

Lead immobilization in simulated polluted soil by Douglas fir biochar-supported phosphate

Arwenyo Beatrice^{a,d}, Jac J. Varco^b, Andrew Dygert^b, Felix S. Atsar^a, Sabrina Solomon^a,
Rooban Venkatesh K.G. Thirumalai^c, Charles U. Pittman Jr.^a, Todd Mlsna^{a,*}

^a Department of Chemistry, Mississippi State University, Mississippi State, MS, 39762, USA

^b Department of Plant and Soil Sciences, Mississippi State University, Mississippi State, MS, 39762, USA

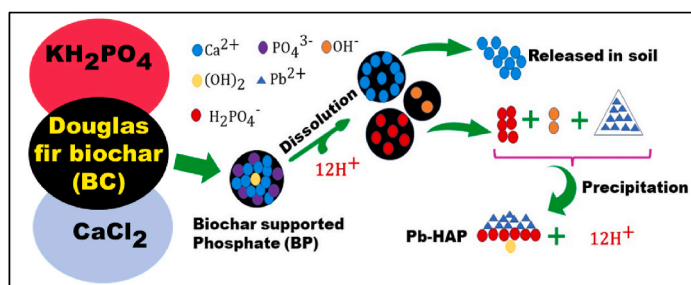
^c Institute for Imaging & Analytical Technologies, Mississippi State University, Starkville, MS, 39762, USA

^d Department of Chemistry, Gulu University, P O Box 166, Gulu, Uganda

HIGHLIGHTS

- Biochar-supported phosphate (BP) effectively immobilized Pb^{2+} from polluted soil.
- A 1% dose of BP removed 87% of the extractable Pb^{2+} originally present within 10 d.
- Pb^{2+} react with $Ca_{10}(PO_4)_6(OH)_2$ to precipitated $Pb_{10}(PO_4)_6 \cdot (OH)_2$ in soil at $pH < 7$.
- After amendment with 5% BP, the residual (immobilized) lead increased by 20.9%.

GRAPHICAL ABSTRACT



ARTICLE INFO

Handling Editor: Grzegorz Lisak

Keywords:

Heavy metals
Biochar supported-phosphate
In-situ-immobilization
Bioavailability
Soil pollution

ABSTRACT

This study compared the lead (Pb^{2+}) immobilization efficacy of biochar-supported phosphate to conventional in-situ heavy metal immobilization methods (with lime, neat biochar and phosphate). The biochar-supported phosphate was obtained by treating Douglas fir biochar (BC) with anhydrous calcium chloride and potassium dihydrogen phosphate. The amount of Pb^{2+} immobilized was determined by comparing the concentration of ammonium nitrate extractable Pb^{2+} lead from lead-spiked soil (without amendment) to that of a 30 d incubation with (a) lead-spiked soil plus 5% (wt./wt.) biochar supported-phosphate, (b) lead-spiked soil plus 5% (wt./wt.) untreated Douglas fir biochar, (c) lead-spiked soil plus 5% (w/w) lime and (d) lead-spiked soil plus 5% (wt./wt.) potassium dihydrogen phosphate. The control (lead-spiked soil without amendment) produced the largest quantity ($96.08 \pm 9.22 \text{ mg L}^{-1}$) of NH_4NO_3 -extractable Pb^{2+} , while lead-spiked soil treated with 5% (wt./wt.) biochar-supported phosphate resulted in the lowest quantity of NH_4NO_3 extractable Pb^{2+} ($0.3 \pm 0.2 \text{ mg L}^{-1}$). The mechanism for immobilization of Pb^{2+} by BP occurs at $pH < 7$ through dissolution of hydroxyapatite embedded in BP during modification, followed by precipitation of insoluble $Pb_{10}(PO_4)_6(OH)_2$. The residual lead fraction in the lead-spiked soil increased by 20.9% following amendment with BP. These results indicate that biochar-

* Corresponding author. Department of Chemistry, Mississippi State University, Box 9573, Mississippi State, MS, 39762-9573, USA.

E-mail address: TMLsna@chemistry.msstate.edu (T. Mlsna).

supported phosphate is a candidate to reduce lead mobility (bioavailability) in polluted soil. This amendment may lower Pb^{2+} uptake into plants while minimizing the potential for water contamination due to Pb^{2+} mobility.

1. Introduction

Over 10 million heavy metal-polluted sites have been reported globally with net economic consequences of nearly US \$10 billion annually (Lwin et al., 2018). Lead, cadmium, and arsenic contaminations constitute more than 50% of the polluted sites. The continued bioaccumulation of heavy metals in soil poses a significant health threat to plants, animals, and humans (Amari et al., 2017; Nelke et al., 2015; UNEP, 2020). Lead (Pb^{2+}) in particular can remain in the environment for more than 150 years (Karnib et al., 2014). Lead uptake by crops has been previously linked to plant phytotoxicity with a consequent adverse impact on humans due to metal transfer through the food chain (Cao et al., 2007; Pinho and Ladeiro, 2012). Ingestion of lead from contaminated food can lead to learning difficulties in children, neuron toxicity, kidney diseases and cancer (Tchounwou et al., 2012; UNEP, 2020). Therefore, the development of an effective and eco-friendly technique for minimizing lead bioavailability from contaminated soil is crucial.

Many methods have been developed for heavy metal remediation from contaminated soil (Basta and McGowen, 2004; Kede et al., 2017; Shareef and Zhao, 2017). Conventional methods include phytoremediation, soil washing, electrokinetic treatment, soil isolation, vitrification, sorption, liming, and precipitation using phosphates (Kede et al., 2017; Sharma et al., 2018; Ullah et al., 2020). Phytoremediation is eco-friendly, but only suitable for moderately contaminated soil. It can also cause secondary pollution (Burlakovs et al., 2012). Electro-kinetic treatment, soil isolation, and vitrification methods are laborious and costly (Khalid et al., 2017). The use of chelating agents for washing poses a significant groundwater contamination risk (Preet and Sidhu, 2016).

In-situ immobilization techniques, using lime, phosphate, and biochar, are considered relatively inexpensive and easy to manage (Zeng et al., 2020). Therefore, they have been used widely. The technique is believed to reduce heavy metal bioavailability (Jiang et al., 2021), and lower plant uptake through adsorption and formation of stable precipitates and complexes (Lwin et al., 2018). Lime application causes an increase in soil pH and co-precipitation hydroxides and carbonates (Hamid et al., 2018). Phosphate use forms insoluble precipitates such as pyromorphite-type minerals [$\text{Pb}_5(\text{PO}_4)_3\text{X}$; $\text{X} = \text{F}, \text{Cl}, \text{B}, \text{or OH}$] which lowers lead mobility (Kede et al., 2017). Biochar immobilizes heavy metals by adsorption, through ion exchange, co-precipitation, complex formation, electrostatic interactions, and physical sorption. (S.Wang et al., 2018; H. Wang et al., 2018; Wang et al., 2019).

It is unclear whether the combination of lime, phosphate, or biochar for lead immobilization is superior to the individual traditional immobilization methods. The phosphate sorption ability of calcium-derived adsorbents has been previously shown (Kong et al., 2020). While phosphate application in isolation could result in eutrophication of water bodies (Zupančič et al., 2012), binding phosphate to calcium treated biochar slows phosphate release into soil (Yao et al., 2013).

In August 2021, the United Nations intergovernmental Panel on Climate Change (IPCC) forecast that adding huge amounts (10^9 tons/yr) of biochar in soil will combat the growth of atmospheric CO_2 while improving soil fertility. Thus, reducing heavy metal mobility by biochar-supported phosphate could further encourage biochar use. Biochar addition to soil sequesters carbon originally obtained from organic biomass that would otherwise release CO_2 by combustion or decay, and serves as a suitable waste management alternative for municipal or agricultural waste (Ye et al., 2019). The oxidative aging of biochar during exposure to water and oxygen generates hydroxyl, carboxyl, and carbonyl functions on BC which readily sorb heavy metals (Hue, 2018; Houben et al., 2013).

Compared with neat biochar, chemically modified-biochars can often exhibit better sorption capacity for contaminants due to improved surface area and modified surface functional groups. Ye et al., 2020, separated Pb from aged microplastic using porous magnetic biochar and obtained ~38 and 34% removal efficiencies in the presence of HPO_4^{2-} and HCO_3^- respectively. They attributed this to the buffering action of HPO_4^{2-} and HCO_3^- causing pH rise, thus promoting precipitation, complexation and reduced Pb^{2+} mobility. Kong et al., 2020 used hydroxyapatite recovered from phosphate absorbed from wastewater onto calcium decorated biochar (BC-HAP) to extract uranium from wastewater. The sorption capacity of neat hydroxyapatite was 935 mg g^{-1} . The biochar's sorption capacity improved with increasing P content, rising from 485 to 917 mg g^{-1} as its PO_4^{3-} amount rose from 50 to 300 mg L^{-1} . Chen et al., 2020 improved heavy metal sorption by biochar in soil by adding hydroxyapatite/calcium silicate hydrate.

The main objective of this study was to assess the lead immobilization efficacy in soil by biochar-supported phosphate from a simulated lead-polluted soil and compare this treatment to the individual use of lime, phosphate, or biochar alone. We thought hydrolysis of biochar-supported phosphate in soil could reduce Pb^{2+} mobility and bioavailability; thereby, diminishing potential transfer to human via the food chain.

2. Materials and methods

2.1. Preparation of Douglas fir biochar (BC) and biochar-supported phosphate (BP)

Douglas Fir biochar (BC) is a commercial product (Black Owl Biochar, Biochar Supreme Inc. Environment Ultra) obtained as a by-product from bio-syngas production. The biochar was prepared according to the method reported by (Bombuwala Dewage et al., 2018). In this method, green Douglas fir wood chips were introduced into an air-fed updraft wood gasifier at ~900–1000 °C for a residence time of ~1–30 s.

Biochar-supported phosphate was obtained by treating BC with anhydrous calcium chloride and potassium dihydrogen phosphate (Fig. S1). BC (50 g) was treated with anhydrous calcium chloride (25 g) to enhance its phosphate sorption ability. All chemicals used were of analytical grade (Aldrich Sigma). Deionized water (400 mL) was added to 50 g of previously ground and sieved BC ($\leq 350 \mu\text{m}$ particle size) in a 1000 mL beaker with continued stirring to form a uniform slurry. The slurry was then heated to about 37 °C. Then, 25 g of anhydrous calcium chloride was added and stirring was continued for 30 min. Then, 25 g of potassium dihydrogen phosphate was added and stirring continued for another 30 min. The pH of the slurry was then adjusted to about 10 using aqueous potassium hydroxide (10 M KOH) and aged for 24 h. The aged slurry was vacuum filtered through Whatman number 1 filter paper. The residue was washed three times, each with 200 mL of deionized water, and oven-dried at ~1 atm pressure for ~48 h to constant weight at 80 °C. The biochar-supported phosphate (BP) was kept in seal containers for later use.

2.2. Characterization of Douglas fir biochar (BC) and biochar-supported phosphate (BP)

The surface area, pore volume and average pore size diameter were analyzed by the BET (Brunauer, Emmet, and Teller) N_2 adsorption method at 77.3K with a Micromeritics Tristar II Plus Version 2.03 surface area analyzer. The morphology of both BC and BP was examined with scanning electron microscopy (JEOL JSM-6500F FE-SEM operated at 5 kV), and the elemental contents were determined using scanning

electron microscopy-electron diffraction energy (SEM-EDX) spectroscopy. Powder x-ray diffraction scrutinized the crystalline content of BC and BP before and after a 30-d incubation with lead-spiked soil using a Rigaku's Smartlab X-ray diffraction system employing Cu-K α ($\lambda = 1.54$ Å) radiation generated at 40 kV and 44mA. The XRD patterns were obtained by scanning 2θ from 3° to 90° with 0.05° steps at a rate of 4° min^{-1} .

The pH and ash contents of BC and BP were determined following the method described by (Domingues et al., 2017) with some modification. The pH was measured using a Hanna pH meter (model HI 3221) after shaking the biochar samples for 1 h in deionized water at a 1:10 (wt./v) ratio. The ash content was determined by heating 1.0 g of oven-dried biochar samples (at 105°C for 24 h) in an open-top porcelain crucible at 650°C in a muffle furnace for 15 h. A (Elementar Unicube, Germany) elemental analyzer was used to determine the elemental composition (C, H, N, S) of BC and BP by combustion. The amount of organic oxygen (% O) was calculated by difference ($\text{O}\% = [100 - (\text{C} + \text{H} + \text{N} + \text{S} + \text{Ash})]$).

2.3. Determining pH point of zero charge (pHpzc)

The solution pH at which the net surface charge on the absorbent (BC and BP) is zero (the PZC) was determined from ~ 0.1 g BC and BP samples, which were placed in 50 mL polypropylene tube and mixed with 20 mL of $50 \text{ mg L}^{-1} \text{ Pb}^{2+}$ solution. The mixtures were then shaken for 24 h in a mechanical shaker (200 rpm) at room temperature. These slurries were then filtered through Whatman number I filter paper. The final pH of the filtrates were measured using the Hanna pH meter for pH values ranging from 1 to 14. The desired solution pH was adjusted using 1 M NaOH or 1 M HCl solutions. The PZC was obtained by plotting ΔpH (initial-final pH) values against the initial solution pH.

2.4. Soil sampling and incubation

These experiments were carried out on Pb-spiked soil. Soil was

$$IP = \frac{\text{NH}_4\text{NO}_3 \text{ extractable lead concentration of the (control - amended soil)} \times 100\%}{\text{NH}_4\text{NO}_3 \text{ extractable lead concentration of the control}}$$

collected at a depth of 0–15 cm from an agricultural field located at Mississippi State University. The soil was sieved to remove roots and stones, air-dried, homogenized, and passed through a 2 mm sieve before use. The initial soil pH (5.64) was measured following the method described by (Thompson and Varco, 2002). An air-dried soil sample (10 g) was added into a 50 mL plastic beaker. Then, 20 mL of deionized water was added using an auto dispenser and the mixture stirred with a glass rod until all soil was suspended. The slurry was then stirred for 1 h and then allowed to stand for a 30 min interval before measuring the pH using a Fisher Scientific Model 25 Accumet pH meter (Denver, CO). In addition, the soil was analyzed for P, Mg, Ca, K and Na contents (Table S1) following the Mississippi State University soil testing (Lancaster method) procedure as described by (Cox, 2001). Air-dried and sieved soil samples (5 g) were placed into 125 mL plastic Erlenmeyer flasks. Then, 5 mL of solution A (0.05 M HCl) was added using an autopipette, and it was left to stand for 10 min. Next, 20 mL of solution B (prepared by mixing 900 mL glacial acetic acid, 65.2 g malonic acid, 120 g malic acid, 12 g ammonium fluoride, 30 g aluminum chloride hexahydrate, 300 mL concentrated ammonium hydroxide and then diluting to 10 L with deionized water after adjusting the solution pH to 4.0) was added to each flask using an autopipette, and the mixture shaken for 10 min on a mechanical shaker ($250 \text{ cycles min}^{-1}$). The suspension was filtered through Whatman number 2 filter paper, and the filtrate analyzed for P, Mg, Ca, K and Na using an Avio 200 ICP-OES

(PerkinElmer, Waltham, MA).

A 3 kg soil sample was spiked with Pb to a 300 mg kg^{-1} level using a $\text{Pb}(\text{NO}_3)_2$ solution. The USEPA maximum acceptable soil lead level for gardening soil is 100 mg kg^{-1} of soil (Schwarz et al., 2016). However, the acceptable soil lead levels set by environmental protection agencies varies by country (Reid and Dirou, 2004; Wade, 2019). The lead-spiked soil was incubated for 14 d at 25°C with a moisture content at 100% field capacity initially, which was then maintained at 80% of water by adding deionized water throughout the duration. After the 14-d incubation, the soil was air dried at 25°C for ~ 7 d and stored at room temperature.

2.5. Determining the lead immobilization efficacy

The lead immobilization efficacy of BP was evaluated in comparison to BC, lime and potassium dihydrogen phosphate, each used individually. The treatments used included: 1) Pb-spiked soil (Control), 2) lead-spiked soil plus 5% BP (wt./wt.), 3) lead-spiked soil plus 5% BC (wt./wt.), 4) lead-spiked soil plus 5% lime (wt./wt.) and 5) lead-spiked soil plus 5% potassium dihydrogen phosphate (wt./wt.). Each treatment was performed in triplicate and arranged in a randomized design. The different treatments were incubated for 30 d at 25°C with their water holding levels maintained at 80% capacity. The sample containers were covered with parafilm with holes pierced into them for aeration. After incubating for 30 d, the soil samples were air-dried and their ammonium nitrate extractable lead concentrations were determined following the procedure described by (Park et al., 2013). This procedure entails weighing 2 g of the air-dried soil samples in a 50 mL polypropylene tube and adding 5 mL of 1 M ammonium nitrate solution to each (soil solution ratio of 1:2.5), followed by shaking in a reciprocating shaker at 200 rpm for 2 h at room temperature. After shaking, the tubes were removed, and their contents filtered using Whatman number one filter paper. The ammonium nitrate extractable lead concentrations of the filtrate were determined using ICP-MS. The lead immobilization percentage (IP) was

obtained from:

The pH and electrical conductivity of the incubated soil samples were determined at a soil-water ratio of 1:2.5 using the Hanna pH meter and a Hanna edge electrical conductivity meter, respectively. The elemental contents of the incubated soil samples were also analyzed using an elemental analyzer (Elementar unicube, Germany).

The effect of BP dose on Pb^{2+} bioavailability was evaluated. The lead-spiked soil (control), lead-spiked soil plus 1% of BP (wt./wt.), lead-spiked soil plus 2.5% of BP (wt./wt.) and lead-spiked soil plus 5% BP (wt./wt.) were each incubated for 10 d with their moisture levels maintained at 80% at 25°C . After incubation, the soil was air-dried, homogenized, and extracted with 1 M NH_4NO_3 . Its NH_4NO_3 -extractable lead concentration was determined by ICP MS as described before.

To evaluate the mobility and bioavailability of lead in lead-spiked soil before and after amendment with BC and BP, the total lead concentration was determined following EPA method 3050B (US EPA, 1996). A modified version of Tessier sequential extraction (Howard et al., 2013; He et al., 2013) was employed to determine the different fraction of lead species in soil. The detailed procedure is shown in Table S1. The concentration of Pb^{2+} in the extract was determined using atomic absorption spectrophotometer (model SHIMADZU AA-7000) at a wavelength of 283.3 nm.

2.6. Immobilization mechanism of Pb^{2+} onto BP

To establish the mechanism of adsorption of Pb^{2+} onto BC and BP, batch experiments were employed. The adsorption kinetics were determined by adding 25 mL of 50 mg L⁻¹ Pb^{2+} solution to ~0.1 g of adsorbent placed in a 50 mL polypropylene tube. The supernatant pH was adjusted to 6.5 to mimic soil pH required for optimum nutrient availability. The samples were shaken at 25 °C at 200 rpm for 0.08, 0.25, 0.5, 1, 2, 4, 6, 9, 12, 24 and 48 h. After filtering the supernatant through Whatman number 41 filter paper, Pb^{2+} concentrations in the filtrates were quantified by atomic absorption spectroscopy. The adsorption isotherm was obtained by adding 25 mL of 5, 10, 15, 20, 30, 40, 50 and 70 mg L⁻¹ Pb^{2+} solution to ~0.1 g of adsorbent placed in a 50 mL polypropylene tube. After adjusting the supernatant pH to 6.5, the tubes were shaken at 200 rpm for 24 h at 25 °C. The supernatant was filtered and Pb^{2+} concentrations determined using atomic absorption spectrophotometer. XPS analyses were performed for BC and BP before and after a 30-d incubation with lead-spiked soil.

3. Results and discussions

3.1. Soil physiochemical properties

The preliminary soil test results showing physiochemical properties are shown in (Table S2). The soil was sandy, acidic with low P, K and Mg concentrations.

3.2. Characterization of Douglas fir biochar (BC) and biochar-supported phosphate (BP)

The pH, ash content, BET surface area, pore volume, pore size diameter, and elemental composition of both BC and BP are shown in (Table S3).

Modifying BC to BP with anhydrous calcium chloride and potassium dihydrogen phosphate lowered the BC surface area from 578.9 to 360.5 m² g⁻¹ in BP. However, both pore volume and average pore size diameter increased from 0.07 in BC to 0.12 cm³ g⁻¹ in BP and 4.55 in BC to 13.40 Å in BP, respectively.

The nitrogen adsorption curve (Fig. 1), for Douglas fir biochar was a type I isotherm indicating formation of micropores, whereas that for BP was a type II adsorption signifying the presence of mesopores and macropores. The nitrogen adsorption capacities of BC and BP increased rapidly when the relative pressure was less than 0.3. This rise at low

relative pressure can be due to monolayer adsorption. Between relative pressures of 0.2–0.7, however, the slopes were small due to multilayer adsorption. At a relative pressure greater than 0.8, a large increment in adsorption capacity was observed in BP which could be from the capillary condensation within mesopores and macropores. According to (Yakout et al., 2015), the decrease in surface area following modification with H₂SO₄ is caused by micropore destruction by modifying agents resulting in greater meso- and macropores fractions. However, the uptake of phosphate to generate BP was done at a very low temperature. Therefore, destruction of micropores originally present in the BC would have been unlikely. Thus, the decrease in surface area after modifying BC to BP could have risen from micropore blockage by the deposition of phosphate compounds on biochar surface. Furthermore, the ash content rose from ~3.4 to ~38.1 wt% after modifying BC to BP (Table S3). This finding agrees with (Leng et al., 2021), who observed a reduced surface area in biochar containing significant ash contents due to extensive pore blockage.

The elemental contents of C, H, O, N and S changed after modifying BC to BP. While there was a reduction in the amount of N% (0.12–0.08), C% (79.4–50.86), H% (1.79–1.37) and O% (15.12–9.5). The quantity of S% and P%, respectively, increased from ~0.01 to ~0.5 in BC to ~0.07 and ~5.8 in BP after the modification. Also, BP had a larger molar H/C ratio (0.32) than BC (0.27). The large increase in P% confirms substantial phosphate surface deposition occurred on BP.

Biochar with O/C ratios of <0.2 meet the criteria for highest stability with a half-life of more than 1000 years in soil irrespective of the production temperatures (300–1000 °C) (Spokas, 2010). Therefore, both BC and BP, with approximate molar O/C ratios of about 0.14 will sequester carbon for a long time in soil. Also, molar O/C ratios are directly correlated to the amount of oxygen groups on biochar surfaces. The molar O/C ratio for BC was slightly less than that of BP. Similarly, BP had a larger molar H/C ratio than BC. The slightly larger molar O/C and H/C ratios in BP are attributed to the addition of phosphate species (HPO₄²⁻, H₂PO₄⁻) to BC during modification. The addition of H₂PO₄⁻ or HPO₄²⁻ to BP resulted in an improved Pb^{2+} immobilization, as Pb^{2+} reacts with H₂PO₄⁻ to form insoluble and immobile lead-phosphate compounds such as Pb₁₀(PO₄)₆(OH)₂ at pH < 7.5. The formation of insoluble lead compounds due to reaction of Pb^{2+} with phosphates species in soil at pH < 7 was reported by (Jensen et al., 2005).

BC and BP have low nitrogen concentrations like many wood-derived biochars. Elemental analysis indicated both BC (751.8) and BP (741.6) had high molar carbon to nitrogen (C/N) ratios. The carbon-to-nitrogen ratio is among the vital chemical parameters considered in soil amendments because it helps to predict biologically controlled nitrogen mineralization and release in soils. A molar C/N ratio in soil greater than 25:1 results in biological N immobilization, where inorganic nitrogen forms (NH₄⁺ and NO₃⁻) are transformed into organic forms (H DeLuca, 2016), making it unavailable for plant use. Similarly, amending soil by switchgrass biochar increased N immobilization but reduced the total inorganic nitrogen due to the high switch grass molar C/N ratio (H DeLuca, 2016). Therefore, the use of significant amounts of BC or BP for soil amendment should be accompanied by the application of nitrogen fertilizer for proper plant growth.

The SEM micrographs of both BC and BP are shown in (Fig. S2). The images of both BC and BP showed the presence of micropores, mesopores and macropores which consisted of tube-like structures much of which reflects the original wood morphology. The morphology of BP exhibited more mesopores and macropores than BC. The larger number of mesopores and macropores found in BP is consistent with the surface area decrease going from BC to BP (BET results) in which both pore diameter and pore volume increased. The decreased surface area in BP could have risen from the blockage of micropores by the deposition of phosphate compounds on the biochar surface. Leng et al. (2021) reported that micropores strongly correlate with biochar's surface area.

The SEM-EDX elemental composition analyses of BC and BP are shown in (Fig. S3).

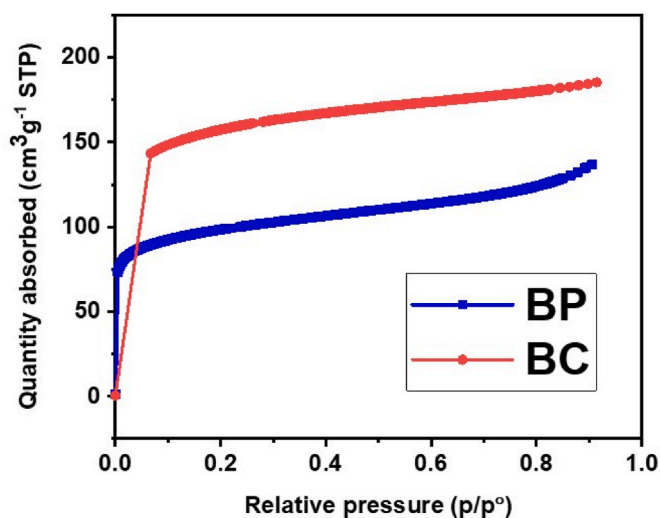


Fig. 1. N₂ adsorption at 77.300 K of Biochar-supported Phosphate (BP) and Douglas Fir Biochar (BC).

Table 1

Selected properties of lead-spiked soil after incubating for 30-d with different amendments verses the control.

Property	Control (Unamended soil)	Soil + BC	Soil + BP	Soil + lime	Soil + KH_2PO_4
pH	5.67 ± 0.15	6.87 ± 0.06	6.20 ± 0.10	7.10 ± 0.10	5.60 ± 0.10
EC (μScm^{-1})	75.93 ± 5.19	176.60 ± 9.52	652.67 ± 14.57	82.73 ± 4.46	2607.67 ± 131.11
C%	0.15 ± 0.02	4.50 ± 0.68	3.10 ± 0.33	0.78 ± 0.09	0.22 ± 0.04
N%	2.17 ± 0.28	0.84 ± 0.27	0.03 ± 0.01	0.36 ± 0.15	1.60 ± 0.23
H%	0.16 ± 0.00	0.24 ± 0.03	0.26 ± 0.03	0.14 ± 0.02	0.21 ± 0.01
S%	0.00 ± 0.00	0.01 ± 0.02	0.07 ± 0.04	0.00 ± 0.00	0.00 ± 0.00
H/C Ratio	12.10 ± 0.86	0.63 ± 0.01	0.99 ± 0.02	2.19 ± 0.05	11.49 ± 2.19
C/N Ratio	0.08 ± 0.02	6.48 ± 1.15	149.80 ± 21.58	3.09 ± 1.88	0.16 ± 0.03

EC = Electrical conductivity, data shown are mean ± SD, n = 3.

SEM-EDX employs a spot diameter of 4–6 μm , penetrating to a depth of $\sim 5.3 \mu\text{m}$. BC and BP surfaces are heterogeneous, so SEM-EDX analyses of many locations are needed to obtain a representative analysis of these surfaces. Analyses of four BC locations gave C (84–87%), O (9.9–12%), P (0.5–1.1%), Ca (0.5–1.6%) and K (0.86–1.75%). After phosphate adsorption, the corresponding analyses of four BP locations exhibited: C (50.8–64.9%), O (20.4–33.0%), P (4.7–5.8), Ca (7.1–8.2%) and K (0.66–1.35%). The molar Ca/P ratio ranged from ~ 1.1 to 1.2. The theoretical molar Ca/P ratio for $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$ and $\text{Ca}_{10}(\text{PO}_4)_6(\text{HO})_2$ are 1.33 and 1.67, respectively (Scapin et al., 2015). These analyses reflect the deposition of phosphate as $(\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O})$, $\text{Ca}_{10}(\text{PO}_4)_6(\text{HO})_2$, CaHPO_4 , $\text{Ca}(\text{H}_2\text{PO}_4)_2$ and related potassium salts on the BP surface. This lowers the contribution by carbon, which was covered partially by deposition and raises the oxygen surface region amount. The bulk analyses in Table 1 are for the entire sample, not just the surface and near surface region which EDX-probed to a depth of $\sim 5.3 \mu\text{m}$. The EDX values confirm successful surface region deposition of phosphorus on BP during the modification of BC with calcium chloride and potassium phosphate.

The XRD patterns for BC and BP before (Fig. 2A) and after a 30-d incubation with lead-spiked soil (Fig. 2B) are shown. The BC XRD spectra revealed two broad peaks comparable to that observed for layered graphene between $2\theta = 20^\circ$ – 30° and 40° – 50° . These peaks are due to graphitic-like diffraction formed as lignocellulositic structures which are partially aromatized during pyrolysis and typical aromatic stacking occurs with d-spacing of ~ 3.4 – $3.5 \text{ \AA}$ (Siburian et al., 2018). The major new peaks exhibited by BP appeared at 2θ approximately 9.4° , 26° and 53° . These result from octacalcium phosphate (OCP, $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$), [ICDD number 261056] (Sakamoto et al., 2016). Further peaks at $2\theta = 28.23^\circ$, 32.05° , 40.28° , 46.66° , 49.41° and 64.18° matched the diffraction pattern of hydroxyapatite (HAP, $\text{Ca}_{10}(\text{PO}_4)_6(\text{HO})_2$) [JCPDS ICDD card Number 09–0432] (Arellano-Jiménez et al., 2009; Sakamoto et al., 2016). These crystalline species form during hydrophosphate

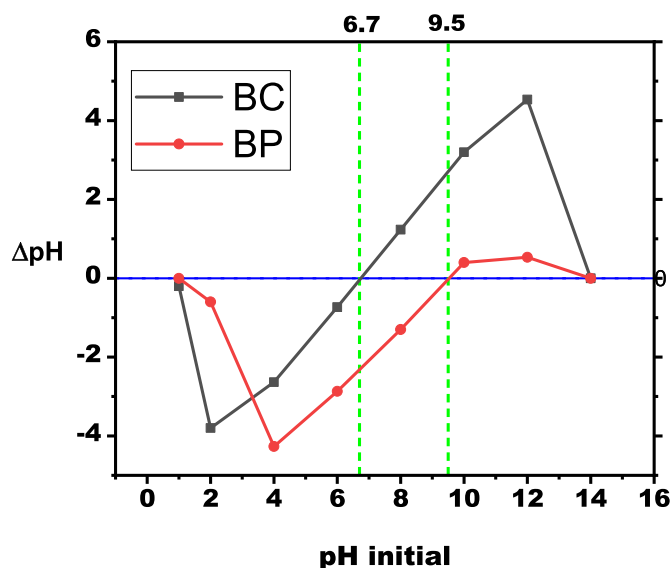


Fig. 3. Point of zero charge (pHpzc) determinations of BP and BC.

deposition in the BP formation. Other amorphous poorly crystallized P-containing species may also be present on BP that are not observed by XRD.

After a 30-d incubation with lead-spiked soil, all XRD peaks matched to lead hydroxyapatite ($\text{Pb}_{10}(\text{PO}_4)_6(\text{OH})_2$, JCPDS card number 01-087-2477) (Zhu et al., 2016). This indicates that both BC and BP immobilize Pb^{2+} in soil through the transformation of HAP ($\text{Ca}_{10}(\text{PO}_4)_6(\text{HO})_2$) into a more stable [hydroxypyromorphite, $\text{Pb}_{10}(\text{PO}_4)_6(\text{OH})_2$].

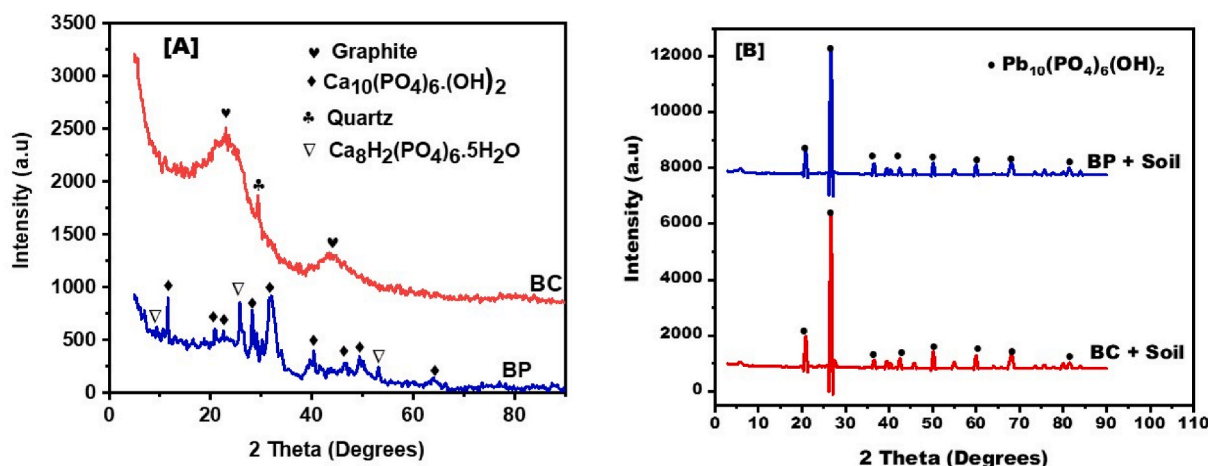
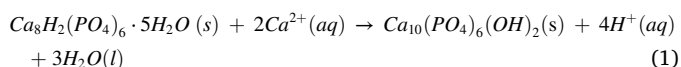


Fig. 2. XRD patterns of BC and BP before (A) and after a 30-d incubation with lead-spiked soil (B).

3.3. pH point of zero charge

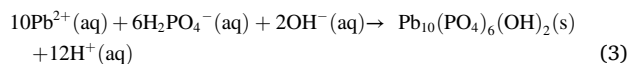
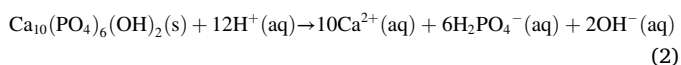
The pH point of zero charge (pHpzc) value corresponds to the point where the net surface charge of the adsorbent is zero (Tran et al., 2015). BC and BP exhibit pH pzc values of 6.7 and 9.5, respectively (Fig. 3). Therefore, anionic species will be attracted, at solution pH values lower than 6.7 to BC surfaces and 9.5 to BP surfaces. Above their pH pzc values, the removal of cationic species will be favored (Iáñez-Rodríguez et al., 2020). Since lead (II) is cationic, its adsorption by BC and BP is favored at pH > 6.7 and 9.5, respectively. However, free ionic Pb^{2+} exists at pH < 7 (Shahid et al., 2012). At pH > 7, hydrolysis to lead hydroxide and hydrous oxides occurs, so Pb^{2+} sorption is typically studied only below pH 6.5 to avoid precipitation. The absorption of Pb^{2+} is suppressed by electrostatic repulsion between the positively charged BP surface (pzc 9.5). Thus, Pb^{2+} immobilization by both BP and BC at pH < 7 occurs by precipitation (PbO and lead hydroxide) and complexation of insoluble octacalcium phosphate, hydroxyapatite and other such species.

The XRD results indicated the phosphorus-containing crystalline species on BP are mostly octacalcium phosphate (OCP $Ca_8H_2(PO_4)_6 \cdot 5H_2O$) and hydroxyapatite (HAP, $Ca_{10}(PO_4)_6 \cdot (OH)_2$). In the presence of water, OCP in soil can be converted to more thermodynamically stable hydroxyapatite (HAP, $Ca_{10}(PO_4)_6 \cdot (OH)_2$) on the release of H^+ as illustrated in equation (1) (Arellano-Jiménez et al., 2009).



(OCP) (Hydroxyapatite).

The use of hydroxyapatite for heavy metal immobilization from contaminated soil has been reported (Ding et al., 2017; Zeng et al., 2020). According to (Zeng et al., 2020), the main Pb^{2+} immobilization mechanisms by hydroxyapatite include surface complexation, precipitation, cation exchange, and adsorption. In acidic soil, hydroxyapatite dissolves (equation (2)), then, Pb^{2+} is adsorbed by the formation of complex lead phosphate precipitates (equation (3)), in which Pb^{2+} reacts with dihydrophosphate. Pb^{2+} also exchanges with Ca^{2+} forming insoluble Pb-containing hydroxyapatites, $Pb_xCa_{10-x}(PO_4)_6(OH)_2$.



3.4. The lead immobilization efficacy

The lead immobilization efficacies of BP, BC, lime and potassium phosphate monobasic are compared to the unamended soil spiked with $Pb(NO_3)_2$ (control) in (Fig. 4a). The control comparison examined NH_4NO_3 extractable lead concentrations from lead-spiked soil shown in (Fig. 4b). This control is compared with lead-spiked soil plus 5% BP (wt./wt.), lead-spiked soil plus 5% BC (wt./wt.), lead-spiked soil plus 5% lime (wt./wt.) and lead-spiked soil plus 5% potassium dihydrogen phosphate (wt./wt.) in (Fig. 4b). All are shown after a 30-d incubation period in (Fig. 4a and b), respectively.

The lowest amount of NH_4NO_3 extractable Pb^{2+} ($0.3 \pm 0.2 \text{ mg L}^{-1}$) was found in the lead-spiked soil amended with 5% BP. The largest amount of extractable Pb^{2+} came from the control ($96.08 \pm 9.22 \text{ mg L}^{-1}$) experiments. Clearly, the BP soil amendment immobilized successfully the Pb^{2+} . The amounts of NH_4NO_3 extractable Pb^{2+} obtained from the lead-spiked soil amended with 5% BC, lime and KH_2PO_4 were 1.7 ± 0.38 , 1.43 ± 0.90 and $1.70 \pm 0.27 \text{ mg L}^{-1}$, respectively. BC, lime and KH_2PO_4 worked well to immobilize Pb^{2+} but not as well as BP. The Pearson correlation coefficient between residual soil pH (pH after incubation) and the NH_4NO_3 extractable Pb^{2+} was negative ($r = -0.5$), indicating that pH played a role in the immobilization of Pb^{2+} in the soil. When soil pH is increased, the negative charge density on soil surfaces and the $[^-OH]$ in the H_2O increases. This in turn, can facilitate immobilization of positively charged Pb^{2+} by precipitation as $Pb(OH)_2$. This enhances adsorption on soil charged sites and or formation of $PbOH^+$ species which is favorably adsorbed compared to Pb^{2+} . Several other studies have reported reduced solubility and mobility of Pb^{2+} with rise in pH (Gholami et al., 2020; Palansooriya et al., 2020).

The incubation of the lead-spiked soil with 5% BC, BP and lime increased soil pH from 5.67 to 6.87, 6.2, and 7.1, respectively (Table 1). The pH was slightly reduced (by ~ 0.07) in soil treated with KH_2PO_4 . The electrical conductivity rose from 75.9 to 82.7, 176.6, 652.7 and $2607.7 \mu\text{Scm}^{-1}$ in lead-spiked soil treated with lime, BC, BP and KH_2PO_4 , respectively. The C/N ratio rose from 0.08 to 0.16, 3.1, 6.45, and 149.8 in lead-spiked soil treated with KH_2PO_4 , lime, BC and BP, respectively. However, H/C ratio was reduced from 12.1 to 11.5, 2.2, 1.0 and 0.6 following amendment with KH_2PO_4 , lime, BP and BC, respectively.

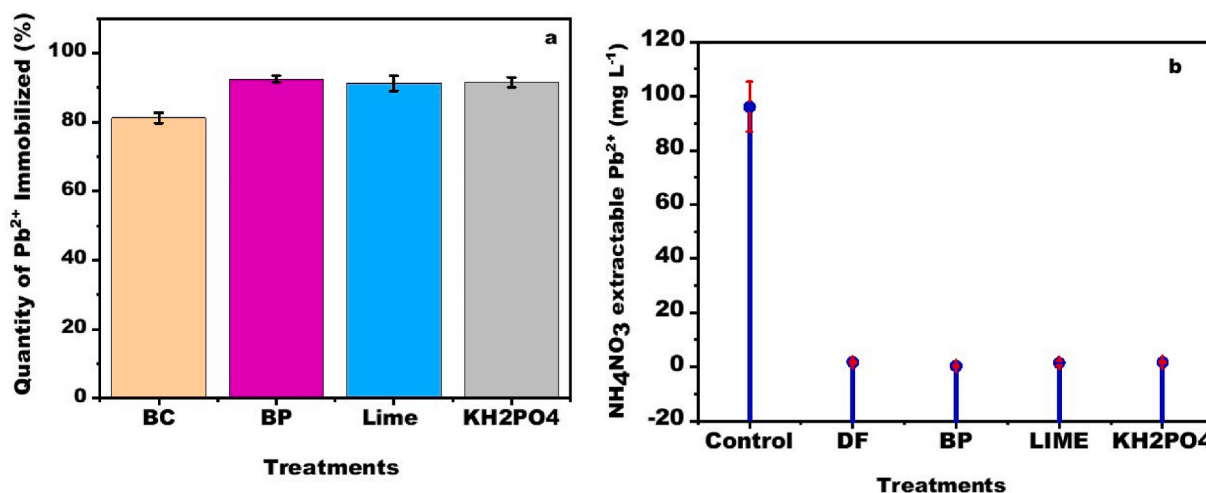


Fig. 4. The Pb^{2+} immobilization efficacy of BP, BC, lime, and potassium dihydrogen phosphate compared to the control (a) and the NH_4NO_3 extractable Pb^{2+} concentration of control (unamended lead -spiked soil), lead-spiked soil plus 5% BC, lead-spiked soil plus 5% BP, lead-spiked soil plus 5% lime and lead-spiked soil plus 5% KH_2PO_4 (b) after a 30 d incubation period at room temperature. (Values are average of $n = 3$, error bars shown as SD).

Information on soil pH, electrical conductivity, molar C/N and H/C ratios are vital parameters for predicting soil productivity because they control nutrient availability. While a reduction in the molar H/C ratio occurred in all treatments, a general increment in the soil pH, electrical conductivity, and the molar C/N ratio was observed. Improvements in both soil electrical conductivity and pH following the application of biochar and lime have been previously reported (Kizito et al., 2019). The rise in the electrical conductivity is attributed to increased salt contents (ash content) following the amendments. The increased soil pH can be ascribed to the presence of oxides, carbonates, and hydroxides of Ca^{2+} , Mg^{2+} , K^+ and Na^+ in the additives used (lime, BC and BP). The increment in soil pH after incubation with BP was much smaller than that induced by unmodified BC. The slight increase in the soil pH treated with BP is likely due to octacalcium phosphate $[(\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O})]$ hydrolysis, which releases H^+ .

Soil microbes' accessibility to C and available N correlates with molar H/C and C/N ratios, respectively. Soil additives with molar C/N ratios values lower than 25:1 indicate their ability to provide sufficient nitrogen needed for microbes to use applied carbon (USDA-NRCS, 2011). The lower molar H/C ratios associated with BC and BP amendments are related to their recalcitrant nature, stability, and ability to mitigate climate change via carbon sequestration. Also, larger molar C/N ratios observed in BP treatment reveal low nitrogen availability, implying nitrogen immobilization (conversion of inorganic nitrogen to organic nitrogen) is more favorable. Increased molar C/N ratios in soil after biochar amendment was previously reported with pine wood biochar because of its low N contents (Ameloot et al., 2015). In the same study however, a reduction in the soil molar C/N ratios was observed after poultry litter biochar amendment based on its greater N contents. So, the effect of biochar application on soil molar C/N ratio depends on the feedstock used. Thus, the use of biochar produced from low N feedstocks, such as BC for soil amendment, should be complemented by nitrogen fertilizer application.

3.5. The effect of BP dose on lead (II) immobilization efficiency

The lead immobilization efficiencies for the control compared to lead-spiked soils amended with different BP doses is shown in (Fig. S4 [A]). The control gave the highest NH_4NO_3 -extractable Pb^{2+} concentrations (98.9 mg L^{-1}). The lead-spiked soils amended with 1%, 2.5%, and 5% of BP had NH_4NO_3 -extractable Pb^{2+} concentrations values of 12.7 mg L^{-1} , 8.4 mg L^{-1} , and 8.1 mg L^{-1} , respectively. The lead (II) immobilization efficacy improved with increasing BP dose. However, the NH_4NO_3 extractable lead (II) concentrations in lead-spiked soil amended with 2.5% (8.4 mg L^{-1}) and 5% (8.1 mg L^{-1}) of BP did not differ significantly. At a BP application rate of only 1% (wt./wt.) to the lead spiked soil, BP reduced Pb^{2+} bioavailable by $\sim 87\%$ versus the

control within 10 d of treatment. Both 2.5% and 5% (wt./wt.) treatment rates lowered Pb^{2+} by $\sim 92\%$ versus the control, suggesting BP's potential to lessen Pb^{2+} accumulation in humans through food chain.

The mobility and bioavailability of lead in lead-spiked soil before and after amendment with BC and BP was examined. Before amendment, Pb was largely in the residual forms (50.2%) and Fe–Mn oxides bound (28.3%). After amendment, the distribution of different lead species changed (Fig. S4 [B]). The organic fraction increased from 6.8% (control) to 28.1% and 9.0% in soil amended by BC and BP, respectively. Similarly, Fe–Mn oxides forms of bound Pb increased from 28.3% (control) to 43% in soil amended by BC. The residual fraction rose by 20.9% in soil amended by BP. In contrast, the exchangeable fraction decreased from 5.4% (control) to 2.7% and 2.5% in soil amended by BC and BP, respectively. Similarly, the carbon-occluded form decreased from 4.5% (control) to 2.0% and 2.5% in soil amended by BC and BP, respectively. This result concurs with earlier study in which, an increase in residual fraction and a decline in exchangeable fractions of Pb^{2+} in soil was reported after amendment with rice hull biochar (Li et al., 2016). Since the residual fraction is associated with the crystalline structure of the mineral and usually not easily extracted, the rise in the residual fraction in soil amended by BP further signifies its potential to immobilize and lower both Pb^{2+} bioavailability and environmental risk.

3.6. Immobilization mechanism of Pb^{2+} onto BP

Pseudo first order and pseudo second order adsorption kinetic models were used to predict the mechanism of Pb^{2+} adsorption onto BC and BP. Kinetic results obtained are shown on Fig. 5. The rate constants, coefficient of determination and adsorption capacities for the adsorbents are shown on Table S4. The adsorption of Pb^{2+} onto both BC and BP were best described by the pseudo second order kinetic model implying that Pb^{2+} adsorption on both BC and BP occurred via chemisorption (Sari et al., 2017).

The adsorption isotherms were fitted by Freundlich and Langmuir models (Fig. 6). Both BC and BP were effective in removing Pb^{2+} . The adsorption capacities of 122.03 mg g^{-1} and 102.75 mg g^{-1} were obtained with BC and BP respectively. The corresponding Langmuir K_L coefficients were 0.065 and 0.12 M mg^{-1} . The sorption of Pb^{2+} ion on BC was best fitted by Langmuir model signifying that sorption on BC surface was a monolayer. However, the adsorption onto BP was best fitted by the Freundlich model (Table S5) indicating the adsorption process was multilayered. The Pb^{2+} adsorption capacities of both BC and BP with other absorbent reported in the literature appear in Table S6 (supporting information). XRD analysis of lead-spiked soil amended with BC or BP confirms that the mechanism of immobilization of Pb^{2+} by BP was due to the dissolution of HAP, followed by the precipitation of lead hydroxyapatite. The XRD diffraction patterns changed from those

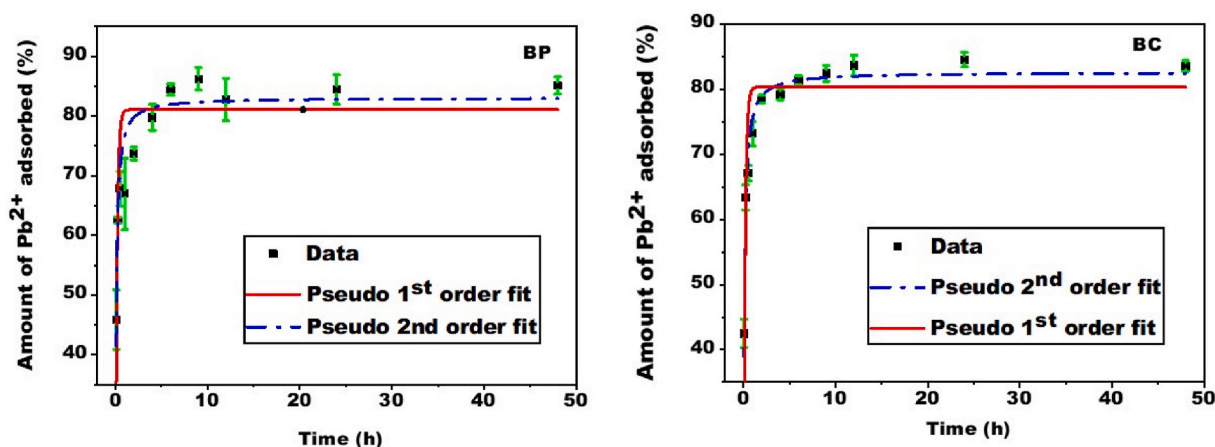


Fig. 5. Adsorption kinetic for Pb^{2+} onto BC and BP at 25°C . Values used are mean of $n = 3$, error bars shown as standard deviation.

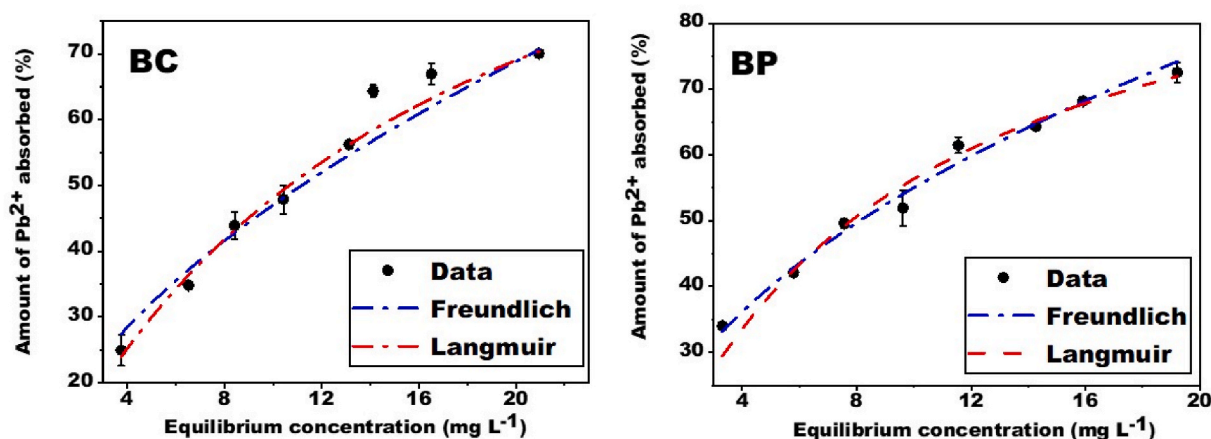


Fig. 6. Adsorption isotherm for Pb^{2+} onto BC and BP at 25 °C. Data presented are average of $n = 3$, error bars are shown as standard deviation.

of OCP and HAP in BP to a phase of $\text{Pb}_{10}(\text{PO}_4)_6(\text{OH})_2$ in soil amended with BP. Therefore, the immobilization of lead ion by BP is mostly resulting from the dissolution of HAP followed by precipitation of lead hydroxyapatite (Pb-HAP). Fig S5 illustrates the mechanism of Pb^{2+} immobilization by BP. Some studies have proposed that lead removal by HAP can occur through an ion exchange process which takes place at the HAP surface (Omar et al., 2019). This mechanism is unlikely for lead immobilization by BP because it occurs at $\text{pH} < 7$. In addition, the increase in oxidizable (organic) lead fraction after amendment with biochar suggest sorption of Pb^{2+} onto biochar surfaces through formation of complexes with the surface functional groups.

XPS spectra were used to further understand the chemistry. The XPS spectra for BC, BP, lead-spiked spoil, lead-spiked soil amended with 5% BC and lead-spiked soil treated with 5% BP are shown in (Fig. S6). Before treatment, the binding energies of O1s, P2p and Ca2p corresponding to BP were 531.6, 134.0 and 348.3 eV respectively, all matching binding energy for HAP (López et al., 2022). The Pb4f peak observed in the lead-spiked soil at binding energy 140.15 eV is consistent with the values for $\text{Pb}(\text{NO}_3)_2$. After treatment with BC and BP, the appearance of Pb 4f peaks shifted to binding energies of 139.74 eV and 139.19 eV, respectively. This confirmed the formation of Pb-OH and Pb- PO_4^{3-} bonds (Lu et al., 2020).

4. Conclusions

The efficacy of using biochar-supported phosphate (BP) over the traditional in-situ heavy metal immobilization methods (lime, potassium dihydrogen phosphate and biochar) was investigated. At an application rate of just 1% wt./wt., BP lowered the Pb^{2+} apparent bioavailability by 87% compared to the control within 10 d. The distribution of different lead species indicated a decline in the exchangeable lead forms from 5.4 to 2.5%, whereas the residual (immobilized) form increased by 20.9% following amendment. The acid-promoted formation of insoluble lead-containing hydroxyapatite was the main mechanism driving Pb^{2+} immobilization by BP. This result confirms that biochar-supported phosphate can effectively reduce Pb^{2+} mobility in contaminated soil, thus minimizing the possibility of Pb^{2+} uptake by plant and the likely transfer to humans through food chain. Besides carbon sequestration due to biochar's recalcitrant nature in soil, binding phosphate to biochar slows phosphate release. This minimizes water contamination and the related economic consequences. Additional research is recommended to establish the long-term effect of BP amendments on soil physical, chemical and biological characteristics. Also, future application of BP for immobilization of Cd, Hg and As should be examined.

Authors contributions

Arwenyo Beatrice: Designed the study, data collection, analyzed the data, wrote the manuscript. Jac J. Varco: Editing and suggested experiment. Andrew Dygert: performed the experiments. Felix S Atsar: contributed to sample preparation. Solomon Sabrina: contributed to sample preparation. Rooban Venkatesh K.G. Thirumalai: Data Formal analysis. Charles U. Pittman Jr.: Editing and worked out almost all the technical details. Todd Mlsna: Editing and technical guidance.

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.

Acknowledgements

Mississippi State University, Department of Chemistry. Fulbright Organization and US Department of State for Scholarship awarded to Beatrice Arwenyo.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chemosphere.2021.133355>.

References

- Amari, T., Ghnaya, T., Abdely, C., 2017. Nickel, cadmium and lead phytotoxicity and potential of halophytic plants in heavy metal extraction. *South Afr. J. Bot.* 111, 99–110. <https://doi.org/10.1016/j.sajb.2017.03.011>.
- Ameloot, N., Sleutel, S., Das, K.C., Kanagaratnam, J., de Neve, S., 2015. Biochar amendment to soils with contrasting organic matter level: effects on N mineralization and biological soil properties. *GCB Bioenergy* 7 (1), 135–144. <https://doi.org/10.1111/gcbb.12119>.
- Arellano-Jiménez, M.J., García-García, R., Reyes-Gasga, J., 2009. Synthesis and hydrolysis of octacalcium phosphate and its characterization by electron microscopy and X-ray diffraction. *J. Phys. Chem. Solid.* 70 (2), 390–395. <https://doi.org/10.1016/j.jpcs.2008.11.001>.
- Basta, N.T., McGowan, S.L., 2004. Evaluation of chemical immobilization treatments for reducing heavy metal transport in a smelter-contaminated soil. *Environ. Pollut.* 127, 73–82. [https://doi.org/10.1016/S0269-7491\(03\)00250-1](https://doi.org/10.1016/S0269-7491(03)00250-1).
- Bombuwala Dewage, N., Liyanage, A.S., Pittman, C.U., Mohan, D., Mlsna, T., 2018. Fast nitrate and fluoride adsorption and magnetic separation from water on A-Fe₂O₃ and Fe₃O₄ dispersed on Douglas fir biochar. *Bioresour. Technol.* 263, 258–265. <https://doi.org/10.1016/j.biortech.2018.05.001>.
- Burlakovs, J., Vircaivs, M., Obszar, T.R., Ci, Z.M., Na, K., Zastosowania, L., Bada, P.N.E. W., 2012. Heavy metal remediation technologies in Latvia: possible applications and preliminary case study results. *Ecol. Chem. Eng. S* 19 (4), 533–547. <https://doi.org/10.2478/v10216-011-0038-3>.

- Cao, Qing, Hu, Qin Hong, Khan, Sardan, Wang, Zi Jian, Lin, Ai Jun, Du, Xin, Zhu, Yong Guan, 2007. Wheat phytotoxicity from arsenic and cadmium separately and together in solution culture and in a calcareous soil. *J. Hazard Mater.* 148 (1–2), 377–382. <https://doi.org/10.1016/j.jhazmat.2007.02.050>.
- Chen, H., Yuan, X., Xiong, T., Jiang, L., Wang, H., Wu, Z., 2020. Biochar facilitated hydroxyapatite/calcium silicate hydrate for remediation of heavy metals contaminated soils. *Water Air Soil Pollut.* 231 (2) <https://doi.org/10.1007/s11270-020-4425-1>.
- Cox, M.S., 2001. The Lancaster Soil Test Method as an Alternative to the Mehlich-3 Soil Test Method, vol. 166, pp. 484–489.
- Ding, Ling, Li, Jianbing, Liu, Wei, Zuo, Qingqing, Liang, Shu-xuan, 2017. Influence of Nano-Hydroxyapatite on the Metal Bioavailability, Plant Metal Accumulation and Root Exudates of Ryegrass for Phytoremediation in Lead-Polluted Soil. *Int. J. Environ. Res. Publ. Health* 14, 532. <https://doi.org/10.3390/ijerph14050532>.
- Domingues, R.R., Trugilho, P.F., Silva, C.A., De Melo, I.C.N.A., Melo, L.C.A., Magriotis, Z. M., Sánchez-Moneder, M.A., 2017. Properties of biochar derived from wood and high-nutrient biomass with the aim of agronomic and environmental benefits. *PLoS One* 12 (5), 1–19. <https://doi.org/10.1371/journal.pone.0176884>.
- Gholami, L., Rahimi, G., Khademi Jolgeh Nezhad, A., 2020. Effect of thiourea-modified biochar on adsorption and fractionation of cadmium and lead in contaminated acidic soil. *Int. J. Phytoremediation* 22 (5), 468–481. <https://doi.org/10.1080/15226514.2019.1678108>.
- H DeLuca, T., 2016. Influence of biochar on soil nutrient transformations, nutrient leaching, and crop yield. *Adv. Plants Agri. Res.* 4 (5), 348–362. <https://doi.org/10.15406/apar.2016.04.00150>.
- Hamid, Y., Tang, L., Wang, X., Hussain, B., Yaseen, M., Aziz, M.Z., Yang, X., 2018. Immobilization of cadmium and lead in contaminated paddy field using inorganic and organic additives. *Sci. Rep.* 8 (1), 1–10. <https://doi.org/10.1038/s41598-018-35881-8>.
- He, M., Shi, H., Zhao, X., Yu, Y., Qu, B., 2013. Immobilization of Pb and Cd in contaminated soil using nano-crystallite hydroxyapatite. *Proc. Environ. Sci.* 18, 657–665. <https://doi.org/10.1016/j.proenv.2013.04.090>.
- Houben, D., Evrard, L., Sonnet, P., 2013. Mobility, bioavailability and pH-dependent leaching of cadmium, zinc and lead in a contaminated soil amended with biochar. *Chemosphere* 92 (11), 1450–1457. <https://doi.org/10.1016/j.chemosphere.2013.03.055>.
- Howard, J.L., Dubay, B.R., McElmurry, S.P., Clemence, J., Daniels, W.L., 2013. Comparison of sequential extraction and bioaccessibility analyses of lead using urban soils and reference materials. *Water Air Soil Pollut.* 224 (10), 1678. <https://doi.org/10.1007/s11270-013-1678-y>.
- Hue, N., 2018. Biochar for Maintaining Soil Health. University of Hawaii. [https://www.ctahr.hawaii.edu/huen/Biochar-Soil-Health-NV-Hue\(pers\)](https://www.ctahr.hawaii.edu/huen/Biochar-Soil-Health-NV-Hue(pers)).
- Iáñez-Rodríguez, I., Calero, M., Blázquez, G., Martín-Lara, M.A., 2020. Greenhouse crop residue and its derived biochar : potential as adsorbent of cobalt from. *MDPI Water* 12 (1282). <https://doi.org/10.3390/w12051282>.
- Jensen, P.E., Ottosen, L.M., Pedersen, A.J., 2005. Speciation of Pb in industrially polluted soils. *Water Air Soil Pollut.* 170 (1–4), 359–382. <https://doi.org/10.1007/s11270-005-9008-7>.
- Jiang, S., Liu, Y., Shu, Y., 2021. Biochar and exogenous calcium assisted alleviation of Pb phytotoxicity in water spinach (*Ipomoea aquatica* Forsk) cultivated in Pb-spiked soil. *Environmental Geochemistry and Health*. <https://doi.org/10.1007/s10653-021-00977-0>.
- Karnib, M., Kabbani, A., Holail, H., Olama, Z., 2014. Heavy metals removal using activated carbon, silica and silica activated carbon composite. *Energy Proc.* 50, 113–120. <https://doi.org/10.1016/j.egypro.2014.06.014>.
- Kede, M.L.F.M., Pérez, D.V., Moreira, J.C., Marques, M., 2017. Effect of phosphates on the bioavailability and phytotoxicity of Pb and Cd in contaminated soil and phytoextraction by vetiver grass. *J. Environ. Eng.* 143 (3), 1–6. [https://doi.org/10.1061/\(ASCE\)EE.1943-7870.0001170](https://doi.org/10.1061/(ASCE)EE.1943-7870.0001170).
- Khalid, S., Shahid, M., Niazi, N.K., Murtaza, B., Bibi, I., Dumat, C., 2017. A comparison of technologies for remediation of heavy metal contaminated soils. *J. Geochem. Explor.* 182, 247–268. <https://doi.org/10.1016/j.jgeexplo.2016.11.021>.
- Kizito, S., Luo, H., Lu, J., Bah, H., Dong, R., Wu, S., 2019. Role of nutrient-enriched biochar as a soil amendment during maize growth: exploring practical alternatives to recycle agricultural residuals and to reduce chemical fertilizer demand. *Sustainability* 11 (11), 3211–3233. <https://doi.org/10.3390/su11113211>.
- Kong, L., Ruan, Y., Zheng, Q., Su, M., Diao, Z., Chen, D., Hou, L., Chang, X., Shih, K., June 2019. Uranium extraction using hydroxyapatite recovered from phosphorus containing wastewater. *J. Hazard Mater.* 382, 120784. <https://doi.org/10.1016/j.jhazmat.2019.120784>, 2020.
- Leng, L., Xiong, Q., Yang, L., Li, H., Zhou, Y., Zhang, W., Jiang, S., Li, H., Huang, H., 2021. An overview on engineering the surface area and porosity of biochar. *Sci. Total Environ.* 763, 144204. <https://doi.org/10.1016/j.scitotenv.2020.144204>.
- Li, H., Liu, Y., Chen, Y., Wang, S., Wang, M., Xie, T., Wang, G., 2016. Biochar amendment immobilizes lead in rice paddy soils and reduces its phytoavailability. *Sci. Rep.* 6, 1–8. <https://doi.org/10.1038/srep31616>.
- López, E.O., Bernardo, P.L., Checca, N.R., Rossi, A.L., Mello, A., Ellis, D.E., Rossi, A.M., Terra, J., 2022. Hydroxyapatite and lead-substituted hydroxyapatite near-surface structures: novel modelling of photoemission lines from X-ray photoelectron spectra. *Appl. Surf. Sci.* 571 (September 2021) <https://doi.org/10.1016/j.apsusc.2021.151310>.
- Lu, X., Zhong, R., Liu, Y., Li, Z., Yang, J., Wang, F., 2020. The incorporation of Pb2+ during struvite precipitation: quantitative, morphological and structural analysis. *J. Environ. Manag.* 276 (August), 111359. <https://doi.org/10.1016/j.jenvman.2020.111359>.
- Lwin, C.S., Seo, B., Kim, H., Owens, G., 2018. Nutrition Application of soil amendments to contaminated soils for heavy metal immobilization and improved soil quality — a critical review. *Soil Sci. Plant Nutr.* 64 (2), 156–167. <https://doi.org/10.1080/00380768.2018.1440938>.
- Nelke, K., Mulak, M., Łuczak, K., Pawlak, W., Nienartowicz, J., Szumny, D., Kochman, M., Gerber, H., 2015. Occurrence and exposure to lead and cadmium and their environmental influence on eyesight. *Pol. J. Environ. Stud.* 24 (4), 1491–1496. <https://doi.org/10.15244/pjoes/39551>.
- Omar, S., Muhamad, M.S., Te Chuan, L., Hadibarata, T., Teh, Z.C., 2019. A review on lead sources, occurrences, health effects, and treatment using hydroxyapatite (HAP) adsorbent made from fish waste. *Water Air Soil Pollut.* 230 (12) <https://doi.org/10.1007/s11270-019-4312-9>.
- Palansooriya, Kumuduni Niroshika, Shaheen, Sabry M, Chen, Season S., Tsang, Daniel C. W., Hashimoto, Yohey, Hou, Deyi, Bolan, Nanthi S, Rinklebe, Jörg, Ok, Yong Sik, 2020. Soil amendments for immobilization of potentially toxic elements in contaminated soils: A critical review. *Environ. Int.* 134, 105046. <https://doi.org/10.1016/j.envint.2019.105046>.
- Park, J.H., Choppala, G., Lee, S.J., 2013. Comparative sorption of Pb and Cd by biochars and its implication for metal immobilization in soils. *Water Air Soil Pollut.* 224, 1711. <https://doi.org/10.1007/s11270-013-1711-1>.
- Pinho, S., Ladeiro, B., 2012. Phytotoxicity by lead as heavy metal focus on oxidative stress. *J. Botany* 1–10. <https://doi.org/10.1155/2012/369572>, 2012.
- Preet, G., Sidhu, S., 2016. Heavy metal toxicity in soils : sources , remediation technologies and challenges. *Adv. Plants Agri. Res.* 5 (1), 445–446. <https://doi.org/10.15406/apar.2016.05.00166>.
- Reid, G., Dirou, J., 2004. How to Interpret Your Soil Test. <https://web.archive.org/web/20150421044903/www.dpi.nsw.gov.au/agriculture/resources/soils/testing/inte rpnet>.
- Sakamoto, T., Inoue, M., Nishimura, T., Yokoi, T., Takashi, S., 2016. Rapid and Topotactic Transformation from Octacalcium Phosphate to Hydroxyapatite (HAP): a New Approach to Self-Organization of Free-Standing Thin-Film HAP-Based Nanohybrids, vol. 18. The Royal Society of Chemistry CrystEngComm, pp. 8388–8395. <https://doi.org/10.1039/c6ce01336h>.
- Sari, A.A., Amriani, F., Muryanto, M., Triwulandari, E., Sudiyani, Y., Barlianti, V., Narrij Lotulung, P.D., Hadibarata, T., 2017. Mechanism, adsorption kinetics and applications of carbonaceous adsorbents derived from black liquor sludge. *J. Taiwan Inst. Chem. Eng.* 77, 236–243. <https://doi.org/10.1016/j.jtice.2017.05.008>.
- Scapin, M.A., Guilhen, S.N., B, M.E., Pires, C.A.F., Pires, M.A.F., 2015. Determination of ca/p molar ratio in hydroxyapatite (ha) by x-ray fluorescence technique. *International Nuclear Atlantic Conference - INAC 2015*. https://inis.iaea.org/collecti on/NCLCollectionStore/_Public/47/013/47013714.pdf, 2015.
- Schwarz, K., Pouyat, R.V., Yesilonis, I., 2016. Legacies of lead in charm city's soil: lessons from the baltimore ecosystem study. *Int. J. Environ. Res. Publ. Health* 13 (2). <https://doi.org/10.3390/ijerph13020209>.
- Shahid, M., Dumat, C., Aslama, M., Pinelli, E., 2012. Assessment of lead speciation by organic ligands using speciation models. *Chem. Speciat. Bioavailab.* 24 (4), 248–252. <https://doi.org/10.3184/095422912X13495331697627>.
- Shareef, Tawheed Mohammed Elhessin, Zhao, Baowei, 2017. The Fundamentals of Biochar as a Soil Amendment Tool and Management in Agriculture Scope: An Overview for Farmers and Gardeners. *J. Agric. Chem. Environ.* 06 (01), 38–61. <https://doi.org/10.4236/jacen.2017.61003>.
- Sharma, S., Tiwari, S., Hasan, A., Saxena, V., Pandey, L.M., 2018. Recent advances in conventional and contemporary methods for remediation of heavy metal - contaminated soils. *3 Biotech* 8 (4), 1–18. <https://doi.org/10.1007/s13205-018-1237-8>.
- Sibirian, R., Sihotang, H., Lumban Raja, S., Supeno, M., Simanjuntak, C., 2018. New route to synthesis of graphene nano sheets. *Orient. J. Chem.* 34 (1), 182–187. <https://doi.org/10.13005/ojc/340120>.
- Spokas, K.A., 2010. Review of the stability of biochar in soils: predictability of O:C molar ratios. *Carbon Manag.* 1 (2), 289–303. <https://doi.org/10.4155/cmt.10.32>.
- Tchounwou, P.B., Yedjou, C.G., Patlolla, A.K., Sutton, D.J., 2012. Heavy metals toxicity and the environment. *Mol. Clin. Environ. Toxicol.* 101, 133–164. <https://doi.org/10.1007/978-3-7643-8340-4>.
- Thompson, M. John, Varco, J.J., 2002. *Soil Fertility and Plant Nutrition Lab Manual*. Mississippi State University.
- Tran, H.N., You, S., Chao, H., May 2016. Effect of Pyrolysis Temperatures and Times on the Adsorption of Cadmium onto Orange Peel Derived Biochar. <https://doi.org/10.1177/0734242X15615698>, 2015.
- Ullah, A., Ma, Y., Li, J., Tahir, N., Hussain, B., 2020. Effective amendments on cadmium , arsenic , chromium and lead contaminated paddy soil for rice safety. *Agronomy* 10 (359), 1–19. <https://doi.org/10.3390/agronomy10030359>.
- UNEP, 2020. Lead and Cadmium | UN Environment. United Nations Environment Programme. <https://www.unenvironment.org/explore-topics/chemicals-waste/wh at-we-do/emerging-issues/lead-and-cadmium>.
- US EPA, 1996. Acid digestion of sediments, sludges, and soils. *J. Japan Soc. bronchol.* 18 (7), 723. <https://doi.org/10.18907/jjsr.18.7.723.2>.
- USDA-NRCS, 2011. Conservation. In: USDA Natural Resources Conservation Service. <http://www.nrcs.usda.gov>.
- Wade, K.M., 2019. Lead in Plants and Soil. <https://plantprobs.net/plant/nutrientl mbalances/lead.html>.
- Wang, H., Yang, X., He, L., Lu, K., 2018. Using Biochar for Remediation of Contaminated Soils. Science Press & Springer Nature Singapore Pte Ltd., pp. 763–783. <https://doi.org/10.1007/978-981-10-6029-8>.
- Wang, S., Xu, Y., Norbu, N., Wang, Z., 2018. Remediation of biochar on heavy metal polluted soils. *IOP Conf. Ser. Earth Environ. Sci.* 108 (2018), 042113.

- Wang, Y., Gu, K., Wang, H., Shi, B., 2019. Remediation of heavy-metal-contaminated soils by biochar : a review. *Environ. Geotech.* 1–14 <https://doi.org/doi.org/10.1680/jenge.18.00091>.
- Yakout, S.M., El, A., Daifullah, H.M., El-reefy, S.A., 2015. Pore structure characterization of chemically modified biochar derived from rice straw. *Environ. Eng. Manag. J.* 14 (2), 473–480. <http://omicron.ch.tuiasi.ro/EEMJ/>.
- Yao, Y., Gao, B., Chen, J., Yang, L., 2013. Engineered biochar reclaiming phosphate from aqueous solutions: mechanisms and potential application as a slow-release fertilizer. *Environ. Sci. Technol.* 47, 8700–8708 <https://doi.org/dx.doi.org/10.1021/es4012977>.
- Ye, S., Cheng, M., Zeng, G., Tan, X., Wu, H., Liang, J., Shen, M., Song, B., Liu, J., Yang, H., Zhang, Y., 2020. Insights into catalytic removal and separation of attached metals from natural-aged microplastics by magnetic biochar activating oxidation process. *Water Res.* 179, 115876. <https://doi.org/10.1016/j.watres.2020.115876>.
- Ye, S., Zeng, G., Wu, H., Liang, J., Zhang, C., Dai, J., Xiong, W., Song, B., Wu, S., Yu, J., 2019. The effects of activated biochar addition on remediation efficiency of co-composting with contaminated wetland soil. *Resour. Conserv. Recycl.* 140 (October 2018), 278–285. <https://doi.org/10.1016/j.resconrec.2018.10.004>.
- Zeng, X., Xu, H., Lu, J., Chen, Q., Li, W., Wu, L., Tang, J., Ma, L., 2020. The immobilization of soil cadmium by the combined amendment of bacteria and hydroxyapatite. *Sci. Rep.* 1–8. <https://doi.org/10.1038/s41598-020-58259-1>.
- Zhu, Y., Huang, B., Zhu, Z., Liu, H., Huang, Y., Zhao, X., Liang, M., 2016. Characterization, dissolution and solubility of the hydroxypyromorphite-hydroxyapatite solid solution [(PbxCa1-x)5(PO4)3OH] at 25 °C and pH 2-9. *Geochem. Trans.* 17 (1), 1–18. <https://doi.org/10.1186/s12932-016-0034-8>.
- Zupančič, M., Lavrič, S., Bukovec, P., 2012. Metal immobilization and phosphorus leaching after stabilization of pyrite ash contaminated soil by phosphate amendments. *J. Environ. Monit.* 14 (2), 704–710. <https://doi.org/10.1039/c2em10798h>.