSL-derived b-BC, when comparing immobilization efficiency over time (T_1 to T_0 variants). For other feedstocks, the reduction of C_{free} $\Sigma 16$ PAHs after six-weeks period of BC application was not statistically significant between n-BC and b-BC. Compared to the control soil, the concentration of C_{free} $\Sigma 16$ PAHs in n-BC/b-BC amendments was lower from 57.7-88.2% after six weeks of incubation (T_1), showing very high effectiveness in C_{free} $\Sigma 16$ PAHs reduction over an extended period of time (Fig. 2f).

The lack of significant differences in immobilization capacity between PB- and RH-derived n-BC and their bulk counterparts over six weeks might be due to the high initial immobilization efficiency of both, leading to an equilibrium that reduced further sorption potential over the extended incubation period (Fig. 2f). While the differences in C_{free} $\Sigma 16$ PAHs immobilization were small, n-BC led to greater reductions in C_{total} $\Sigma 16$ PAHs, suggesting it may stimulate PAHs degradation, possibly by enhancing microbial activity or catalytic processes (Sarma et al., 2019). Initially only naphthalene (NAP), phenanthrene (PHE), anthracene (ANT), and fluoranthene (FLA) showed significant reduction with b-BC/n-BC amendments, with n-BC

showing higher reductions (Fig. S5). For the remaining C_{free} PAHs, both decreases and increases in concentrations were noted, depending on the type of b-BC/n-BC used. However, this trend changed over time (T_1), as the prolonged incubation period led to the immobilization of all C_{free} 16 PAHs. Regarding feedstock, it was observed that SSL-derived b-BC and n-BC were the least effective in immobilizing PAHs, both at the beginning and over time (Fig. 2f-g).

The mass transfer of PAHs molecules to b-BC/n-BC particles can be described by a three-step process where (1) PAHs molecules are desorbed from the contaminated soil matrix to soil solution; (2) molecules diffuse through the aqueous phase toward the b-BC/n-BC particles and (3) molecules are sequestered by the b-BC/n-BC (Werner et al., 2006). The smaller particle size of n-BC reduces the diffusion path length that PAHs must pass through, facilitating more efficient adsorption. The enhanced reduction of C_{free} $\Sigma 16$ PAHs content observed for n-BC particle compared to conventional b-BC observed at the beginning (T_0) and (in some cases) at the end of the experiment (T_1) aligns with previously documented behaviour patterns for various organic contaminants in solid matrices (McLeod et al., 2007; Tomaszewski et al., 2007). Maintaining a constant mass-based dosage while decreasing particle size results in an exponential increase in the number of individual n-BC particles per unit soil volume (Wang et al., 2013). This optimization leads to two critical advantages. First, a significantly enhanced surface area (S_{BET}) available for PAHs binding interactions emerges. Second, there is an increased probability of successful collision events between n-BC particles and target PAHs molecules, ultimately maximizing the system's overall sorption capacity. The enhanced surface area-to-volume ratio of nano-scale particles facilitates more efficient mass transfer and stronger binding kinetics compared to their conventional bulk counterparts. In addition, n-BC contributes higher amounts of TOC and DOC to the soil system, which further expands the pool of sorption domains and promotes stronger interactions with hydrophobic PAHs, reinforcing their immobilization.

The reduction of C_{free} $\Sigma 16$ PAHs content during six-week period (T_1) can be related to besides of adsorption also to physical (photodegradation, volatilization, leaching) and biological degradation of PAHs, which confirms significant correlation between C_{free} and C_{total} $\Sigma 16$ PAHs reduction, calculated relative to the control soil at the respective time points (Fig. 3a). We observed the strong positive correlations between carbon (C), hydrogen (H), and oxygen (O) content in both b-BC and n-BC and their ability to immobilize in C_{free} $\Sigma 16$ PAHs (Fig. 3a). Furthermore, the PCA analysis confirmed these observations, highlighting that elemental composition plays a significant role in the reduction of C_{free} $\Sigma 16$ PAHs content (Fig. 3c). These findings suggest that the mechanisms governing C_{free} PAHs binding play a more crucial role than physical or biological-related PAHs losses. Higher oxygen content indicates the presence of functional groups such as hydroxyl, carboxyl, and carbonyl, which enhance PAHs adsorption through hydrogen bonding and dipole interactions (Ambaye et al., 2021; Tan et al., 2024), while increased hydrogen content suggests the presence of aliphatic structures that may contribute to interactions with PAHs molecules (Elvati and Violi, 2013), which is confirmed by FT-IR and XPS spectra (Fig. S1 and S2). The carbon-rich nature of b-BC and n-BC provides a hydrophobic surface that facilitates the sorption of hydrophobic PAHs molecules (Geng et al., 2024), reducing their mobility and bioavailability. Additionally, higher carbon content is associated with greater microporosity and specific surface area, enhancing PAHs immobilization through π - π interactions. The balance between oxygen-containing groups, which introduce some hydrophilicity, and carbon structures, which promote hydrophobic interactions, is crucial for optimizing PAHs sorption efficiency. Moreover, as previously observed for C_{total} $\Sigma 16$ PAHs, statistical correlation analysis also revealed a strong positive relationship between the percentage changes in soil properties (TOC, DOC, pH, EC) after b-BC/n-BC amendments and the reduction in C_{free} $\Sigma 16$ PAHs content over time (Fig. 3c). This consistency suggests that modifications in soil characteristics induced by BC amendments play

a fundamental role in enhancing both PAHs immobilization and/or degradation, reinforcing the importance of these interactions in both bound and freely available PAHs fractions.

To date, no research has specifically investigated the effect of n-BC on the persistence (C_{total}) and availability (C_{free}) of PAHs in contaminated soils. There are only a few studies examining the persistence of C_{free} $\Sigma 16$ PAHs in soils amended with BC or AC (Hale et al., 2012; Kołtowski et al., 2016; Oleszczuk et al., 2016). For example, Kołtowski et al. (2016) reported that adding BC derived from willow and wheat straw required doses of up to 2.5-significantly reduce by 44-86% in BC-amended soils. Similarly, Godlewska et al. (2022b) reported that the addition of 2% SSL-derived BC resulted in a 36-43% reduction in C_{free} $\Sigma 16$ PAHs. Our previous studies (Oleszczuk et al., 2014) have shown that the smaller particles of BC (<300 μm) were slightly more effective in C_{free} $\Sigma 16$ PAHs immobilization in SSL than bigger ones. However, this research did not investigate the nano-size particles and focused on different matrix, which cannot be comparable with present studies. Zand and Grathwohl (2016) investigated the effect of BC's size (crushed and pulverised) on PAHs mobility. The addition of pulverized BC to soil remarkably reduced mobilization and leaching of the studied PAHs in most cases compared to crushed BC. In the case of AC-amendments, Hale et al. (2012) observed reductions of 67-93% and 17-56% for powdered and granular AC in amended soils, respectively. Cornelissen et al. (2006) demonstrated that a 2% addition of AC to different sediments effectively decreased the C_{free} $\Sigma 16$ PAHs content, with phenanthrene (PHE) reductions ranging from 91-97% and pyrene (PYR) reductions from 98-99%. AC is more effective and faster in contaminant removal, as confirmed by the previous research (Kołtowski et al., 2016; Oleszczuk et al., 2019, 2017) . However, the use of AC is constrained by its tendency to bind essential nutrients and DOC, thereby reducing their availability in soil and potentially impairing plant growth (Oleszczuk et al., 2019). In contrast, BC not only avoids these drawbacks but also gradually supplies nutrients and DOC, offering long-term benefits for soil functioning and sustainability.

3.4. Evaluation of log K_{TOC} coefficients

The log K_{TOC} coefficient was calculated to quantify the binding affinity of each PAHs in both control and b-BC/n-BC-amended soil (Table S8). The sorption strength of specific PAHs varied according to the feedstock materials used to produce b-BC/n-BC as an amendment, as evidenced by distinct log K_{TOC} values. Generally, the addition of b-BC/n-BC resulted in increased log K_{TOC} values across most PAHs compounds (Table S8), indicating enhanced sorption capacity. To provide a comprehensive analysis of changes in log K_{TOC} values across treatment variants, representative PAHs compounds with varying number of aromatic rings were selected for detailed discussion (Fig. 4). Depending on the compound (Fig. 4), the log K_{TOC} coefficient increased after amendment with b-BC/n-BC from 1.6-32.7%. No specific trend was observed in changes in log K_{TOC} after the addition of b-BC/n-BC for the evaluated PAHs. It is noteworthy that the sorption behavior of phenanthrene (PHE), fluoranthene (FLA), and benzo[k]fluoranthene (BkF) showed compound-specific variations when exposed to SSL-derived b-BC. These compounds exhibited slightly lower log K_{TOC} coefficients in BC-amended soil compared to unamended soil. The relatively weak PHE, FLA BkF sorption observed for SSL derived b-BC may be linked to its high ash and mineral content (Table S1), which can block or mask aromatic sorption domains and thus reduce effective sorption affinity. Although, the abundance of mineral phases played a dominant role in limiting sorption efficiency, resulting in lower log K_{TOC} coefficients (A. Tomczyk et al., 2020; B. Tomczyk et al., 2020). Across all selected compounds (Fig. 4), n-BC amendments consistently resulted in higher log K_{TOC} than conventional b-BC, suggesting n-BC's enhanced sorption affinity for the investigated compounds. While SSL-derived b-BC amendments did not significantly increase in log K_{TOC} values, n-BC treatment demonstrated a marked increase in log K_{TOC} values relative to the control soil. Feedstock type played a critical role in b-BC/n-BC performance. PB- and RH-derived b-BC and n-BC achieved the highest log K_{TOC} in most cases. In contrast, SSL-

derived b-BC and n-BC amended soils consistently displayed lower sorption capacities, likely due to differences in porosity, surface chemistry, or carbon content inherent to SSL-derived materials (Table S1). Temporal trends suggested potential aging effects, as inferred from slight declining log K_{TOC} values over sequential sampling periods, possibly linked to competitive interactions between native soil PAHs and those released from b-BC/n-BC over time (Table S8) (Ukalska-Jaruga et al., 2020). The n-BC enhanced performance in select cases emphasizes its utility for targeted remediation, though its application must account for PAH-specific hydrophobicity and molecular structure. However, long-term stability of biochar-PAH interactions remains uncertain, necessitating further research into aging processes and bioavailability dynamics. Optimizing feedstock selection, BC form (bulk vs. nano), and application strategies will be essential to maximize sorption efficiency and ensure sustainable contaminant mitigation in diverse environmental contexts.

3.5. Ecological safety of b-BC/n-BC amended soil

Analysis of the Nemerow pollution index (NIPI) showed distinct patterns across treatments (Table 1). At T_0 (initial stage), the highest NIPI value (0.1084) was observed in unamended soil, indicating elevated pollution levels from PAHs contamination. The addition of b-BC and n-BC consistently reduced NIPI values. Notably, PB-derived n-BC and SSL-derived n-BC demonstrated superior performance, achieving the lowest NIPI values of 0.07877 and 0.07523 respectively. This suggests these materials were most effective in mitigating PAHs-related soil pollution. Comparative analysis showed that b-BC amendments, regardless type of feedstock, consistently produced slightly higher NIPI values than corresponding n-BC (Table 1). Following a six-weeks incubation period (T_1), all treatments exhibited further decline in NIPI values, with the most pronounced reduction occurred for RH-derived n-BC-amended soil (from 0.08118 to 0.04236). Generally, 5 levels can be classified as follows: $NIPI \leq 0.7$, safe; $NIPI$ 0.7–1.0, warning-line; $NIPI$ 1.0–2.0, weakly polluted; $NIPI$ 2.0–3.0, moderately polluted; and

NIPI > 3.0, heavily polluted (Xu et al., 2021). According to this classification, all samples were within safe levels (Table 10). These findings indicate that soils from the tested industrial area, based on the European Union (EU) regulation regarding total PAHs in soils, can be considered almost unpolluted (Ministry of Climate and Environment, 2024). However, this status may change over time due to pollutants released from the abandoned industrial plant. Therefore, appropriate control measures and strict management are required to minimize future contamination. In this context, it is important to note that the addition of n-BC can effectively reduce this risk.

3.6. Economic aspects of n-BC production

The economic feasibility of n-BC production appears promising, particularly when considering its relatively low costs compared to other advanced carbon-based materials such as carbon nanotubes (CNTs) or graphene nanoplatelets (GNPs) (Isaacs et al., 2010; Safian et al., 2021). Although no comprehensive large-scale techno-economic analysis has yet been conducted, recent studies suggest that production costs of n-BC can range from only a few to several tens of dollars per kilogram, depending on the preparation method (Anupama and Khare, 2021; Chaubey et al., 2024). Among these, ball milling emerges as the least expensive, sustainable, and reproducible approach (Kumar et al., 2020). The reported values are generally lower than the market price of AC or graphene oxide (GO), highlighting its potential as a cost-effective alternative (Chausali et al., 2021). Moreover, since n-BC is derived from renewable biomass through environmentally friendly processes, it offers an economically and ecologically attractive option for large-scale applications. In comparison to b-BC, the production of n-BC requires additional processing steps, yet it still remains relatively inexpensive and can complement b-BC by offering enhanced functional properties at a moderate additional cost.

3.7. Limitation and future needs

This study offers new insights into the comparative performance of n-BC and b-BC for PAHs immobilization, some limitations should be noted. Moreover, six-week pot experiment, conducted under controlled pot conditions, may not fully reflect longer-term processes such as biochar aging, weathering, or interactions with soil microbiota (Amusat et al., 2021; Sarma et al., 2024). Only three feedstocks and one pyrolysis temperature were tested, and the study focused solely on PAHs, while contaminated soils often contain complex mixtures of pollutants. Moreover, the results may vary with soil type, pH, texture, and organic matter content, which can strongly influence immobilization efficiency (Emoyan et al., 2018). The ecological risk assessment was also limited to NIPI, which does not capture all ecological endpoints. Therefore, complementary ecotoxicological assays would be valuable to obtain a more comprehensive evaluation of potential environmental risks. In addition, the environmental fate of n-BC remains uncertain, and it is essential to investigate how its properties and interactions evolve over time when applied to soils as an amendment.

Future work should therefore include long-term field trials across different soil types and environmental conditions to confirm the persistence of n-BC effects. Broader feedstocks, pyrolysis conditions, and post-processing methods need to be assessed to optimize n-BC properties. Studies should also address multi-contaminant systems and apply advanced molecular techniques to clarify the mechanisms of PAHs immobilization. Finally, life-cycle and techno-economic evaluations are required to ensure sustainable large-scale n-BC applications.

4. Conclusion

This study demonstrated that both b-BC and n-BC amendments significantly affected the content and bioavailability of PAHs in contaminated soil, as well as their associated ecological risk. The impact varied considerably based on the type of feedstock and particle size. The results showed that n-BC was more effective than b-BC in reducing both total (C_{total} $\Sigma 16$ PAHs) and freely available PAHs (C_{free} $\Sigma 16$ PAHs), contributing to lower contamination levels and ecological risks over time. The ecological risk assessment revealed that n-BC amendments led to greater reductions in the Nemerow Pollution Index (NIPI). The strong correlation between reductions in PAHs content and BC physicochemical properties, such as specific surface area (S_{BET}), total pore volume (V_p), and functional group composition and TOC and DOC content highlights the role of enhanced surface characteristics and other factor in improving PAH immobilization. These findings emphasize that n-BC amendments, particularly those derived from PB and RH, offer a promising strategy for long-term PAHs immobilization and risk mitigation in contaminated soils.

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Table 1. Nemerow multifactor pollution index (NIPI) for soil and soil amendment with b-BC/n-BC.

Sample	NIPI	PAHs with the highest concentration-to-permissible value ratio
T ₀ term		
Soil (S)	0.1084	ACY
S+b-BC-PB	0.1040	ACY
S+n-BC-PB	0.07877	PHE
S+b-BC-RH	0.08369	ACE
S+n-BC-RH	0.08118	ACE
S+b-BC-SSL	0.09469	ACY
S+n-BC-SSL	0.07523	ANT
T ₁ term		
Soil (S)	0.07337	FLA
S+b-BC-PB	0.07967	PHE
S+n-BC-PB	0.05837	PHE
S+b-BC-RH	0.06010	ACE
S+n-BC-RH	0.04236	ACY
S+b-BC-SSL	0.06623	ACE
S+n-BC-SSL	0.06338	PHE

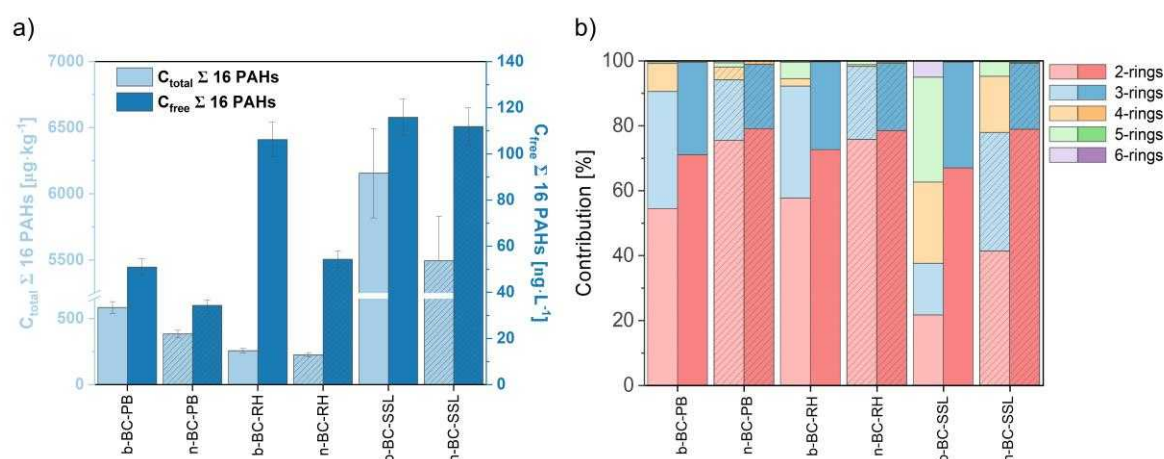


Figure 1. The $C_{total} \Sigma 16 \text{ PAHs}$ (light blue) and $C_{free} \Sigma 16 \text{ PAHs}$ (dark blue) content in b-BC and n-BC (a) and contribution of $C_{total} \Sigma 16 \text{ PAHs}$ (lighter shades) and $C_{free} \Sigma 16 \text{ PAHs}$ (darker shades) based on the number of rings (b). Dashed bars represent n-BC. Error bars represent the standard deviation of the mean ($n = 3$).

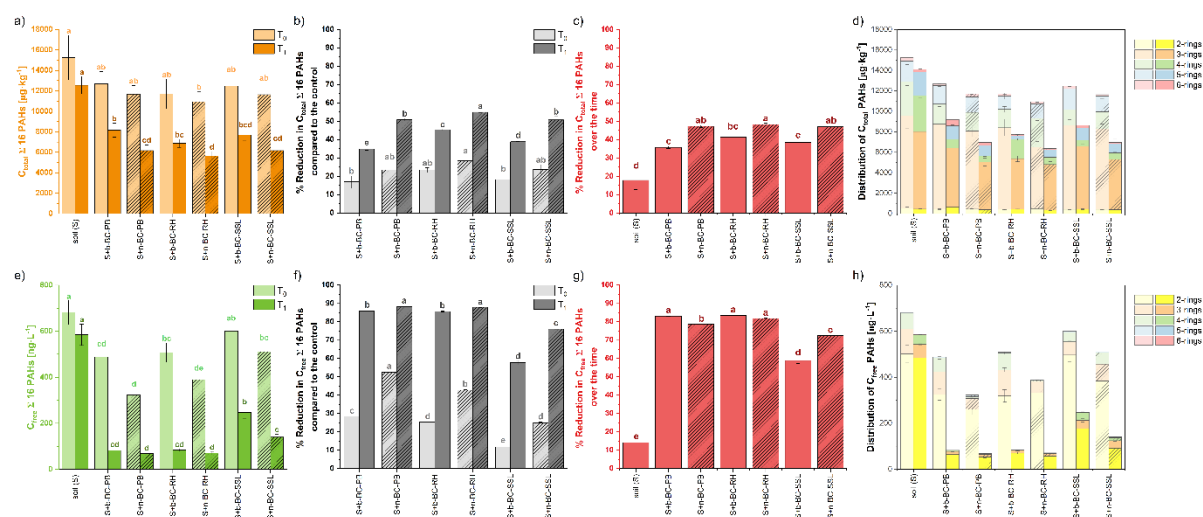


Figure 2. The $C_{total} \Sigma 16 \text{ PAHs}$ content (a) its percentage reduction compared to control (b) its percentage reduction over the time (c) and its distribution based on the number of rings (right plot) (d), as well as the $C_{free} \Sigma 16 \text{ PAHs}$ content (e) and its percentage reduction compared to control (f) and its percentage reduction over the time (g) and its distribution based on the number of rings (h), in soil and/or soil amended with 2% of b-BC/n-BC at the beginning of the experiment (T_0) (light shades) and after 6 weeks (T_1) (darker shades). Dashed bars represent n-BC amendment. Different letters denote statistically significant differences ($p \leq 0.05$) among treatments at T_0 and T_1 .

Error bars represent the standard deviation of the mean ($n = 3$).

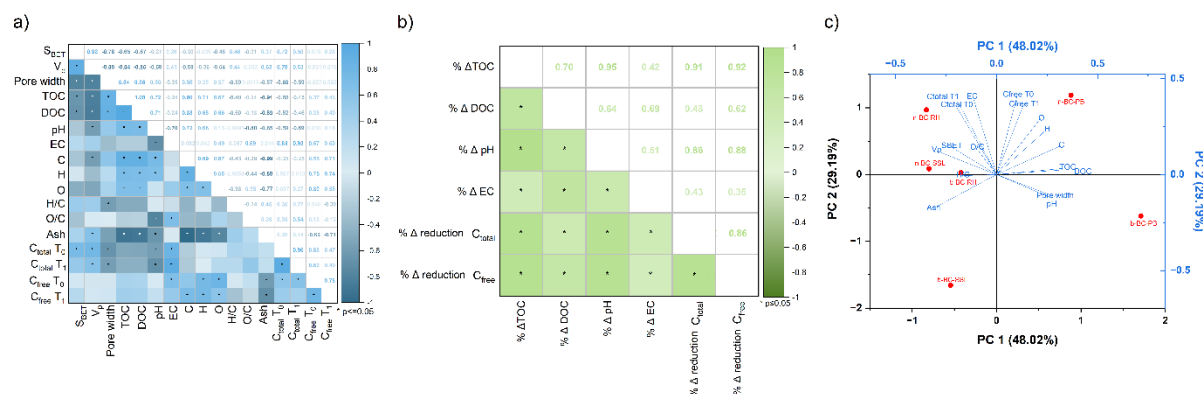


Figure 3. Pearson's correlation plot between (a) physicochemical properties of b-BC and n-BC and C_{total} $\Sigma 16$ PAHs and C_{free} $\Sigma 16$ PAHs reductions calculated in reference to the control soil (b) % changes in selected properties of soil and soil amended with b-BC/n-BC and % changes C_{total} $\Sigma 16$ PAHs and C_{free} $\Sigma 16$ PAHs reduction over the time and (c) principal component analysis (PCA) biplots illustrating the influence of physicochemical properties of b-BC/n-BC on C_{total} $\Sigma 16$ PAHs and C_{free} $\Sigma 16$ PAHs reductions calculated in reference to the control soil where PC1 explains 48.02% of the variance and PC2 explains 28.19% of the variance. * means statistically significant correlation ($p \leq 0.05$).

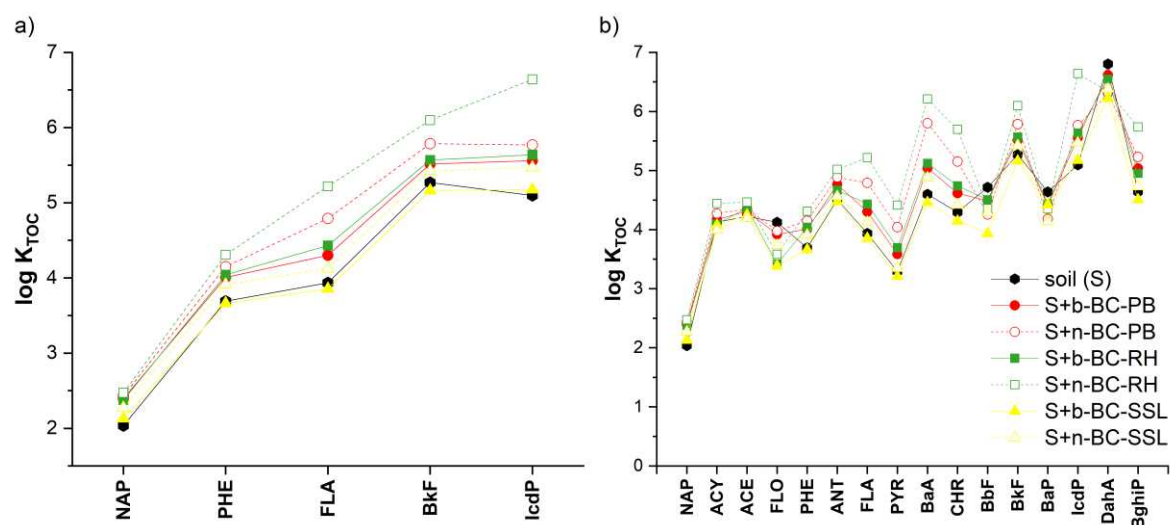


Figure 4. $\log K_{TOC}$ values for in (a) a subset of five representative PAHs based on number of aromatic rings and (b) all 16 priority PAHs in soil and/or soil amended with 2% of b-BC/n-BC. The points represent mean values ($n=3$).

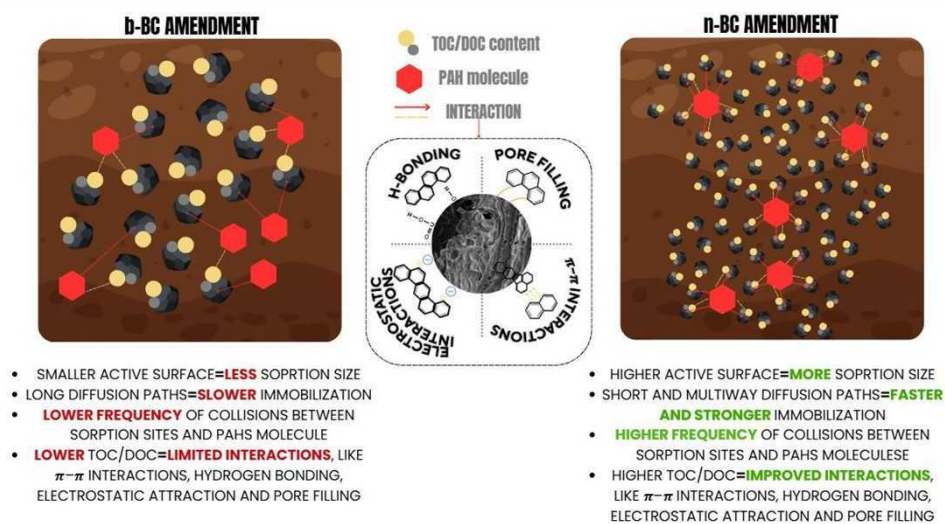
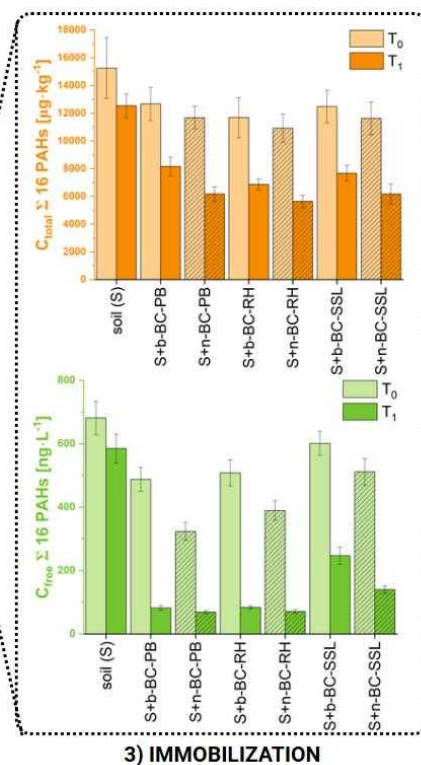
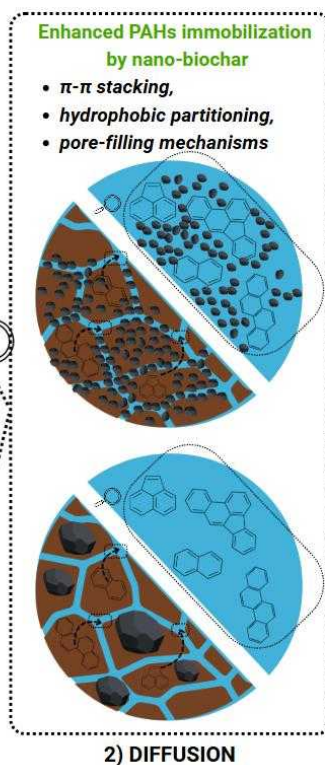
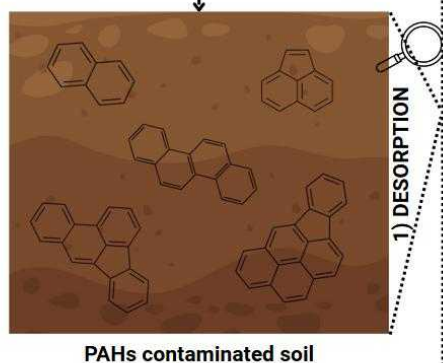
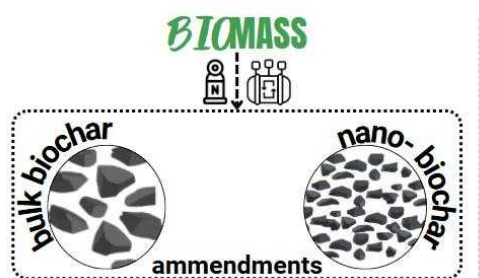
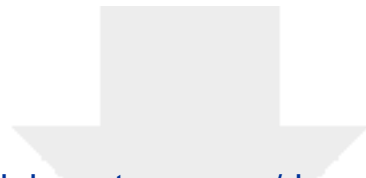


Figure 5. Conceptual mechanisms driving stronger PAHs immobilization by n-BC vs b-BC.



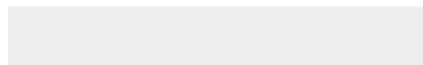
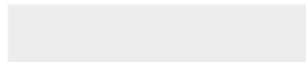
- Nano-biochar (n-BC) outperformed conventional biochar in immobilizing PAHs
- N-BC amendments led to a substantial rapid decrease in PAHs in contaminated soil
- PB- and RH-derived n-BC demonstrated the highest immobilization capacity



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Supplementary Material

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Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Supplementary material for:

Nanoscale biochar outperforms conventional biochar in enhancing soil health and reducing risks from polycyclic aromatic hydrocarbon-contaminated soil

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This file includes:

Supplementary text
Tables S1 to S8
Figure S1 to S8
References

1. Physicochemical properties of biochars

The porous structure of bulk biochar (b-BC) and nano-biochars (n-BC) was analyzed using low-temperature nitrogen adsorption-desorption isotherms at 77.3 K with an ASAP 2420 analyzer (Micromeritics, USA). Key parameters, including specific surface area (S_{BET}), micropore surface area (S_{micro}), total pore volume (V_p), micropore volume (V_{micro}), and average pore diameter, were derived from nitrogen desorption data, as described elsewhere (Michot and Villieras, 2013). The pH and electrical conductivity (EC) of the BC samples were assessed following the procedure (Kończak et al., 2020). Briefly, suspensions were prepared by mixing 1 g of BC with 10 mL of deionized water, achieving a solid-to-liquid ratio of 1:10 (w/v). Measurements of pH and EC were taken using a digital meter (HQ430d Benchtop Single Input, HACH, USA or CP-401, ELMETRON, Poland). The hydrodynamic diameter of n-BCs was determined by dynamic light scatterings (DLS) using a Zetasizer Nano ZS90 analyzer (Malvern Instrument Inc., UK) equipped with a universal dip cell. The intensity-weighted hydrodynamic particle size was determined assuming the Stokes-Einstein equation for spherical particles. Elemental composition, including carbon, hydrogen, and nitrogen, was determined using a Perkin–Elmer 2400 CHN analyzer (Akron, OH, USA). The ash content was measured according to the ASTM D3176 standard by combusting dried samples at 760°C for 6 hours (Abdel-Fattah et al., 2015). The ash content was calculated using the following equation:

$$\% \text{ Ash} = \frac{\text{weight after ashing} - \text{weight of crucible}}{\text{dry weight of BC sample}} \cdot 100\% \quad (1)$$

The total content of O was determined by the following equation:

$$\% \text{ O} = 100\% - \% \text{ Ash} - \% \text{ C} - \% \text{ H} - \% \text{ N} \quad (2)$$

The physicochemical properties of b-BC and n-BC are presented in Table S1.

The functional groups of b-BC and n-BC were determined by FTIR-ATR spectra in the region of 400–4000 cm^{-1} with a resolution of 1 cm^{-1} (Nicolet 8700A, Thermo Scientific, USA) and X-ray photoelectron spectroscopy (XPS) (analytical system UHV Prevac, Poland).

Table S1. Physicochemical properties of bulk (BC) and nano-biochars (n-BC) used in the experiment.

Sample	S _{BET} [m ² /g]	V _p [cm ³ /g]	Pore width [nm]	TOC [mg/g]	DOC [mg/L]	pH	EC [μS/cm]	C [%]	H [%]	O [%]	H/C [molar ratio]	O/C [molar ratio]	Ash [%]	Average particle size [nm]
b-BC-PB	1.14	0.004	13.08	464.9	460.6	10.8	623	67.46	1.54	9.1	0.274	0.101	20.66	
	±	±	±	±	±	±	±	±	±	±	±	±	±	-
	0.0616	0.0002	0.6974	23.71	24.394	0.5655	32.92	3.471	0.0817	0.4888	0.0149	0.0054	1.074	
n-BC-PB	11.54	0.020	6.8	308.8	299.8	9.53	1312	59.66	1.7	15.1	0.342	0.19	22.40	81.7
	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	0.529	0.0008	0.3832	16.68	11.95	0.4746	65.09	2.894	0.0645	0.8284	0.0178	0.0067	1.178	5.21
b-BC-RH	175.8	0.110	2.2	12.50	1.8	9.41	968	43.77	1.18	5.65	0.324	0.097	48.50	
	±	±	±	±	±	±	±	±	±	±	±	±	±	-
	7.877	0.005	0.0967	0.5597	0.0959	0.4553	33.51	2.097	0.05921	0.2518	0.0138	0.0047	2.32	
n-BC-RH	195.6	0.141	3.12	96.00	0.10	9.11	1236	35.81	1.36	6.53	0.455	0.137	55.37	89.6
	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	10.09	0.007	0.1585	4.999	0.0053	0.4751	63.26	1.837	0.07156	0.3497	0.0239	0.00707	2.806	6.22
b-BC-SSL	41.61	0.055	5.27	5.27	6.95	9.55	552	20.53	0.74	3.69	0.433	0.135	72.31	
	±	±	±	±	±	±	±	±	±	±	±	±	±	-
	2.459	0.003	0.303	0.3035	0.4056	0.5318	31.98	1.188	0.0428	0.2145	0.02523	0.0076	4.285	
n-BC-SSL	51.23	0.090	7.03	4.36	15.30	7.75	1358	23.06	0.53	7.83	0.276	0.255	65.54	30.0
	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	2.674	0.005	0.3584	0.2294	0.7997	0.3897	72.5	1.197	0.0278	0.4131	0.0145	0.01332	3.392	3.02

Where: b-BC- bulk biochar, n-BC-nano-biochar, PB-palm bunch, RH-rice husk, SSL-sewage sludge, S_{BET}-specific surface area, V_p-pore volume, TOC- total organic carbon, DOC-dissolved organic carbon, pH- in H₂O, EC- electrical conductivity, C, H, O, Ash [%] total content, O/C, H/C- molar ratios. The values are mean of three repetitions.

2. FT-IR analysis

The FT-IR spectra of the analyzed BCs reveal complexity due to the presence of multiple bands associated with various functional groups on the BC surface, as noted by Ilić et al. (2022). However, there is no significant distinction between the spectra of b-BCs and n-BCs other than differences in peak intensities. More pronounced variations in FT-IR spectra are observed between b-BCs and n-BCs derived from different feedstocks. Across all b-BCs and n-BCs, several characteristic peaks are consistently identified: a broad peak near 3400 cm⁻¹ (corresponding to -OH stretching vibrations from carboxylic groups or adsorbed water in pores), a weak peak at 2920 cm⁻¹ (associated with aliphatic -C-H bands and =C-H stretching in aromatic compounds) and strong peaks in the range of 1430–1621 cm⁻¹ (attributed to -C=C and -C=O stretching vibrations in aromatic rings, indicating the presence of carboxylic and phenol groups) (Ternero-Hidalgo et al., 2016; Gromov et al., 2017; Cordero-Lanzac et al., 2018). Notably, the -C=C and -C=O stretching vibrations in aromatic rings exhibit a slight shift to higher wavenumbers after ball milling, likely due to a reduced conjugation effect. Peaks within the 1200–1000 cm⁻¹ range correspond to -C-H deformation vibrations in

aromatic structures, while those in the 1060–783 cm^{-1} range are attributed to -Si-O vibrations from inorganic materials in BCs. Additionally, the bands between 950–600 cm^{-1} represent -C-H bending in aromatic compounds (Barroso-Bogeat et al., 2015). These findings indicate that n-BCs retain diverse functional groups and aliphatic chains similar to BCs, highlighting their high environmental reactivity.

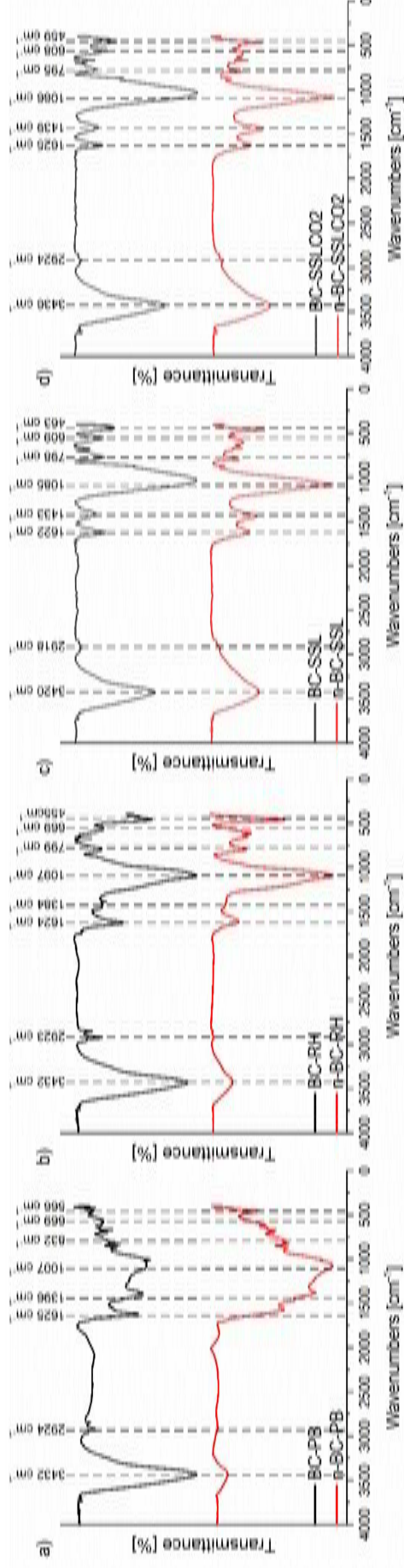


Figure S1. FT-IR spectra of obtained bulk-(BC) and nano-biochars (n-BC).

3. XPS analysis

The X-ray Photoelectron Spectroscopy (XPS) analysis of b-BC and n-BC derived from different feedstock- PB, RH and SSL-revealed notable differences in surface chemistry, particularly in the distribution of carbon- and oxygen-containing functional groups. In all cases, b-BC and n-BC exhibited a dominant C-C/C-H peak (~285 eV), characteristic of graphitic structures, with n-BC showing an increased presence of oxygenated functional groups (C-OH, C=O, COOR), indicating higher surface oxidation. Among the feedstocks, PB-derived b-BC had the highest degree of carbonization, as evidenced by the most intense C-C/C-H signal and minimal oxygen-containing groups, whereas RH-derived b-BC exhibited a more pronounced presence of oxygenated species, suggesting higher surface reactivity. SSL-derived BC displayed the highest intensities of C-OH, C=O, and COOR peaks, along with a distinct carbonate signal (~290–292 eV), likely due to inorganic residues from sewage sludge. These trends were further supported by the XPS O1s spectra, which showed significant variations in oxygen-containing surface functionalities. The transition from b-BC to n-BC led to a substantial increase in surface oxygenation across all feedstocks, with SSL-derived b-BC and n-BC exhibiting the highest intensity of oxygen-related peaks, particularly in the Me-O/carbonates/O=C (~530–531 eV) and Me-OH/O=C/O=C-O-R (~532–533 eV) regions, suggesting an abundance of inorganic residues. RH-derived b-BC showed a prominent C-OH/Si-O peak (~533–534 eV), reflecting its naturally high

silica content, while PB-derived b-BC had a more balanced distribution of oxygen functionalities, indicating moderate oxidation. The transition to n-BC amplified oxidation across all feedstocks, with SSL-derived n-BC displaying the most pronounced increase in oxygenated groups, including hydroxyls, esters, and carbonates. The enhanced presence of these functionalities suggests that n-BC, particularly from SSL, possesses improved surface reactivity and a higher potential for adsorption interactions. These findings indicate that SSL-derived n-BC may exhibit superior sorption properties, making it a promising material for environmental applications, especially in pollutant removal.

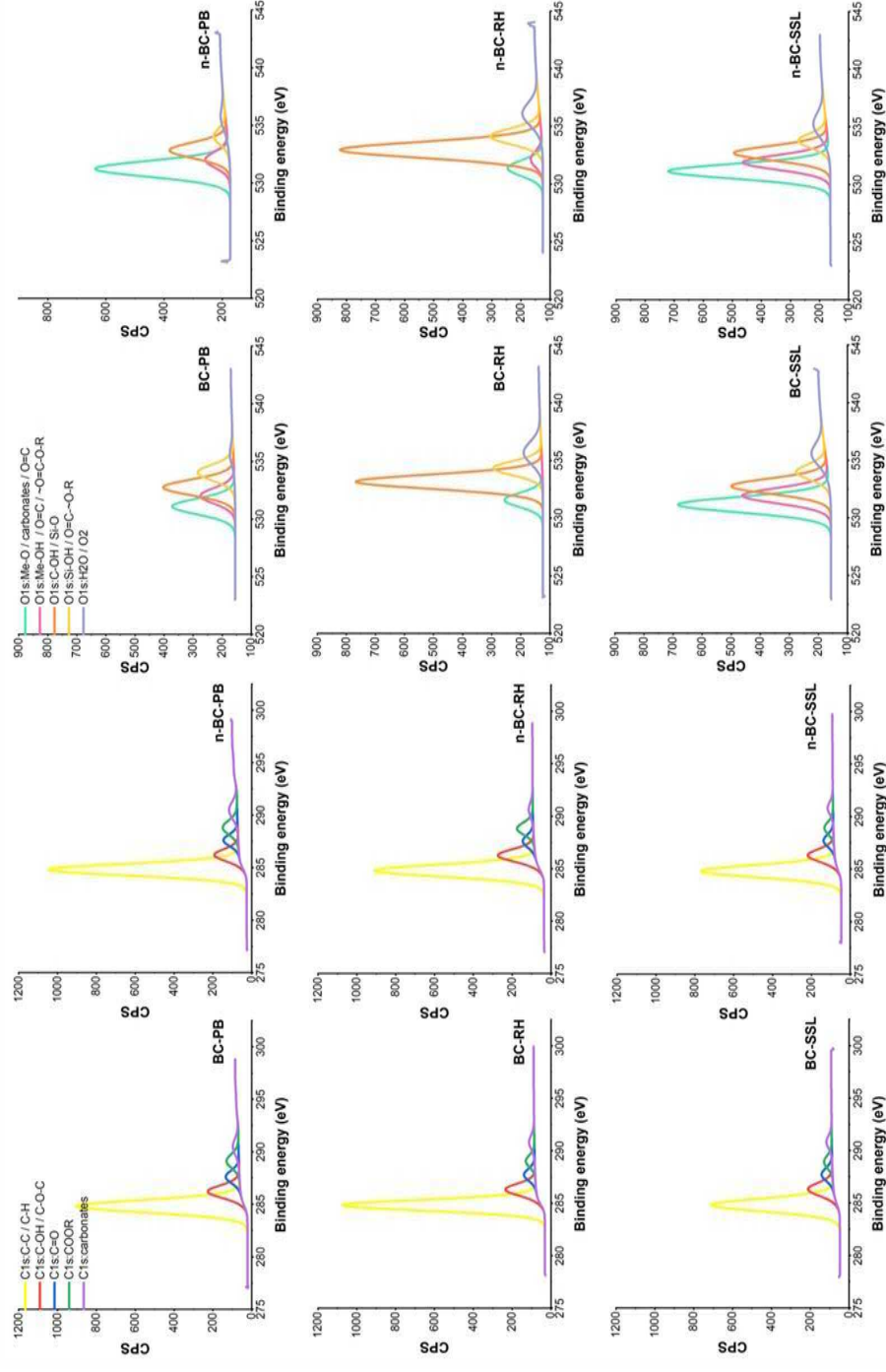


Figure S2. XPS spectra of obtained bulk- (b-BC) and nano-biochars (n-BC).

4. C_{total} and C_{free} $\Sigma 16$ PAHs content in b-BC and n-BC

The type of feedstock influences the content of C_{total} $\Sigma 16$ PAHs in b-BC and n-BC. The content of individual C_{total} PAHs is presented in Table S2. The C_{total} $\Sigma 16$ PAHs ranged from 257 ± 15.9 to $6,156 \pm 339 \mu\text{g}\cdot\text{kg}^{-1}$ for b-BC and from 226 ± 16.3 to $5,494 \pm 338 \mu\text{g}\cdot\text{kg}^{-1}$ for n-BC showing constantly lower content of PAHs in n-BC than b-BC. The reduction of particle size to the nanoscale decreased C_{total} $\Sigma 16$ PAHs content by 10.8% to 34.0% compared to b-BC. The SSL-derived b-BC and n-BC contained the highest C_{total} $\Sigma 16$ PAHs. These concentrations were several times higher than those observed in BC produced from other feedstocks. In contrast, BCs (b-BC and n-BC) produced from RH exhibited the lowest C_{total} $\Sigma 16$ PAHs concentrations. Analysis of the PAHs composition showed that 2- and 3-ring PAHs, particularly naphthalene (NAP) and phenanthrene (PHE), were the predominant compounds in both b-BC and n-BC samples, regardless of the feedstock used. Previous research showed (Buss et al., 2022; Lehmann and Stephen, 2024) that, regardless of BC, the dominance of both 2- and 3-ring PAHs is typical for these materials. All n-BC were characterized by the higher contribution of 2-rings PAHs and lower 3-rings PAHs than b-BC. Some discrepancies were observed for SSL-derived n-BC which showed higher 3-rings PAHs contribution than b-BC.

The C_{free} $\Sigma 16$ PAHs content ranged from 50.9 ± 3.6 to $116 \pm 7.9 \text{ ng}\cdot\text{L}^{-1}$ for b-BC and from 34.3 ± 2.3 to $112 \pm 8.2 \text{ ng}\cdot\text{L}^{-1}$ for n-BC, consistently showing lower PAHs concentrations in n-BC compared to their corresponding b-BC as it was observed for C_{total} $\Sigma 16$ PAHs. The content of individual C_{free} PAHs is presented in Table S2. Specifically, n-BC exhibited from 32.5% to 48.9% less C_{free} $\Sigma 16$ PAHs than b-BC. The only exception was SSL-derived n-BC, where there was no statistically significant ($p > 0.05$) difference in C_{free} $\Sigma 16$ PAHs content between n-BC and b-BC. Moreover, SSL-derived b-BC and n-BC were characterized by the highest absolute concentrations of C_{free} $\Sigma 16$ PAHs among tested BC. The C_{free} $\Sigma 16$ PAHs were predominantly composed of 2- and 3-rings PAHs, with naphthalene (NAP) and phenanthrene (PHE) being the most abundant compounds regardless of the type of feedstock and BC's particle size. In b-BC and n-BC samples, 2-rings PAHs accounted from 67.0% to 72.6% (b-BC) and from 78.5% to 79.1% (n-BC), respectively, while the 3-ring PAHs from 27.1% to 32.7% (b-BC) and 19.9% to 20.8% (n-BC), respectively. The observed reduction in C_{free} $\Sigma 16$ PAHs with decreasing particle size implies that the structural modifications occurring during the transformation of b-BC into n-BC, such as increased surface area, porosity (Table S1), and altered surface chemistry, directly impact the desorption and mobility of PAHs. However, no statistically significant change (in SSL-derived n-BC) in C_{free} $\Sigma 16$ PAHs after size reduction compared to C_{total} $\Sigma 16$ PAHs (where decrease was observed after size reduction) may suggest the specific feedstock characteristics influence on the binding strength and accessibility of PAHs in BC (Keilluweit et al., 2012). Moreover, the strong negative correlation between both PAHs (C_{total} and C_{free}) content and TOC and DOC indicates that increased

mobile organic carbon concentrations enhance PAHs desorption resulting in decreased C_{total} and C_{free} PAHs concentrations in both b-BC and n-BC matrices (Kristoffer Næs,* et al., 1998) (Fig. S3).

The log K_d values calculated for the b-BC and n-BC based on C_{total} $\Sigma 16$ PAHs and C_{free} $\Sigma 16$ PAHs were within a similar range determined by other researchers (Hale et al., 2016; Kupryianchuk et al., 2016). This parameter may help predict how effectively the b-BC or n-BC will bind with PAHs in contaminated soil (Amstaetter et al., 2012; Mayer et al., 2016a). The log K_d values determined for specific PAHs for particular b-BC/n-BC are presented in Table S3. The log K_d values for b-BC and n-BC varied depending on the feedstock used. Figure S4 presents scatter plots with the log K_d values for b-BC versus n-BC. Data points above the 1:1 line with a 5% confidence range (shaded gray area) indicate a higher affinity of individual PAHs for n-BC, while points below this line show that b-BC has a higher affinity for the investigated compounds. These values were closely aligned for PB-derived b-BC/n-BC, indicating that changes in PB-derived BC size did not significantly affect most PAH's affinity to these materials (Fig. S4a). In contrast, the RH-derived b-BC/n-BC exhibited high variability, with several PAHs (Fig. S4b) showing significantly lower values for n-BC than b-BC. These differences suggest that size reduction did not enhance directly BC's affinity in certain cases (Fig. S4b). The SSL-derived b-BC/n-BC, despite having limited data points, generally followed the same trend as PB-derived BC, with most PAHs showing similar behaviour between b-BC and n-BC (Fig. S4c). These observations confirm that the nature of the feedstock determines the affinity of PAHs to BC changes with particle size. While the affinity of most PAHs for BCs remains relatively consistent regardless of type, specific PAHs may exhibit enhanced retention in particular BCs, potentially affecting their environmental persistence and mobility upon soil application.

Table S2. The content of individual C_{total} PAHs [$\mu\text{g}\cdot\text{kg}^{-1}$] and C_{free} PAHs [$\text{ng}\cdot\text{L}^{-1}$] in b-BCs and n-BCs.

Sample	NAP	ACY	ACE	FLO	PHE	ANT	FLA	PYR	BaA	CHR	BbF	BkF	BaP	IcdP	DahA	BghiP	$\Sigma 16$ PAHs
C_{total} PAHs [$\mu\text{g}\cdot\text{kg}^{-1}$]																	
b-BC-PB	317.0	11.38	12.92	22.88	149.6	13.78	17.72	16.88	8.463	7.185	0.4487 $\pm$ 0.04134	0.1577 $\pm$ 0.01128	1.28 $\pm$ 0.09338	0.8961 $\pm$ 0.0846	0.9682 $\pm$ 0.09727	0.9881 $\pm$ 0.08106	582.5 $\pm$ 45.64
	27.70	1.008	0.8783	1.543	9.378	1.012	1.341	1.083	0.7216	0.648							
n-BC-PB	290.2	6.345	9.435	13.67	36.73	5.314	7.723	5.726	0.8081	0.9369	1.256 $\pm$ ± 0.1248	0.267 $\pm$ 0.02397	2.295 $\pm$ 0.1519	1.637 $\pm$ 0.1137	1.287 $\pm$ 0.08226	0.6464 $\pm$ 0.04921	384.3 $\pm$ 26.94
	22.07	0.464	0.7569	1.051	2.63	0.3624	0.5487	0.4231	0.0617	0.07288							
b-BC-RH	148.4	3.419	4.978	14.02	65.28	1.146	4.077	1.645	0.04624	0.1491	1.625 $\pm$ ± 0.1176	1.461 $\pm$ ± 0.1251	9.376 $\pm$ 0.6657	0.1009 $\pm$ 0.007028	0.4365 $\pm$ 0.04644	1.094 $\pm$ ± 0.06544	257.2 $\pm$ 15.93
	8.855	0.1974	0.4203	1.044	4.612	0.09092	0.2911	0.1378	0.004589	0.01447							
n-BC-RH	171.3	2.225	1.74	5.995	12.59	28.34	0.5978	0.5614	0.03083	0.02225	0.6944 $\pm$ ± 0.05018	0.4594 $\pm$ 0.04412	1.022 $\pm$ 0.0816	0.0798 $\pm$ 0.004466	0.1786 $\pm$ 0.01444	0.2022 $\pm$ 0.01166	226.1 $\pm$ 16.32
	12.49	0.1703	0.1403	0.3522	0.7869	2.385	0.03621	0.03416	0.002411	0.001986							
b-BC-SSL	1332	359.9	47.97	184.8	150.9	237.4	109.1	330.6	520.4	580.2	498.5 $\pm$ ± 46.48	561.3 $\pm$ 49.96	449.3 $\pm$ 26.44	149.9 $\pm$ 10.78	483.5 $\pm$ 46.12	160.3 $\pm$ 12.64	6156 $\pm$ 338.5
	85.22	23.66	3.644	14.23	11.42	18.37	7.223	30.62	37.26	53.5							

n-BC-SSL		2274 ± 160.2	15.31 ± 1.209	76.00 ± 4.403	1585 ± 114.4	314.5 ± 22.17	15.15 ± 0.9119	235.7 ± 16.63	165.6 ± 13.08	111.1 ± 8.044	441 ± 28.74	12.11 ± 1.22	10.44 ± 0.8302	210.4 ± 19.88	8.281 ± 0.8232	11.80 ± 1.168	7.505 ± 0.705	5494 ± 337.5
C _{free} PAHs [ng·L ⁻¹]																		
	NAP	ACY	ACE	FLO	PHE	ANT	FLA	PYR	BaA	CHR	BbF	BkF	BaP	IcdP	DabA	BghiP	Σ16 PAHs	
b-BC-PB	36.15 ±	0.1274 ±	0.5663 ±	6.266 ±	7.438 ±	0.1053 ±	0.05991 ±	0.1093 ±	<LOD	<LOD	<LOD	<LOD ⁻⁶	0.05823 ±	0.0001169 ±	0.0001174 ±	0.0001209 ±	50.88 ±	
	2.693	0.008233	0.04214	0.3185	0.5285	0.006228	0.004385	0.009351					0.005375	1.005·10 ⁻⁵	7.466·10 ⁻⁶	1.277·10 ⁻⁵	3.594	
n-BC-PB	27.15 ±	0.07745 ±	0.3082 ±	2.324 ±	3.825 ±	0.2861 ±	0.1151 ±	0.2529 ±	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	34.34 ±	
	1.822	0.006796	0.019	0.1337	0.2618	0.018	0.008845	0.0212									2.289	
b-BC-RH	77.12 ±	0.2315 ±	1.795 ±	2.416 ±	24.25 ±	0.05214 ±	0.1164 ±	0.1810 ±	0.0002494 ±	0.0001261 ±	0.01965 ±	0.007103 ±	0.0119 ±	0.0002452 ±	0.0002459 ±	0.0001225 ±	106.2 ±	
	4.559	0.01855	0.1341	0.1925	1.677	0.004179	0.008594	0.01565	1.775·10 ⁻⁵	1.189·10 ⁻⁵	0.001754	0.0007484	0.0008526	2.058·10 ⁻⁵	1.665·10 ⁻⁵	8.31·10 ⁻⁶	7.463	
n-BC-RH	42.63 ±	0.1041 ±	0.7838 ±	3.417 ±	6.401 ±	0.5905 ±	0.08085 ±	0.114 ±	0.0005723 ±	0.0001855 ±	0.1615 ±	0.0001918 ±	0.02909 ±	0.0003804 ±	0.0001982 ±	<LOD	54.31 ±	
	2.399	0.006774	0.04656	0.2692	0.5104	0.03485	0.004679	0.009234	4.513·10 ⁻⁵	1.361·10 ⁻⁵	0.01293	1.261·10 ⁻⁵	0.002175	3.391·10 ⁻⁵	1.124·10 ⁻⁵		3.555	
b-BC-SSL	77.58 ±	0.1438 ±	1.426 ±	14.3 ±	21.72 ±	0.237 ±	0.1292 ±	0.2709 ±	<LOD	<LOD	<LOD	<LOD	0.0255 ±	<LOD	<LOD	<LOD	115.8 ±	
	6.170	0.01109	0.08022	0.9581	1.688	0.01391	0.01019	0.02631					0.001714				7.893	
n-BC-SSL	88.23 ±	0.193 ±	1.287 ±	1.943 ±	19.33 ±	0.01526 ±	0.08158 ±	0.1392 ±	<LOD	<LOD	<LOD	<LOD	0.5606 ± 0.0398	<LOD	<LOD	<LOD	111.8 ±	
	6.697	0.01468	0.06582	0.1476	1.135	0.00123	0.005952	0.01259									8.155	

Values are mean of three repetitions. <LOD – below limit of detection. NAP - naphthalene, ACY - acenaphthylene, ACE - acenaphthene, BbF - benzo(b)fluoranthene, BkF - benzo(k)fluoranthene, BaP - benzo(a)pyrene, IcdP - indeno(1.2.3-cd)pyrene, FLA - fluoranthene, PYR - pyrene, BaA - benz(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(g,h,i)perylene.

Table S3. Coefficients of *log* K_d for individual PAHs in the b-BC and n-BC used in the experiment.

Sample	NAP	ACY	ACE	FLO	PHE	ANT	FLA	PYR	BaA	CHR	BbF	BkF	BaP	IcdP	DahA	BghiP
b-BC-PB	3.94	4.95	4.36	3.56	4.30	5.12	5.47	5.19	8.84	8.80	7.57	7.13	4.34	6.88	6.92	6.91
n-BC-PB	4.03	4.91	4.49	3.77	3.98	4.27	4.83	4.35	7.84	7.91	8.03	7.34	8.30	8.15	8.04	7.73
b-BC-RH	3.28	4.17	3.44	3.76	3.43	4.34	4.54	3.96	5.27	6.07	4.92	5.31	5.90	5.61	6.25	6.95
n-BC-RH	3.60	4.33	3.35	3.24	3.29	4.68	3.87	3.69	4.73	5.08	3.63	6.38	4.55	5.32	5.95	6.32
b-BC-SSL	4.23	6.40	4.53	4.11	3.84	6.00	5.93	6.09	-	-	-	-	7.25	-	-	-
n-BC-SSL	4.41	4.90	4.77	5.91	4.21	6.00	6.46	6.08	-	-	-	-	5.57	-	-	-

NAP - naphthalene, ACY - acenaphthylene, ACE - acenaphthene, FLO - fluorene, PHE - phenanthrene, ANT - anthracene, FLA - fluoranthene, PYR - pyrene, BaA - benz(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(g,h,i)perylene.

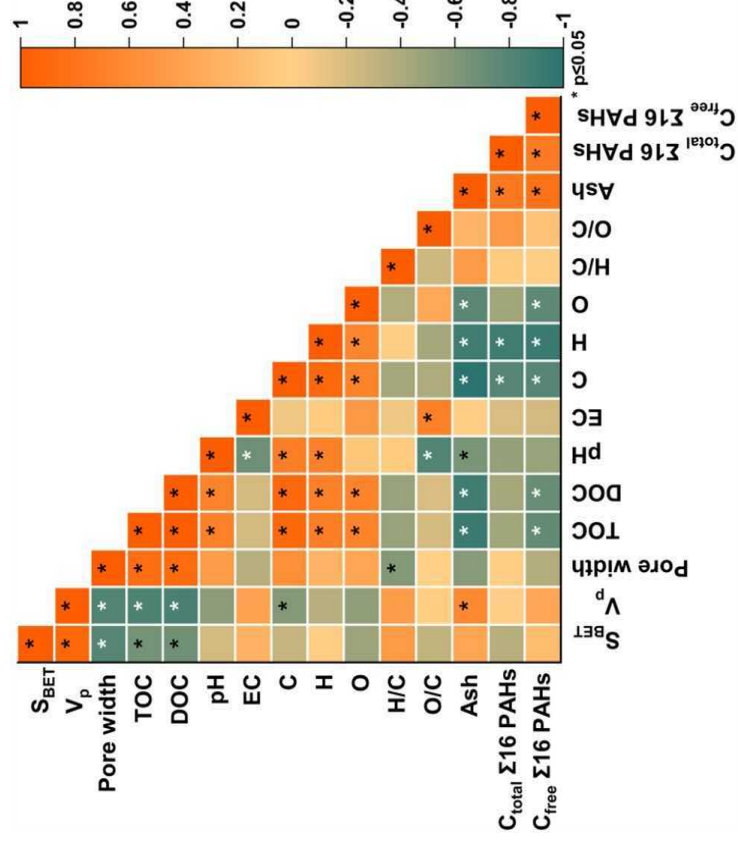


Figure S3. Pearson's correlation plot between physicochemical properties of b-BC and n-BC and $C_{free} \Sigma 16 \text{ PAHs}$ and $C_{total} \Sigma 16 \text{ PAHs}$ content, where * means statistically significant correlation ($p \leq 0.05$).

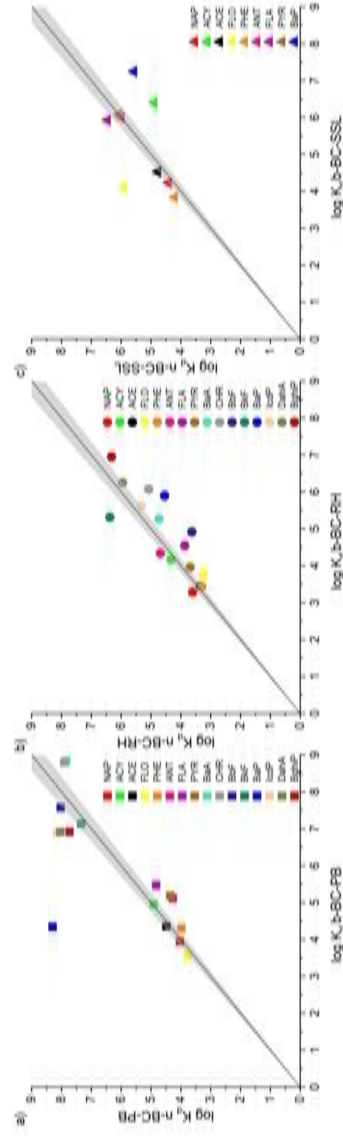


Figure S4. Log $K_d \text{ b-BC}$ vs log $K_d \text{ n-BC}$ plots for individual PAHs for different feedstock (a) PB-, (b) RH- and (c) SSL-derived b-BC/n-BC.

Table S4. Physicochemical properties of soil amendment with 2% of bulk biochar (b-BC) or nano-biochar (n-BC) at T₀ and T₁ term.

Sample	Term	TOC [mg/g]	DOC [mg/L]	pH	EC [μS/cm]
Soil (S)	T ₀	8.602 ± 0.2043	13.48 ± 0.1480	6.75 ± 0.2524	151.9 ± 7.931
	T ₁	7.952 ± 0.3360	12.52 ± 0.2228	6.76 ± 0.2501	150.9 ± 6.426
S+b-BC-PB	T ₀	12.74 ± 0.1209	22.01 ± 0.1220	8.65 ± 0.2071	330.7 ± 13.34
	T ₁	13.60 ± 0.3130	25.42 ± 0.1254	9.24 ± 0.1411	352.6 ± 18.52
S+n-BC-PB	T ₀	13.35 ± 0.1130	19.33 ± 0.1933	9.33 ± 0.2032	423.3 ± 24.01
	T ₁	14.60 ± 0.2230	22.62 ± 0.3226	9.87 ± 0.1904	454.2 ± 23.22
S+b-BC-RH	T ₀	11.78 ± 0.2090	14.55 ± 0.3455	7.58 ± 0.1509	146.5 ± 4.431
	T ₁	13.20 ± 0.1816	16.58 ± 0.2165	8.12 ± 0.2669	145.7 ± 7.706
S+n-BC-RH	T ₀	10.36 ± 0.2346	14.00 ± 0.2440	7.49 ± 0.1152	149.7 ± 5.214
	T ₁	11.28 ± 0.2465	13.53 ± 0.1653	8.03 ± 0.2895	151.7 ± 5.613
S+b-BC-SSL	T ₀	10.65 ± 0.1166	9.906 ± 0.1991	7.49 ± 0.1023	152.0 ± 7.206
	T ₁	11.59 ± 0.1704	11.34 ± 0.1340	8.04 ± 0.1028	163.0 ± 7.466
S+n-BC-SSL	T ₀	12.02 ± 0.1015	16.44 ± 0.1644	7.72 ± 0.1221	196.4 ± 11.91
	T ₁	13.24 ± 0.2993	18.37 ± 0.1837	8.34 ± 0.1383	210.9 ± 10.98

Where: b-BC- bulk biochar, n-BC-nano-biochar, PB-palm bunch, RH-rice husk, SSL-sewage sludge, TOC- total organic carbon, DOC-dissolved organic carbon, pH- in H₂O, EC- electrical conductivity. The values are mean of three repetitions.

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

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

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

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

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

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

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 total PAH content in the biochar (μg kg⁻¹) and C_{free} PAH concentration in the aqueous phase (ng L⁻¹), were used to calculate the solid-water distribution coefficient for each PAH (K_d) (Mayer et al., 2016b) was calculated by the following equation (3):

$$\text{Log } K_d \text{ (L kg}^{-1}\text{)} = \frac{C_{total} \text{ (μg/kg)} * 1000}{C_{free} \text{ (μg/L)}} \quad (3)$$

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

6. Limit of detection and RFI of the PAHs

To ensure the accuracy and precision of the PAHs analysis in this investigation, a five-point calibration standard (15 to 500 ng/mL) was prepared in solution, encompassing detection limits, procedural blanks, check standards, sample duplicates and matrix spike standards. The limit of detection (LOD) for each PAH is presented in Table S3. Extraction blanks (below 3 times LOD) were systematically analyzed within each sample batch and deducted from all sample measurements. The precision of both the extraction procedure and GC-MS analysis was evaluated by measuring three replicates of each samples, yielding a precision level below 20%. To evaluate the recovery rate after evaporation, each extract was spiked with 20 μL of a 1,3,5-tri-tert-butylbenzene (TTB) solution in isooctane at a known concentration, serving as a recovery standard (RS). The absolute recoveries achieved through accelerated solvent extraction ranged from 75% to 89%, while those obtained from the POM spiking experiment ranged from 82% to 99% for all 16 PAHs.

Table S5. The limit of detection (LOD) of PAHs for GC-MS analysis.

Polycyclic aromatic hydrocarbons (PAHs)	LOD [ng·L⁻¹]	RFI
Naphthalene [NAP]	0.00357	0.8786
Acenaphthylene [ACY]	0.00242	0.9004
Acenaphthene [ACE]	0.00256	0.8606
Fluorene [FLO]	0.00217	0.8967
Phenanthrene [PHE]	0.00240	0.7478
Anthracene [ANT]	0.00174	0.6604
Fluoranthene [FLA]	0.00124	0.6743
Pyrene [PYR]	0.00073	0.6422
Benzo[a]anthracene [BaA]	0.00042	0.7459
Chrysene [CHR]	0.00012	0.8239
Benzo[b]fluoranthene [BbF]	0.00010	0.7499
Benzo[k]fluoranthene [BkF]	0.00058	0.7333
Benzo[a]pyrene [BaP]	0.00192	0.7243
Indeno[1.2.3-c,d]pyrene [IcdP]	0.000631	0.6823
Dibenz[a,h]anthracene [DahA]	0.000815	0.7079
Benzo[g,h,i]perylene [BghiP]	0.000464	0.8422

Table S6. The recovery rates of all 16 PAHs for GC-MS analysis.

C _{total} PAHs recovery [%]																
Sample	NAP	ACY	ACE	FLO	PHE	ANT	FLA	PYR	BaA	CHR	BbF	BkF	BaP	IcdP	DahA	BghiP
T ₀ term																
Soil (S)	84 ± 5	85 ± 5	83 ± 5	86 ± 5	82 ± 5	89 ± 5	83 ± 5	84 ± 5	87 ± 5	85 ± 5	87 ± 5	85 ± 5	82 ± 5	80 ± 5	81 ± 5	84 ± 5
S+b-BC-PB	82 ± 5	89 ± 5	85 ± 5	89 ± 5	84 ± 5	84 ± 5	89 ± 5	86 ± 5	85 ± 5	89 ± 5	86 ± 5	87 ± 5	76 ± 5	84 ± 5	86 ± 5	82 ± 5
S+n-BC-PB	88 ± 5	84 ± 5	84 ± 5	88 ± 5	87 ± 5	75 ± 5	86 ± 5	85 ± 5	83 ± 5	83 ± 5	89 ± 5	81 ± 5	80 ± 5	86 ± 5	88 ± 5	88 ± 5
S+b-BC-RH	79 ± 5	87 ± 5	76 ± 5	85 ± 5	83 ± 5	88 ± 5	85 ± 5	83 ± 5	88 ± 5	86 ± 5	81 ± 5	83 ± 5	79 ± 5	81 ± 5	82 ± 5	89 ± 5
S+n-BC-RH	87 ± 5	83 ± 5	87 ± 5	77 ± 5	85 ± 5	86 ± 5	84 ± 5	88 ± 5	86 ± 5	87 ± 5	85 ± 5	82 ± 5	81 ± 5	82 ± 5	85 ± 5	87 ± 5
S+b-BC-SSL	86 ± 5	86 ± 5	88 ± 5	83 ± 5	86 ± 5	83 ± 5	87 ± 5	89 ± 5	89 ± 5	84 ± 5	88 ± 5	80 ± 5	83 ± 5	85 ± 5	87 ± 5	86 ± 5
S+n-BC-SSL	83 ± 5	88 ± 5	89 ± 5	84 ± 5	88 ± 5	87 ± 5	88 ± 5	87 ± 5	84 ± 5	82 ± 5	84 ± 5	86 ± 5	87 ± 5	83 ± 5	80 ± 5	83 ± 5
T ₁ term																
Soil (S)	87 ± 5	76 ± 5	84 ± 5	78 ± 5	78 ± 5	78 ± 5	76 ± 5	80 ± 5	79 ± 5	80 ± 5	77 ± 5	81 ± 5	80 ± 5	87 ± 5	77 ± 5	87 ± 5
S+b-BC-PB	78 ± 5	88 ± 5	77 ± 5	75 ± 5	84 ± 5	81 ± 5	78 ± 5	81 ± 5	77 ± 5	77 ± 5	85 ± 5	80 ± 5	82 ± 5	78 ± 5	76 ± 5	78 ± 5
S+n-BC-PB	79 ± 5	79 ± 5	76 ± 5	77 ± 5	82 ± 5	79 ± 5	85 ± 5	77 ± 5	78 ± 5	79 ± 5	80 ± 5	75 ± 5	78 ± 5	80 ± 5	81 ± 5	79 ± 5
S+b-BC-RH	80 ± 5	80 ± 5	78 ± 5	87 ± 5	76 ± 5	80 ± 5	77 ± 5	75 ± 5	82 ± 5	84 ± 5	75 ± 5	76 ± 5	76 ± 5	77 ± 5	78 ± 5	80 ± 5
S+n-BC-RH	86 ± 5	85 ± 5	75 ± 5	81 ± 5	77 ± 5	75 ± 5	79 ± 5	78 ± 5	80 ± 5	86 ± 5	79 ± 5	87 ± 5	79 ± 5	86 ± 5	79 ± 5	86 ± 5
S+b-BC-SSL	75 ± 5	75 ± 5	80 ± 5	80 ± 5	80 ± 5	86 ± 5	81 ± 5	76 ± 5	75 ± 5	78 ± 5	88 ± 5	77 ± 5	77 ± 5	76 ± 5	80 ± 5	75 ± 5
S+n-BC-SSL	77 ± 5	77 ± 5	83 ± 5	79 ± 5	79 ± 5	76 ± 5	75 ± 5	84 ± 5	83 ± 5	75 ± 5	78 ± 5	78 ± 5	83 ± 5	75 ± 5	82 ± 5	77 ± 5
C _{free} PAHs recovery [%]																
Sample	NAP	ACY	ACE	FLO	PHE	ANT	FLA	PYR	BaA	CHR	BbF	BkF	BaP	IcdP	DahA	BghiP
T ₀ term																
Soil (S)	82 ± 5	90 ± 5	91 ± 5	92 ± 5	94 ± 5	94 ± 5	92 ± 5	97 ± 5	91 ± 5	91 ± 5	92 ± 5	94 ± 5	92 ± 5	91 ± 5	89 ± 5	90 ± 5
S+b-BC-PB	85 ± 5	85 ± 5	92 ± 5	92 ± 5	94 ± 5	90 ± 5	93 ± 5	99 ± 5	95 ± 5	95 ± 5	91 ± 5	93 ± 5	90 ± 5	89 ± 5	87 ± 5	86 ± 5
S+n-BC-PB	82 ± 5	88 ± 5	93 ± 5	89 ± 5	97 ± 5	93 ± 5	93 ± 5	97 ± 5	91 ± 5	96 ± 5	93 ± 5	90 ± 5	87 ± 5	92 ± 5	87 ± 5	91 ± 5
S+b-BC-RH	86 ± 5	90 ± 5	92 ± 5	90 ± 5	93 ± 5	91 ± 5	95 ± 5	93 ± 5	93 ± 5	92 ± 5	94 ± 5	92 ± 5	93 ± 5	91 ± 5	86 ± 5	91 ± 5
S+n-BC-RH	84 ± 5	87 ± 5	93 ± 5	91 ± 5	91 ± 5	91 ± 5	97 ± 5	94 ± 5	93 ± 5	91 ± 5	93 ± 5	91 ± 5	89 ± 5	87 ± 5	87 ± 5	89 ± 5
S+b-BC-SSL	88 ± 5	88 ± 5	88 ± 5	94 ± 5	95 ± 5	92 ± 5	92 ± 5	95 ± 5	95 ± 5	96 ± 5	92 ± 5	93 ± 5	92 ± 5	88 ± 5	90 ± 5	87 ± 5
S+n-BC-SSL	83 ± 5	89 ± 5	91 ± 5	89 ± 5	96 ± 5	89 ± 5	97 ± 5	93 ± 5	95 ± 5	96 ± 5	94 ± 5	92 ± 5	90 ± 5	90 ± 5	85 ± 5	90 ± 5
T ₁ term																
Soil (S)	89 ± 5	91 ± 5	89 ± 5	97 ± 5	89 ± 5	93 ± 5	94 ± 5	92 ± 5	92 ± 5	91 ± 5	93 ± 5	90 ± 5	92 ± 5	87 ± 5	86 ± 5	89 ± 5
S+b-BC-PB	90 ± 5	93 ± 5	95 ± 5	91 ± 5	91 ± 5	93 ± 5	94 ± 5	91 ± 5	96 ± 5	94 ± 5	89 ± 5	89 ± 5	91 ± 5	85 ± 5	89 ± 5	90 ± 5
S+n-BC-PB	91 ± 5	92 ± 5	90 ± 5	96 ± 5	93 ± 5	93 ± 5	96 ± 5	95 ± 5	92 ± 5	90 ± 5	82 ± 5	87 ± 5	89 ± 5	84 ± 5	89 ± 5	91 ± 5
S+b-BC-RH	86 ± 5	89 ± 5	89 ± 5	92 ± 5	91 ± 5	92 ± 5	98 ± 5	91 ± 5	97 ± 5	90 ± 5	92 ± 5	90 ± 5	92 ± 5	88 ± 5	91 ± 5	86 ± 5
S+n-BC-RH	86 ± 5	88 ± 5	95 ± 5	92 ± 5	93 ± 5	95 ± 5	94 ± 5	92 ± 5	96 ± 5	92 ± 5	94 ± 5	93 ± 5	88 ± 5	87 ± 5	88 ± 5	86 ± 5
S+b-BC-SSL	85 ± 5	89 ± 5	93 ± 5	97 ± 5	89 ± 5	96 ± 5	98 ± 5	96 ± 5	92 ± 5	93 ± 5	90 ± 5	90 ± 5	86 ± 5	89 ± 5	85 ± 5	85 ± 5
S+n-BC-SSL	88 ± 5	91 ± 5	92 ± 5	96 ± 5	88 ± 5	93 ± 5	94 ± 5	92 ± 5	91 ± 5	92 ± 5	90 ± 5	91 ± 5	90 ± 5	85 ± 5	87 ± 5	88 ± 5

7. Changes in $C_{\text{total}} \Sigma 16$ PAHs and in $C_{\text{free}} \Sigma 16$ PAHs contribution

The addition of b-BC/n-BC to the soil influenced not only the content of $C_{\text{total}} \Sigma 16$ PAHs and $C_{\text{free}} \Sigma 16$ PAHs but also the contribution of PAHs categorized based on the number of aromatic ringss. Comparing the contribution of $C_{\text{total}} \Sigma 16$ PAHs based on the number of aromatic ringss, the contribution shifted following the amendment with BC/n-BC compared to the untreated soil (control), both at the beginning of the experiment (T_0) and after six weeks (T_1). Immediately after the addition of 2% b-BC/n-BC (T_0), 3-rings PAHs became dominant, accounting for 55.2% to 68.4%, depending on the type of b-BC/n-BC used. Compared to the control, the contribution of 3-rings PAHs increased by 10.2% to 15.4%, depending on the type of b-BC/n-BC used. In the case of 4- and 6-rings a significant decrease in the contribution was noted, ranging from 29.0% to 45.1% and 6.81% to 42.2%, respectively. When comparing feedstocks, the highest increase in the contribution of 3-rings PAHs and the highest decrease in 4-rings PAHs was observed for SSL-derived b-BC and n-BC. Regarding particle size, a trend was noted where all n-BCs exhibited higher contributions of 2-rings and 6-rings (except for SSL-derived n-BC) PAHs compared to b-BC. After six weeks (T_1), a also a dominance of 3-rings PAHs was observed in the contribution of $C_{\text{total}} \Sigma 16$ PAHs, ranging from 63.0% to 71.2%, depending on the type of b-BC/n-BC applied. Compared to the control, an increase in the contribution of 2-, 3- and 6-rings PAHs was observed, ranging from 43.3% to 101%, from 36.9% to 43.4% and from 6.47% to 215%, respectively depending on the type of b-BC/n-BC. However, a decrease in the contribution of 4- and 5-rings PAHs was noted, ranging from 9.14% to 74.0% and 19.9% to 74.4%, depending on the type of b-BC/n-BC. In terms of feedstock, the greatest changes in the contribution of 2-, 4-, and 6-rings PAHs were observed for PB-derived b-BC and n-BC. Comparing particle size, n-BC derived from PB and RH showed lower contributions of 2-, 4-, and 6-rings PAHs and higher contributions of 3- and 5-rings PAHs. For SSL-derived n-BC, this trend was reversed (excluding 6-rings PAHs).

In the case of the contribution of $C_{\text{free}} \Sigma 16$ PAHs, changes depended on both the particle size (b-BC or n-BC) and the feedstock type and contact time. At the beginning (T_0), for 2-rings PAHs, a decrease in their contribution to the $C_{\text{free}} \Sigma 16$ PAHs content was observed for PB- and RH-derived

b-BC, ranging from 9.61% to 14.8%, while an increase was recorded for PB- and RH-derived n-BC, ranging from 9.49% to 15.6%. In contrast, for SSL-derived b-BC and n-BC, an increase (2.1% to 12.3%) in the contribution of 2-ringss PAHs was noted. Regarding 3- and 4-ringss PAHs, an increasing trend was observed for PB- and RH-derived b-BC, with contributions increasing by 27.5% to 39.4% and 22.6% to 41.6%, respectively. However, the opposite trend was noted for their nano-counterparts, where reductions in 3- and 4-rings PAHs contributions were recorded, ranging from 7.86% to 19.6% and 78.6% to 86.4%, respectively. In this case, SSL-derived b-BC and n-BC exhibited a different behavior, with both forms showing a decline in 3- and 4-rings PAHs content, ranging from 10.9% to 40.4% and 2.08% to 31.9%, respectively. However, SSL-derived n-BC exhibited lower reductions in contribution compared to its bulk counterpart. For all BC/n-BC amendments an increase in contribution of 5-ringss PAHs was recorded by 101% to 740%. It should be noted that for PB- and RH-derived n-BC, this increase was on average 283% higher compared to BC. In contrast, for SSL-derived n-BC, the trend was reversed, with the contribution of 5-rings PAHs decreasing by 43.8% compared to SSL-derived BC. Regarding 6-rings PAHs, an increase in contribution ranging from 61.5% to 110% was observed for PB- and RH-derived BC, whereas their n-BC counterparts exhibited a decrease in contribution by 79.8% to 86.9%. In this case, SSL-derived BC and n-BC again showed an opposite trend, where BC exhibited a 12.5% decrease in 6-rings PAHs contribution, while n-BC showed an increase of 38.8%. After six weeks (T_1), the contribution of 2-rings PAHs in all BC/n-BC amendments decreased from 2.21% to 34.1%. An increasing trend was identified for 3-rings PAHs, where contribution increased by 37.2% to 171% over this period. In contrast, the contribution of 4-rings and 6-rings PAHs declined by 22.0% to 77.7% and 60.9% to 83.0%, respectively. Consistent with observations at T_0 term, the contribution of 5-rings PAHs increased across all BC/n-BC amendments, with the magnitude of this increase averaging 3.0-fold compared to T_0 term. These observations suggest that the feedstock type and particle size significantly influence the redistribution and immobilization efficiency of PAHs in soil. The decreased contribution of 2-rings PAHs after prolonged time may indicate a preferential sorption of lower molecular weight PAHs, potentially due to competitive adsorption dynamics. Over time, a stabilization trend emerged, with low molecular weight PAHs decreasing, intermediate PAHs increasing, and high molecular weight PAHs either stabilizing or undergoing sequestration, suggesting that BC and n-BC influence both PAH retention and potential transformation in soil. The distinct behavior of SSL-derived BC and n-BC, may be attributed to variations in surface

chemistry and sorption mechanisms specific to SSL-derived materials, potentially influencing the long-term stability and sequestration of PAHs in the soil matrix.

Table S7. The content of individual C_{total} PAHs [$\mu\text{g}\cdot\text{kg}^{-1}$] and C_{free} PAHs [$\text{ng}\cdot\text{L}^{-1}$] in soil and soil amended with b-BCs and n-BCs.

Sample	C _{total} PAHs [μg·kg ⁻¹]																Σ16 PAHs
	NAP	ACY	ACE	FLO	PHE	ANT	FLA	PYR	BaA	CHR	BbF	BkF	BaP	IcdP	DahA	BghiP	
T ₀ term																	
Soil (S)	508.0	2915	1923	124.9	2126	1946	2082	592.2	383.9	287.1	301.2	692.4	314.1	271.6	716.0	74.91	15260
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
S+b-BC-PB	108.2	403	281.1	40.79	317.1	190.2	179.8	96.68	62.47	40.35	50.86	60.88	177.2	98.48	66.62	14.78	2189
	403.5	2834	1621	104.1	2096	1672	1059	380.7	286.8	244.1	176.7	623.4	312.4	100.6	703.9	60.23	12680
S+n-BC-PB	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	44.11	240.6	82.17	14.13	250.9	220.7	192.9	± 25.65	5.958	18.84	28.21	25.59	13.65	10.36	26.28	5.078	1205
S+n-BC-PB	432.8	1609	1867	123.3	2105	1922	1100	207.4	326.1	185.1	147.9	440.3	247.9	112.0	690.0	166.5	11680
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
S+b-BC-RH	30.39	93.24	148.4	11.80	147.7	143.8	79.88	15.35	19.99	8.288	12.06	9.563	18.56	11.05	64.40	13.11	827.5
	396.4	1804	2252	152.0	2163	1617	1308	112.9	274.8	115.2	196.9	215.6	194.7	128.6	685.7	70.32	11690
S+b-BC-RH	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	25.32	155.7	234.7	93.54	200.8	135.2	101.1	56.84	86.59	19.26	191.2	49.21	12.00	12.30	66.30	6.919	1447
S+n-BC-RH	466.0	1683	2192	118.2	1449	583.9	2016	320.0	124.0	358.7	214.1	409.2	109.2	163.6	653.0	57.91	10920
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
S+b-BC-SSL	41.62	158	177.9	20.85	126.2	56.13	161.0	22.68	10.61	25.08	18.80	28.89	76.08	22.44	56.55	12.15	1015
	387.8	2562	1806	123.3	1747	2011	129.3	366.1	781.7	227.0	338.2	591.7	457.0	177.3	693.5	86.92	112490
S+b-BC-SSL	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	30.58	179.7	169.9	10.56	49.75	122.0	22.94	2.557	5.069	6.072	14.45	38.17	351	58.97	122.1	± 2.829	1187
S+n-BC-SSL	385.6	1943	1858	102.9	1977	2000	154.8	330.3	660.6	535.0	175.6	400.7	281.1	110.1	679.9	42.76	11640
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
S+n-BC-SSL	23.39	72.04	170.9	8.573	141.9	94.17	12.21	16.35	12.27	99.56	17.10	11.64	53.9	415.0	25.22	6.505	1181
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
T ₁ term																	
Soil (S)	384.2	1831	1519	41.08	1575	1758	1946	186.1	330.0	667.7	264.4	865.1	325.4	192.9	601.5	51.34	12540
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
Soil (S)	12.84	126.1	156.6	13.32	151.3	124.2	121.3	16.55	16.42	16.45	10.33	37.69	18.4	15.77	18.95	5.831	862.1
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±

S+b-BC-PB	545.2	1342	781.7	202.5	2195	612.4	47.95	384.9	151.1	183.4	175.5	224.5	255.2	426.3	508.3	118.1	8154
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
S+n-BC-PB	51.64	94.21	76.66	12.34	201.7	55.37	3.685	27.54	9.206	15.00	14.91	18.45	13.96	35.44	43.99	8.407	682.5
	333.8	1215	754.7	156.0	1605	410.7	22.62	286.3	81.68	149.4	104.5	107.7	218.2	144.8	476.7	102.1	6169
S+b-BC-RH	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	22.31	98.98	53.37	16.17	137.3	35.9	1.800	29.46	5.331	12.28	8.406	8.080	20.01	35.79	39.37	8.571	533.1
S+b-BC-RH	368.9	289.0	1645	128.1	1308	1018	1048	6.222	512.5	124.2	106.5	38.26	150.0	91.85	17.91	8.827	6862
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
S+n-BC-RH	25.47	18.11	14.06	13.05	121.1	83.02	19.55	0.4599	46.15	8.030	8.061	3.674	28.68	3.721	1.232	0.863	395.2
	269.5	1145	1013	67.05	1046	720.9	93.55	437.3	72.28	31.92	225.4	369.9	11.68	89.86	16.06	37.4	5646
S+b-BC-SSL	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	18.43	86.85	62.55	5.281	19.98	68.16	7.748	26.55	4.032	2.486	54.58	76.72	0.1836	6.827	1.371	2.677	444.4
S+b-BC-SSL	366.3	7	1811	109.3	1529	1258	67.52	151.2	88.7	234.0	68.74	111.1	255.7	162.0	626.6	81.59	7675
	±	54.5 ±	±	±	±	±	±	±	±	±	±	±	±	±	±	±2	±
S+n-BC-SSL	36.95	71.22	15.1	7.988	13.25	103.2	5.769	12.53	9.222	61.98	5.282	9.267	22.98	6.396	172	3.69	555.5
	318.4	487.5	768.9	117.6	1751	1239	17.19	197.4	137.8	221.4	14.57	66.75	108.7	59.09	634.1	38.23	6177
S+n-BC-SSL	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	20.09	45.44	73.84	7.07	17.52	10.56	1.277	20.46	53.76	20.83	1.573	6.185	43.84	33.27	364.6	2.797	723.1

C _{free} PAHs [ng·L ⁻¹]																	
Sample	NAP	ACY	ACE	FLO	PHE	ANT	FLA	PYR	BaA	CHR	BbF	BkF	BaP	IcdP	DahA	BghiP	Σ16 PAHs

T ₀ term																	
Soil (S)	501.1	15.79	12.89	0.9892	68.77	10.25	38.05	26.81	1.234	2.948	0.645	0.5065	0.9348	0.2086	0.01136	0.1608	681.3
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
S+b-BC-PB	38.51	1.477	1.273	0.08326	4.793	0.6188	3.226	2.179	0.1067	0.2807	0.0472	0.04112	0.07447	0.01631	0.0009144	0.01528	52.74
	324.3	15.51	27.37	9.163	39.85	7.279	32.25	23.42	1.291	3.636	1.011	0.6884	1.565	0.1816	0.01615	0.2454	487.7
S+n-BC-PB	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	24.17	1.378	2.435	0.8725	2.934	0.5338	3.177	1.914	0.1037	0.2089	0.09432	0.06251	0.1387	0.01631	0.001347	0.02336	38.07
S+n-BC-PB	260.5	9.288	11.82	5.920	17.67	2.856	3.520	3.296	0.02077	0.1877	3.545	0.1082	4.633	0.01633	0.07994	0.01915	323.5
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
S+b-BC-RH	23.07	0.9749	0.9739	0.391	1.016	0.1833	0.2153	0.3207	0.001771	0.0102	0.2952	0.007967	0.4207	0.001543	0.007694	0.001766	27.89
	318.4	22.56	29.16	9.018	44.85	7.393	37.45	29.19	1.801	4.435	1.027	0.8387	1.259	0.2528	0.02189	0.3243	508.0
S+n-BC-RH	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	26.61	2.064	2.566	0.6998	3.376	0.6343	2.889	1.940	0.1399	0.3637	0.07934	0.07132	0.09716	0.01433	0.001627	0.03015	41.58
S+b-BC-SSL	331.2	10.77	13.80	5.526	16.86	2.980	2.539	2.658	0.03374	0.1406	1.37	0.1064	1.407	0.007969	0.02753	0.01974	389.5
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
S+n-BC-SSL	26.39	0.9233	1.233	0.384	1.600	0.2885	0.1905	0.1618	0.002649	0.01062	0.1022	0.00876	0.1036	0.000613	0.002483	0.001607	31.41
	496.4	17.33	3.222	0.7711	30.78	5.050	21.61	16.56	0.8385	2.459	3.001	0.4873	2.429	0.1272	0.02324	0.1582	601.2
S+b-BC-SSL	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	29.69	1.324	0.1928	0.07305	2.077	0.4643	1.681	1.592	0.05163	0.2006	0.2752	0.03566	0.1661	0.009115	0.001884	0.01404	37.85

S+n-BC-SSL		383.7 ±	18.57 ±	6.106 ±	0.6238 ±	40.6 ±	6.742 ±	26.57 ±	20.21 ±	1.005 ±	2.916 ±	1.090 ±	0.4571 ±	1.960 ±	0.2048 ±	0.02116 ±	0.1797 ±	511.0 ±
		33.07	1.236	0.482	0.03524	3.506	0.5482	1.720	1.365	0.06543	0.1535	0.09049	0.03571	0.1526	±0.01771	0.001454	±0.01217	±42.49
T₁ term																		
Soil (S)		483.8 ±	12.42 ±	11.82 ±	0.4279 ±	29.41 ±	4.709 ±	20.97 ±	15.59 ±	0.9632 ±	2.450 ±	0.6646 ±	0.5007 ±	0.8449 ±	0.2349 ±	0.01364 ±	0.1821 ±	585.0 ±
S+b-BC-PB		38.61 ±	0.7269 ±	0.875 ±	0.03912 ±	1.721 ±	0.4182 ±	2.063 ±	1.091 ±	0.07429 ±	0.2206 ±	0.04668 ±	0.04609 ±	0.05887 ±	0.0224 ±	0.001267 ±	0.01716 ±	±46.03
S+n-BC-PB		63.93 ±	4.636 ±	1.671 ±	0.05317 ±	5.344 ±	0.916 ±	2.052 ±	2.074 ±	0.0529 ±	0.2035 ±	0.5462 ±	0.05307 ±	0.6515 ±	0.0143 ±	0.01023 ±	0.008646 ±	82.21 ±
S+b-BC-PB		5.956 ±	0.4504 ±	0.124 ±	0.003422 ±	0.2925 ±	0.08987 ±	0.1843 ±	0.1957 ±	0.004875 ±	0.01931 ±	0.04692 ±	0.00435 ±	0.05903 ±	0.001136 ±	0.0009238 ±	0.0006836 ±	7.433 ±
S+n-BC-RH		52.32 ±	3.824 ±	2.516 ±	0.04927 ±	4.865 ±	1.035 ±	1.539 ±	1.427 ±	0.07799 ±	0.261 ±	0.3545 ±	0.0759 ±	0.4908 ±	0.04787 ±	0.00681 ±	0.03559 ±	68.93 ±
S+b-BC-RH		3.587 ±	0.3609 ±	0.1303 ±	0.003673 ±	0.4011 ±	0.09413 ±	0.1164 ±	0.09781 ±	0.006025 ±	0.01977 ±	0.0323 ±	0.007109 ±	0.03808 ±	0.003555 ±	0.0006297 ±	0.002883 ±	4.902 ±
S+n-BC-RH		69.03 ±	4.487 ±	1.200 ±	0.5337 ±	3.976 ±	1.370 ±	0.9488 ±	0.9907 ±	0.02568 ±	0.09102 ±	0.4868 ±	0.0335 ±	0.6967 ±	0.007057 ±	0.01017 ±	0.009552 ±	83.9 ±
S+b-BC-RH		3.873 ±	0.4165 ±	0.09652 ±	0.04978 ±	0.4016 ±	0.1111 ±	0.0687 ±	0.08752 ±	0.002383 ±	0.008597 ±	0.0473 ±	0.002805 ±	0.06267 ±	0.0006088 ±	0.0009011 ±	0.0008196 ±	5.231 ±
S+n-BC-SSL		57.09 ±	2.514 ±	2.108 ±	0.5433 ±	4.094 ±	0.902 ±	0.4953 ±	0.5224 ±	0.01215 ±	0.04717 ±	0.9501 ±	0.03046 ±	1.247 ±	0.002928 ±	0.02813 ±	0.005631 ±	70.59 ±
S+b-BC-SSL		5.281 ±	0.2237 ±	0.1844 ±	0.03494 ±	0.2249 ±	0.07183 ±	0.04429 ±	0.0459 ±	0.001155 ±	0.004502 ±	0.08189 ±	0.00234 ±	0.1158 ±	0.0002629 ±	0.002581 ±	0.0005136 ±	6.321 ±
S+n-BC-SSL		176.4 ±	8.120 ±	10.33 ±	0.1906 ±	11.31 ±	6.135 ±	19.66 ±	10.45 ±	0.4863 ±	2.218 ±	0.6896 ±	0.4686 ±	0.4917 ±	0.1481 ±	0.01306 ±	0.1907 ±	247.3 ±
S+b-BC-SSL		14.86 ±	0.6662 ±	2.191 ±	0.7943 ±	3.372 ±	0.5903 ±	2.135 ±	1.761 ±	0.1278 ±	0.2537 ±	0.1922 ±	0.04312 ±	0.04537 ±	0.0101 ±	0.003613 ±	0.01463 ±	27.06 ±
S+n-BC-SSL		91.45 ±	8.384 ±	12.13 ±	1.743 ±	7.748 ±	2.403 ±	5.384 ±	6.923 ±	0.1373 ±	0.6041 ±	1.216 ±	0.1171 ±	1.724 ±	0.0184 ±	0.02177 ±	0.04642 ±	140.1 ±
		7.573 ±	0.5544 ±	1.121 ±	0.1267 ±	0.5572 ±	0.2600 ±	0.528 ±	0.6409 ±	0.008868 ±	0.03554 ±	0.0747 ±	0.009461 ±	0.1840 ±	0.00138 ±	0.002158 ±	0.002886 ±	11.68 ±

NAP - naphthalene, ACY - acenaphthylene, ACE - acenaphthene, FLO - fluorene, PHE - phenanthrene, ANT - anthracene, FLA - fluoranthene, PYR - pyrene, BaA - benz(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(g,h,i)perylene.

Table S8. Coefficients of *log* K_{TOC} for individual PAHs in the control soil and b-BC/n-BC-amended soil.

PAHs	Soil (S)			S+b-BC-PB			S+n-BC-PB			S+b-BC-RH			S+n-BC-RH			S+b-BC-SSL			S+n-BC-SSL		
	T ₀ term	T ₁ term	Average term	T ₀ term	T ₁ term	Average term	T ₀ term	T ₁ term	Average term	T ₀ term	T ₁ term	Average term	T ₀ term	T ₁ term	Average term	T ₀ term	T ₁ term	Average term	T ₀ term	T ₁ term	Average term
NAP	2.07	1.99	2.04	2.13	2.66	2.40	2.16	2.70	2.43	2.62	2.38	2.47	2.78	2.47	1.98	2.27	2.04	2.5	2.27	2.04	2.5
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
ACY	0.0928	0.0625	0.0359	0.0928	0.1115	0.27	0.0982	0.0673	0.269	0.09	0.119	0.247	0.0822	0.132	0.303	0.046	0.0726	±0.146	0.0778	0.108	0.23
	4.33	3.93	4.13	4.20	4.14	4.17	4.37	4.17	4.27	4.04	4.15	4.09	4.42	4.47	4.44	4.2	3.95	4.07	4.12	3.87	4.00
ACE	0.163	0.132	0.203	0.154	0.116	0.0324	0.182	0.178	0.0992	0.178	0.137	0.0535	0.146	0.214	0.025	0.115	0.173	0.126	0.12	0.18	0.121
	4.24	4.21	4.22	3.78	4.84	4.31	4.09	4.62	4.35	3.75	4.98	4.36	4.13	4.81	4.47	4.75	3.75	4.25	4.42	3.98	4.20
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	0.205	0.115	0.0154	0.107	0.116	0.534	0.124	0.154	0.265	0.154	0.208	0.617	0.164	0.214	0.338	0.17	0.125	0.500	0.105	0.14	0.221

FLO	4.17	4.08	4.12	3.07	4.77	3.92	3.20	4.76	3.98	3.07	3.77	3.42	3.34	3.58	4.18	2.59	3.38	4.22	3.25	3.74
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
PHE	0.166	0.152	0.0426	0.129	0.148	0.854	0.145	0.155	0.779	0.0878	0.165	0.348	0.115	0.129	0.244	0.138	0.0944	0.798	0.147	0.486
	3.56	3.83	3.69	3.66	4.36	4.01	3.95	4.35	4.15	3.60	4.48	4.04	4.09	4.53	4.31	3.81	3.52	3.66	3.64	4.19
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
ANT	0.147	0.145	0.136	0.156	0.17	0.349	0.113	0.103	0.196	0.15	0.13	0.436	0.179	0.182	0.224	0.124	0.0965	0.147	0.172	0.274
	4.34	4.67	4.51	4.36	5.17	4.76	4.71	5.07	4.89	4.35	4.99	4.67	4.8	5.24	5.02	4.56	4.39	4.48	4.38	4.56
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
FLA	0.173	0.113	0.164	0.171	0.128	0.406	0.157	0.18	0.179	0.126	0.178	0.319	0.216	0.221	0.219	0.115	0.171	0.0827	0.125	0.181
	3.80	4.07	3.94	3.74	4.86	4.30	4.65	4.94	4.79	3.67	5.19	4.43	4.90	5.54	5.22	3.96	3.75	3.85	3.81	4.13
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
PYR	0.164	0.139	0.132	0.149	0.205	0.561	0.158	0.204	0.146	0.134	0.181	0.759	0.229	0.146	0.322	0.148	0.161	0.102	0.0914	0.311
	3.41	3.18	3.29	3.33	3.84	3.59	4.13	3.95	4.04	3.24	4.15	3.69	4.33	4.5	4.42	3.53	2.89	3.21	3.39	3.31
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
BaA	0.145	0.124	0.117	0.09	0.096	0.253	0.117	0.151	0.0891	0.13	0.134	0.459	0.167	0.188	0.0834	0.161	0.119	0.315	0.0925	0.106
	4.56	4.63	4.60	4.40	5.68	5.04	6.14	5.46	5.80	4.26	5.99	5.12	6.04	6.38	6.21	4.63	4.28	4.46	4.50	5.26
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
CHR	0.128	0.204	0.241	0.102	0.166	0.787	0.191	0.192	0.0923	0.163	0.168	1.00	0.166	0.274	0.402	0.134	0.176	0.107	0.175	0.504
	4.73	4.70	4.72	4.40	4.57	4.49	3.80	4.71	4.26	4.40	4.61	4.51	4.33	4.39	4.36	3.97	3.90	3.94	4.36	4.29
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
BbF	0.143	0.187	0.0178	0.148	0.222	0.0833	0.106	0.168	0.452	0.142	0.177	0.109	0.188	0.187	0.0327	0.121	0.123	0.039	0.191	0.0729
	5.20	5.34	5.27	4.93	6.1	5.52	5.68	5.89	5.79	4.85	6.29	5.57	5.80	6.4	6.10	5.13	5.20	5.16	5.10	5.42
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
BkF	0.157	0.209	0.0679	0.209	0.244	0.583	0.243	0.217	0.106	0.13	0.246	0.723	0.221	0.264	0.301	0.167	0.233	0.0385	0.171	0.259
	4.59	4.69	4.64	4.23	4.58	4.41	3.71	4.66	4.18	4.33	4.55	4.44	4.33	4.36	4.35	4.08	4.76	4.42	4.13	4.14
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
BaP	0.145	0.158	0.0467	0.181	0.178	0.176	0.156	0.182	0.476	0.134	0.188	0.111	0.163	0.13	0.0155	0.176	0.199	0.336	0.174	0.0145
	5.18	5.01	5.10	5.11	6.02	5.56	6.10	5.44	5.77	4.96	6.32	5.64	6.52	6.77	6.64	5.3	5.05	5.18	5.04	5.9
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
IcdP	0.151	0.21	0.0831	0.15	0.207	0.455	0.248	0.124	0.327	0.205	0.252	0.678	0.255	0.221	0.125	0.154	0.185	0.126	0.181	0.205
	6.86	6.74	6.8	6.58	6.66	6.62	5.83	6.78	6.30	6.44	6.65	6.55	6.40	6.28	6.34	6.46	5.99	6.23	6.45	6.38
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
DahA	0.202	0.195	0.0605	0.162	0.254	0.0393	0.16	0.201	0.478	0.276	0.32	0.104	0.18	0.227	0.061	0.246	0.245	0.235	0.154	0.269
	4.73	4.55	4.64	4.42	5.66	5.04	5.47	4.99	5.23	4.29	5.61	4.95	5.56	5.91	5.74	4.65	4.37	4.51	4.54	4.92
	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
BghiP	0.129	0.177	0.0919	0.14	0.132	0.622	0.218	0.199	0.236	0.101	0.196	0.659	0.213	0.198	0.172	0.205	0.149	0.141	0.15	0.191

NAP - naphthalene, ACY - acenaphthylene, ACE - acenaphthene, FLO - fluorene, PHE - phenanthrene, ANT - anthracene, FLA - fluoranthene, PYR - pyrene, BaA - benz(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(g,h,i)perylene.

Table S9. The Toxic Equivalency Factor (TEF) of 16 priority PAHs recognized by the US EPA compared to benzo[a]pyrene (BaP).

Polycyclic aromatic hydrocarbons (PAHs)	TEF [US EPA]
Naphthalene [NAP]	0.001
Acenaphthylene [ACY]	0.001
Acenaphthene [ACE]	0.001
Fluorene [FLO]	0.001
Phenanthrene [PHE]	0.001
Anthracene [ANT]	0.01
Fluoranthene [FLA]	0.001
Pyrene [PYR]	0.001
Benzo[a]anthracene [BaA]	0.1
Chrysene [CHR]	0.01
Benzo[b]fluoranthene [BbF]	0.1
Benzo[k]fluoranthene [BkF]	0.1
Benzo[a]pyrene [BaP]	1.0
Indeno[1.2.3-c.d]pyrene [IcdP]	0.1
Dibenz[a,h]anthracene [DahA]	1.0
Benzo[g,h,i]perylene [BghiP]	0.01

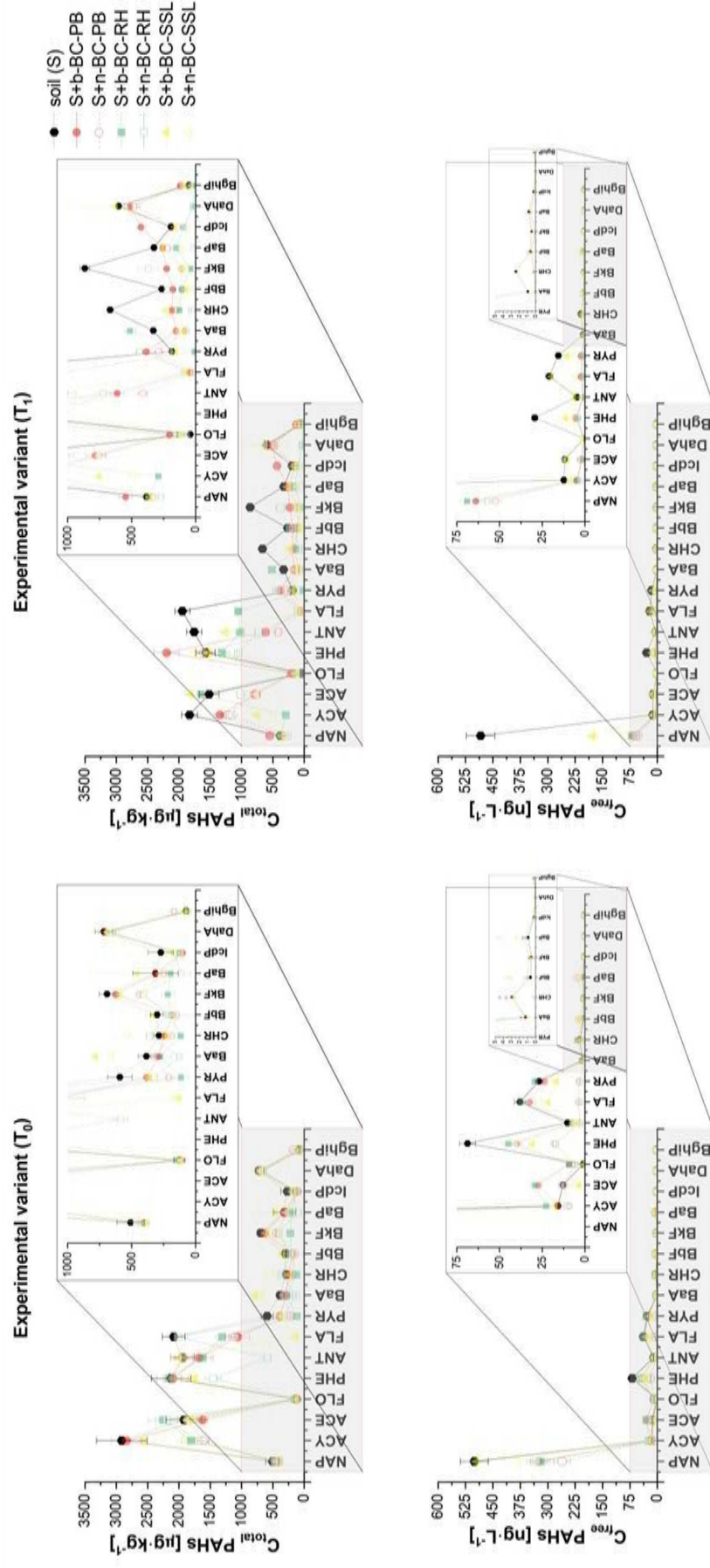
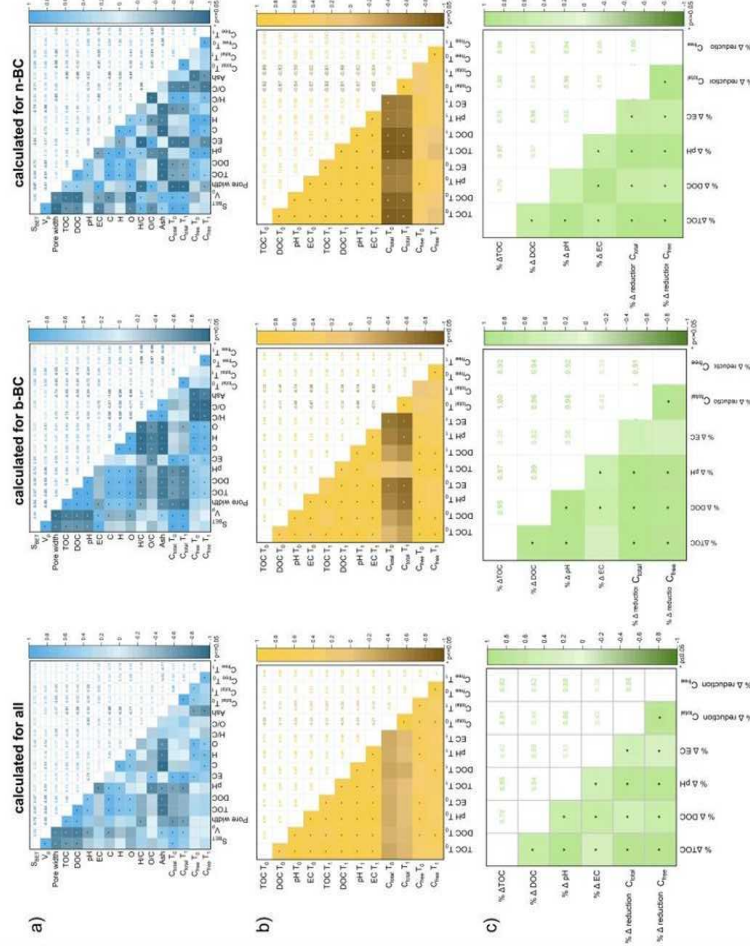


Figure S5. The profile of changes in individual C_{total} $\Sigma 16$ PAHs and C_{free} $\Sigma 16$ PAHs in control (soil) and different b-BC/n-BC amendments in T_0 and T_1 term.



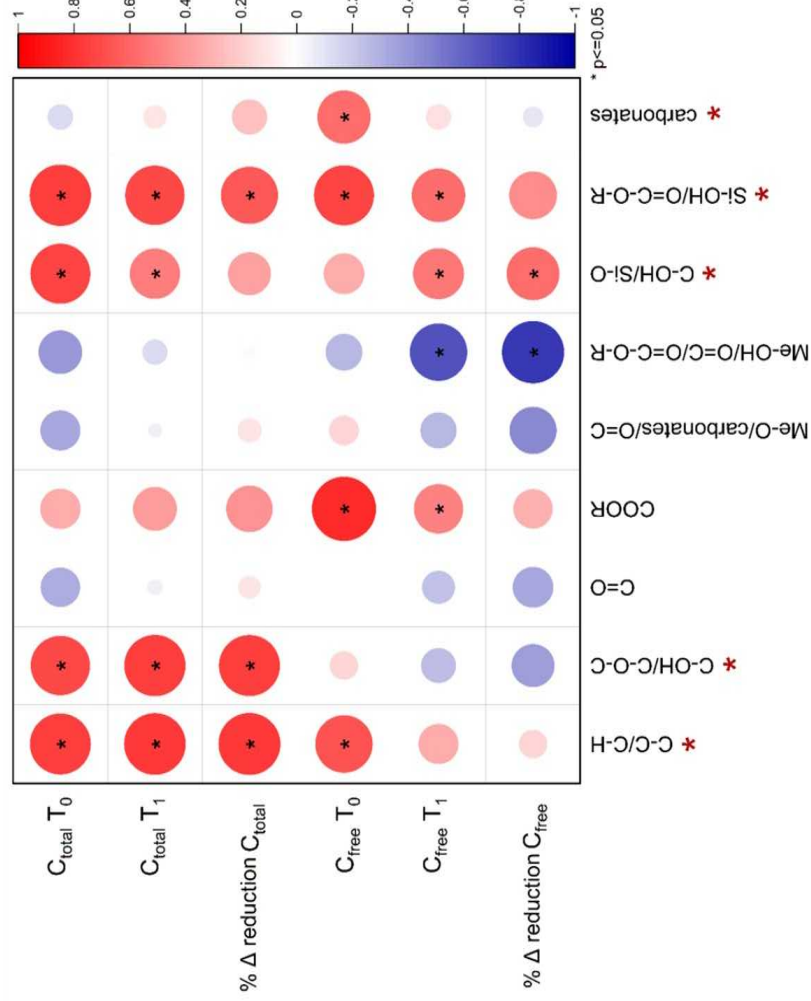


Figure S7. Pearson's correlation plot between amount of functional groups present on b-BC/n-BC and C_{total} $\Sigma 16$ PAHs and C_{free} $\Sigma 16$ PAHs reduction. Reductions were calculated for different b-BC/n-BC amendments in reference to control soil at T_0 (immediately after amendment) and T_1 (after six weeks) and over the time (Δ reduction), where * means statistically significant correlation ($p \leq 0.05$) and * means key functional groups.

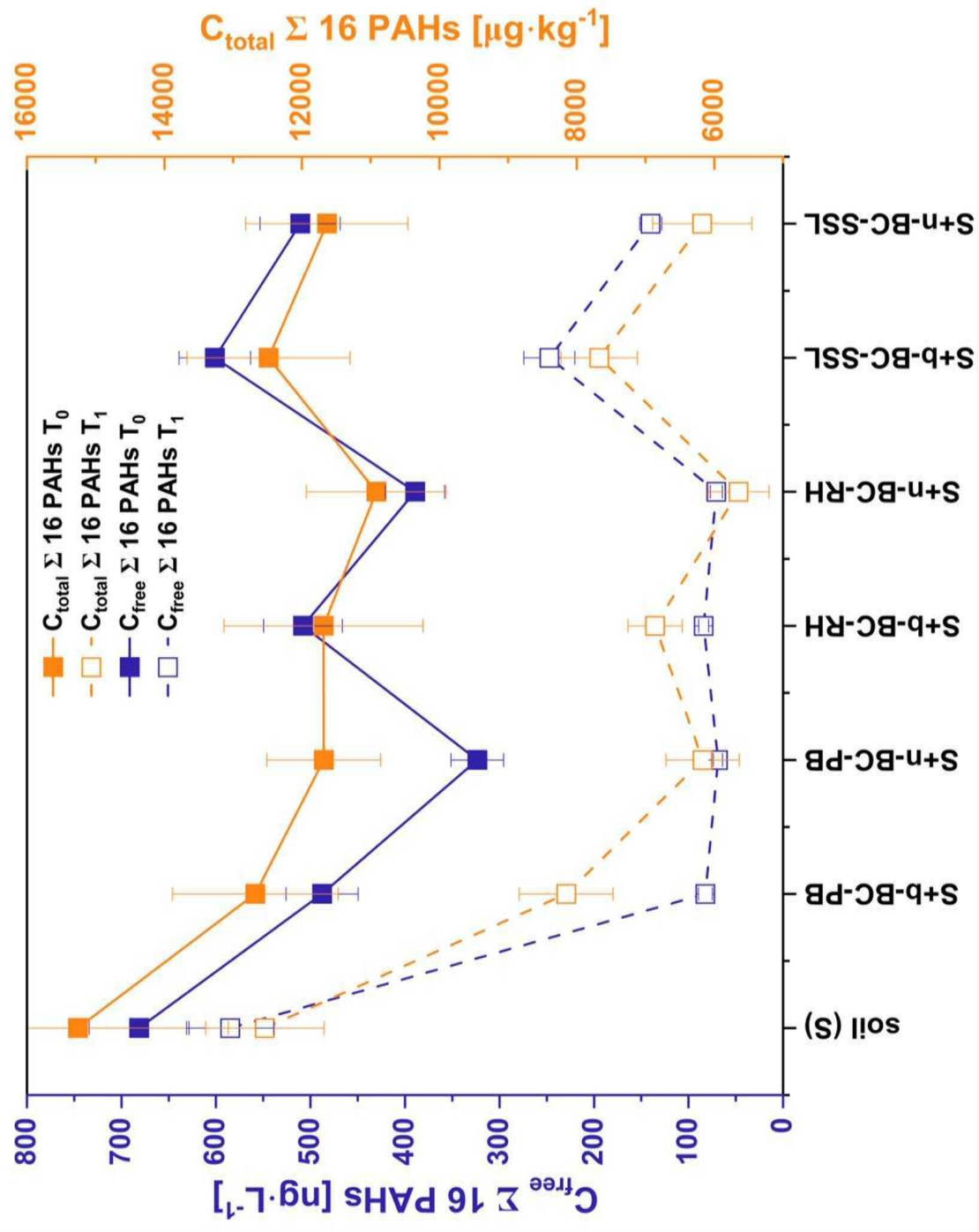


Figure S8. The profile of changes in $C_{total} \Sigma 16 \text{ PAHs}$ and $C_{free} \Sigma 16 \text{ PAHs}$ in control (soil) and different b-BC/n-BC amendments.

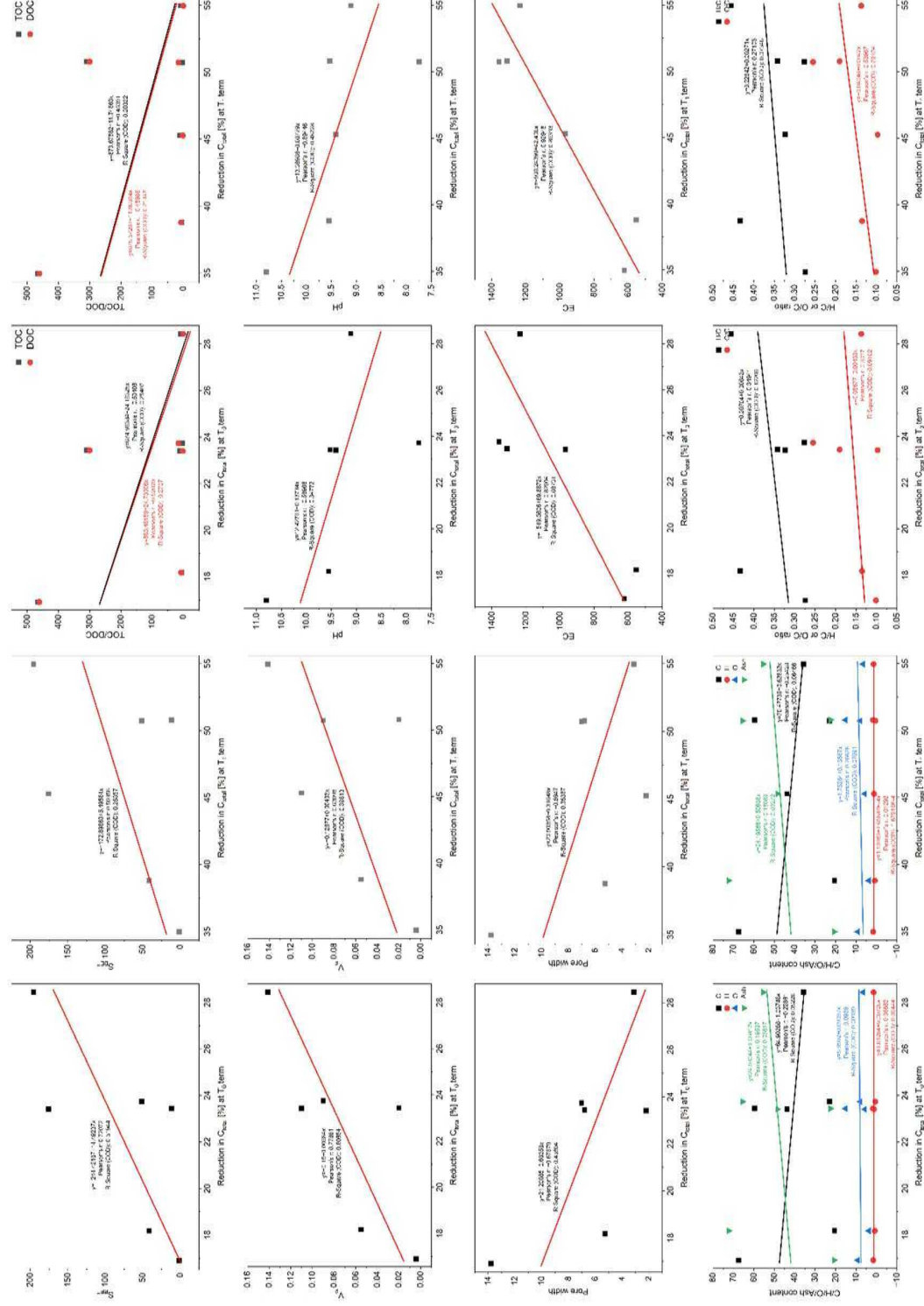


Figure S9. Linear regression analyses between selected physicochemical properties of b-BC and n-BC and the reduction of $C_{\text{total}} \Sigma 16$ PAHs at two sampling times (T_0 and T_1). Regression equations, R^2 values, and significance levels are shown on each panel.

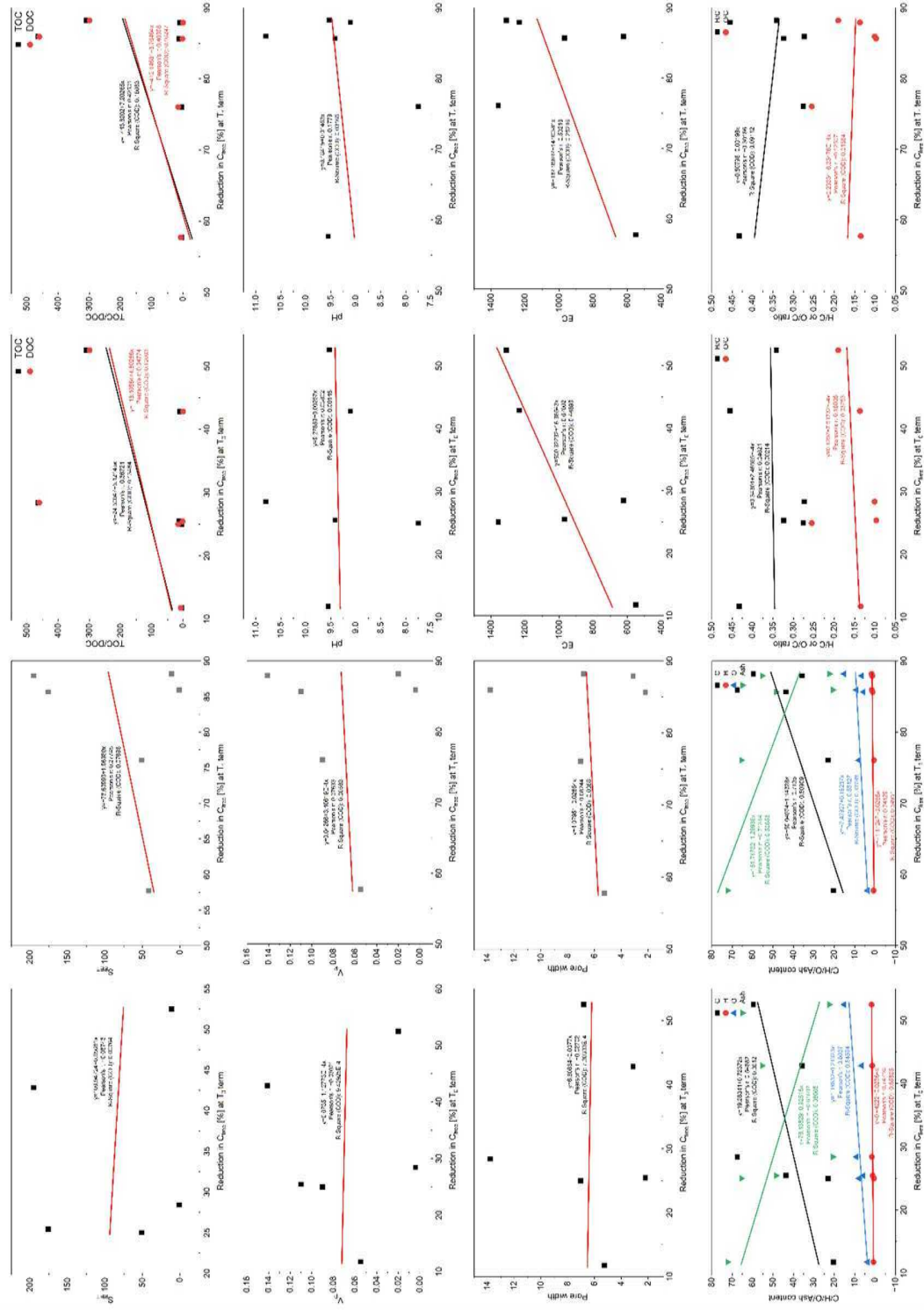


Figure S10. Linear regression analyses between selected physicochemical properties of b-BC and n-BC and the reduction of $C_{\text{free}} \Sigma 16$ PAHs at two sampling times (T_0 and T_1). Regression equations, R^2 values, and significance levels are shown on each panel.

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