

University of Texas Rio Grande Valley

ScholarWorks @ UTRGV

Earth, Environmental, and Marine Sciences
Faculty Publications and Presentations

College of Sciences

10-3-2017

Nitrate Leaching from Sand and Pumice Geomedia Amended with Pyrogenic Carbon Materials

Jihoon Kang

The University of Texas Rio Grande Valley

Marissa Davila

Harlingen Waterworks System

Sergio Mireles

The University of Texas Rio Grande Valley

Jungseok Ho

The University of Texas Rio Grande Valley, jungseok.ho@utrgv.edu

Follow this and additional works at: https://scholarworks.utrgv.edu/eems_fac



Part of the [Earth Sciences Commons](#), [Environmental Sciences Commons](#), and the [Marine Biology Commons](#)

Recommended Citation

Kang, J.; Davila, M.; Mireles, S.; Ho, J. Nitrate Leaching from Sand and Pumice Geomedia Amended with Pyrogenic Carbon Materials. *Environments* 2017, 4, 70. <https://doi.org/10.3390/environments4040070>

This Article is brought to you for free and open access by the College of Sciences at ScholarWorks @ UTRGV. It has been accepted for inclusion in Earth, Environmental, and Marine Sciences Faculty Publications and Presentations by an authorized administrator of ScholarWorks @ UTRGV. For more information, please contact justin.white@utrgv.edu, william.flores01@utrgv.edu.

Article

Nitrate Leaching from Sand and Pumice Geomedia Amended with Pyrogenic Carbon Materials

Jihoon Kang ^{1,*} , Marissa Davila ², Sergio Mireles ¹ and Jungseok Ho ³

¹ School of Earth, Environmental and Marine Sciences, University of Texas Rio Grande Valley, Edinburg, TX 78539, USA; sergio.mireles01@utrgv.edu

² Harlingen Waterworks System, Harlingen, TX 78550, USA; mldavilaz13@gmail.com

³ Civil Engineering, University of Texas Rio Grande Valley, Edinburg, TX 78539, USA; jungseok.ho@utrgv.edu

* Correspondence: jihoon.kang@utrgv.edu; Tel.: +1-956-665-3526

Received: 1 September 2017; Accepted: 30 September 2017; Published: 3 October 2017

Abstract: There is increasing interest in using pyrogenic carbon as an adsorbent for aqueous contaminants in stormwater. The objective of this study was to investigate pyrogenic carbon materials as an amendment to geomedia to reduce nitrate leaching. Batch adsorption and column experiments were conducted to evaluate the performance of a commercial activated carbon and two biochars incorporated (5% by weight) into sand and pumice columns. The batch adsorption with 50 mg L⁻¹ of nitrate solution showed that only activated carbon resulted in a substantial adsorption for nitrate up to 41%. Tested biochars were not effective in removing aqueous nitrate and even released nitrate (<1%) with 1 h reaction time. Column experiment with a pulse input of nitrate solution (50 mg L⁻¹) confirmed that the sand or pumice columns amended with biochars were not as effective as those amended with activated carbon for reducing nitrate leaching. Our results suggested that net negatively charged surfaces of biochar may inhibit nitrate anion adsorption while activated carbon has reactive sites containing acidic functional groups to improve nitrate retention. There was no difference between sand and pumice for nitrate retention in any of the carbon amendments. Additional surface activation process during biochar production may be needed to improve adsorptive capacity of biochar for aqueous nitrate removal.

Keywords: activated carbon; adsorption; biochar; bioswale; nitrate; pumice

1. Introduction

Urban stormwater has become an important source of nitrogen (N) to receiving waters [1]. Excess N inputs to aquatic ecosystems can cause overstimulation of aquatic plant and algae growth, leading to eutrophication [2]. Excessive nitrate (NO₃⁻) in drinking water can cause critical health issues in young infants and young livestock such as blue baby syndrome [3]. The United States Environmental Protection Agency (USEPA) has set the Maximum Contaminant Level (MCL) of nitrate-nitrogen (NO₃-N) at 10 mg L⁻¹ (=44.2 mg NO₃⁻ L⁻¹) for the safety of drinking water.

Conventionally aqueous nitrates can be removed via biological and/or physicochemical methods [4,5]. A physicochemical method viable to stormwater nitrate reduction is the addition of adsorptive materials into existing and new porous media. Activated carbon (AC) has been the most common adsorptive material in water and wastewater treatments but it remains to be expensive [6,7]. Recently biochar has been viewed as a low-cost sorbent for remediating soil and water media contaminated with organic and inorganic contaminants [8,9]. Biochar is a charcoal derived from the thermal conversion of a wide range of biomass materials such as wood, grass, and other agricultural and forestry residue via pyrolysis. When the resulting biochar is applied to soils, the carbon can be effectively sequestered while improving soil structure and fertility [10]. Reducing leaching of nutrients

(nutrient retention) or even providing nutrients to plants with increase in microbial activity are claimed to be the benefits of commercially available biochars sold in USA.

There have been increasing adoptions of raingardens, bioswales, and infiltration basins in urban landscapes, which are designed to slow and filter stormwater runoff as a part of low impact development (LID) practices [11]. For example, the Lower Rio Grande Valley (LRGV) in South Texas has increasingly adopted bioswale systems and more are expected to be built in parking lots and driveways as a green infrastructure projects [12]. The objective of this study was to investigate the performance of pyrogenic carbon materials (AC and biochars) as an additive to selected geomedia (sand and pumice) for reducing nitrate leaching. Specific objectives were to (1) determine nitrate adsorption capacity of the carbon materials, and (2) evaluate nitrate retention in sand and pumice columns amended with the carbon materials (5% by weight).

2. Materials and Methods

2.1. Filter Media and Pyrogenic Carbon Materials

Commercially available play sand (Quikrete International Inc., Atlanta, GA, USA) and pumice (Nature's Footprint, Inc., Bellingham, WA, USA) were used in this study as geomedia (Figure 1). The play sand was determined to be fine sand having 97.5% sand, 1.8% silt, and 0.7% clay according to the particle size analysis by hydrometer method [13]. The pumice was a felsic volcanic rock with highly vesicular rough texture and it is often found to float on the beaches of the Texas coast due to its low density (0.2 to 0.6 g cm⁻³) [14]. No textural analysis was performed with pumice as it contained grain sizes coarser than 2 mm in diameter. Both materials were air-dried before use.



Figure 1. Geomedia tested in this study.

Three pyrogenic carbon materials—a commercial AC and two biochar products, Hoffman biochar (HB) and Wakefield biochar (WB)—were used as amendments to the sand or pumice geomedia in this study (Figure 2). The AC (CR610A, Carbon Resources LLC, Oceanside, CA, USA) was a granular product sold for water purification in aquaculture and it was produced from selected coal by a high temperature steam activation process according to the manufacturer. The HB (A.H. Hoffman Inc., Lancaster, NY, USA) was a pelletized charcoal product sold as a soil conditioner and it was claimed to improve drainage and adsorb harmful impurities according to the manufacturer. The WB (Wakefield Agricultural Carbon LLC, Columbia, MO, USA) was a USDA-certified soil conditioner and it was mainly claimed to contribute to healthier soils and improve drought resistance according to the manufacturer. The WB was not a pelletized product and it was much finer than other two carbon products. All carbon materials were analyzed for pH (1:80 solid to solution ratio) using a pH meter (Extech Instruments, Waltham, MA, USA) and elemental composition via Energy-dispersive X-ray spectroscopy equipped in ZEISS EVO LS10 Scanning Electron Microscope (Hitachi, Japan). The results for elemental composition were expressed in weight % (Table 1).



Figure 2. Pyrogenic carbon materials tested in this study.

Table 1. pH and elemental composition (weight %) of pyrogenic carbon materials.

Carbon Material	pH	C (%)	O (%)	Fe (%)	Al (%)
Activated carbon	8.03	73.1	11.6	13.7	1.6
Hoffman biochar	7.88	86.7	13.3	0	0
Wakefield biochar	10.23	89.8	10.2	0	0

2.2. Batch Adsorption Experiment

Batch sorption experiments were conducted in 50-mL centrifuge tubes at room temperature. Nitrate solution was prepared in deionized (DI) water containing 50 mg L⁻¹ as potassium nitrate (KNO₃). The carbon materials (0.45 g) were weighted to the tubes and equilibrated with 36 mL of the nitrate solution. The concentration of nitrate at 50 mg NO₃⁻ L⁻¹ were equivalent to 11.3 mg L⁻¹ NO₃-N in elemental basis. Samples with sand or pumice only were included as experimental control. The tubes were shaken for 1 h on an end-to-end shaker at 90 oscillations min⁻¹, and supernatants were filtered through 0.2-µm membrane filters. It is important to note that the 1 h reaction time in this study may have not reached a chemical equilibrium of nitrate adsorption onto the carbon materials. Our previous zinc (Zn) adsorption experiment with biochars up to 48-h equilibration showed that 54–98% of Zn adsorption occurred after 1 h relative to the amount of Zn adsorbed after 48 h [15]. The filtrates were analyzed colorimetrically using a HACH UV-vis spectrophotometer (DR3900, Loveland, CO, USA) according to dimethylphenol method for nitrate (HACH method 10206). The amount of nitrate adsorbed by the tested materials (q) was calculated by Equation (1):

$$q = (C_0V - CV)/M \quad (1)$$

where C_0 is the concentration of nitrate in input solution (mg L⁻¹), V is the volume of liquid (L), C is the concentration of nitrate in solution after 1-h equilibration, and M is dry weight of carbon material (kg). Nitrate adsorption (removal) capacity of the carbon materials was calculated in % relative to the initial amount of aqueous nitrate added (1.8 mg NO₃⁻).

2.3. Column Experiment

A total of 16 columns (metal cylinder in 7.62-cm diameter by 7.62-cm depth) were packed to a depth of 4.56 cm with sand or pumice. Sixteen columns corresponded to our experimental treatments consisting of two geomedia and four different carbon amendments, including control in duplicates. During our initial trials, maximum amounts of sand and pumice needed for packing the columns (i.e., control columns) were determined to be 318 g and 165 g, respectively. These given amounts were determined to have a bulk density of 1.67 g cm⁻³ for sand columns and 0.86 g cm⁻³ for pumice columns. Sand or pumice with and without carbon amendment (5% by weight) was dry-packed into the columns (Table 2). The bottom of each column was covered with cheese cloth to prevent the geomedia loss.

Table 2. The amount of geomeedia used for column packing with and without carbon amendment (5% by weight).

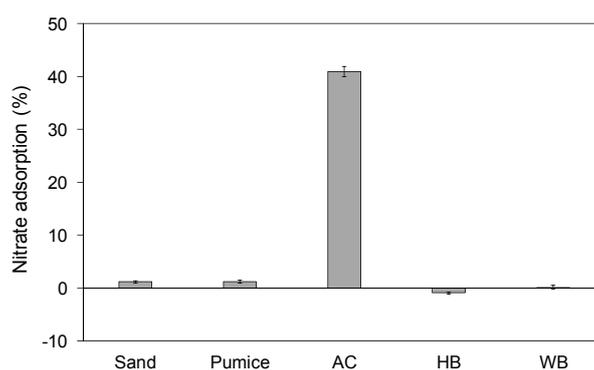
Column	Sand or Pumice (g)	Amendment (g)
Sand only	318	0
Sand + amendment	302	16
Pumice only	165	0
Pumice + amendment	157	8

Each column received 500 mL of DI water for initial flushing on the first day (day 1). The water was irrigated by 100 mL increments manually through a circular plastic pan that was placed on the top of column. The plastic pan had multiple small holes, allowing uniform dripping of the water and minimizing surface disturbance on the geomeedia. On the second day (day 2), columns were leached with nitrate solution (50 mg L^{-1}) five times by 100 mL increments, totaling 500 mL of aqueous nitrate throughput. The amount of nitrate applied was 25 mg in total. On the third day (day 3), columns were leached again with DI water only (100 mL increments, totaling 500 mL of DI water) to assess desorption of the nitrate. Collected column effluents were analyzed for nitrate in the aforementioned method. The effluent samples were also determined for turbidity using NEP 260 turbidity probe (McVan Instruments, Melbourne, Australia) in nephelometric turbidity units (NTU) and for pH using a portable pH meter (Extech Instruments, Waltham, MA, USA).

3. Results and Discussion

3.1. Batch Adsorption of Nitrate

Only AC had ability to adsorb nitrate up to 41% (Figure 3) while biochars showed none to even release (negative %) of nitrate (0.93% from HB and 0.13% from WB). DI water extracts (1:80 solid-to-solution ratio) with the carbon materials (i.e., DI water only with no aqueous nitrate) confirmed that an appreciable concentration was found for HB ($1.46 \text{ mg NO}_3^- \text{ L}^{-1}$) and WB ($1.55 \text{ mg NO}_3^- \text{ L}^{-1}$). Commercial pumice for gardening use is often claimed to hold nutrients but it was not the case in the batch adsorption with 1 h reaction time, accounting for only 1.3% of nitrate adsorption. Sand also showed limited adsorption ability (1.2%), indicating a lack of reactive surfaces for nitrate.

**Figure 3.** Adsorption from aqueous nitrate affected by geomeedia and carbon materials. Error bars represent standard error of the mean.

It is often claimed that biochar can be used as a soil amendment to retain nutrients in soils [16,17]. However, the biochar materials tested in this study showed no adsorption (even release) of nitrate in a batch-scale experiment. Our result is in agreement with Yao et al. (2012) who found limited adsorption of nitrate (<4%) onto biochars made from various feedstock materials such as sugarcane bagasse, peanut hull, pepperwood, and bamboo [18]. In general, biochars were found to be more effective at

removing cationic species from solution as most biochars were found to have a net negative surface charge [15]. While both AC and biochars have carbonaceous materials as feedstock materials and convert them to stable carbon through pyrolysis, AC typically receives an additional activation process where it undergoes oxidation to increase adsorptive capacities [19].

It is important to note that feedstock materials for the biochars were plant-based while the AC was produced from selected coal according to manufacturer. Detailed information on the feedstock types and manufacturing processes of these commercial products are proprietary and unknown. However, it was notable that the AC contained substantial amount of Fe (13.7%) and Al (1.6%) in its elemental composition (Table 1), indicating mineral inclusion of Fe and Al and possibly leading to better adsorption of nitrate. The Fe minerals such as hematite, goethite, and iron-oxide have been found to increase oxyanion adsorption such as arsenite and arsenate in the Fe-impregnated carbon materials through surface complexation between the Fe-O surface and oxyanion [20,21].

3.2. Nitrate Leaching in Column Experiment

Overall turbidity in effluent samples were 3- to 4-times greater in pumice columns (17 to 27 NTU) compared to sand columns (5 to 7 NTU) (Table 3). Coarse grains in pumice were likely to contribute to the higher turbidity by eluting fine suspended solids from pumice and the carbon materials. The pH values in column effluents ranged from 7.6 to 8.7 and the columns amended with WB showed the highest pH (7.9 to 8.7), indicating the presence of mineral ash in the WB [22].

Under the pulse input of nitrate solution ($50 \text{ mg NO}_3^- \text{ L}^{-1}$), nitrate in column effluents increased with nitrate solution throughput (leaching event 1–5 in Figure 4) up to 11 mg L^{-1} in both sand and pumice columns (Figure 4). The lowest concentration was found with sand column amended with AC (7 mg L^{-1}) and pumice column amended with AC (8 mg L^{-1}), suggesting AC outperformed biochars in the flow-through experiment as well. Under the desorption stage (leaching event 6–10), the nitrate concentrations gradually decreased with AC amendment in both geomeedia being the lowest (1 to 3 mg L^{-1}) suggesting that AC amendment was more effective than biochar amendment in retaining nitrate during desorption stage.

Table 3. Average pH and turbidity (mean \pm standard error) in column effluents over the entire leaching events.

Column ^a	pH	Turbidity (NTU)
Sand only	7.84 ± 0.18	7.29 ± 0.85
Sand + AC	7.92 ± 0.06	5.38 ± 0.59
Sand + HB	7.93 ± 0.08	7.43 ± 0.56
Sand + WB	8.73 ± 0.16	7.35 ± 1.72
Pumice only	7.60 ± 0.08	23.86 ± 5.57
Pumice + AC	7.50 ± 0.06	24.61 ± 4.22
Pumice + HB	7.58 ± 0.05	26.80 ± 3.80
Pumice + WB	7.91 ± 0.17	17.41 ± 2.46

^a AC = activated carbon; HB = Hoffman biochar; WB = Wakefield biochar.

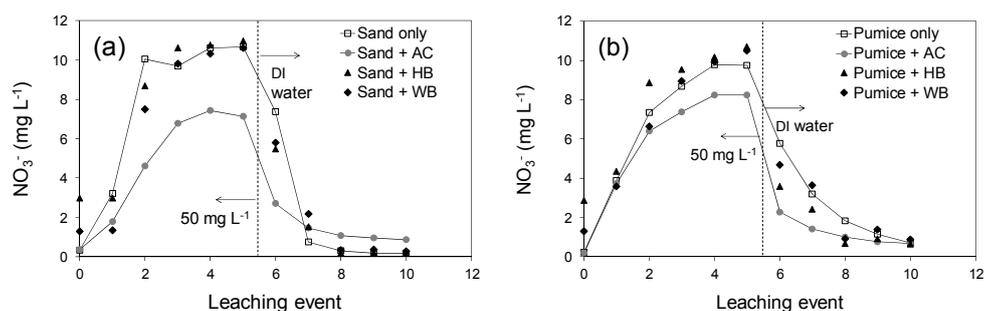


Figure 4. Concentrations of nitrate (NO_3^-) in column effluents: (a) sand and (b) pumice.

Cumulative amount of nitrate leached (Figure 5) showed a clear separation of AC-amended sand or pumice geomeedia being the lowest (3.6 to 4 mg as NO_3^-) after the entire leaching event. In both sand and pumice columns, HB resulted in higher nitrate leaching (6.3 mg) than control columns (5.2–5.3 mg) while WB-amended columns showed similar amount of nitrate leached with control columns. The higher amount of nitrate leached from the columns amended with HB was in agreement with the greater release of nitrate from batch adsorption (Figure 3). There was no substantial difference in the amount of nitrate leached between sand-only (5.3 mg) and pumice-only columns (5.2 mg).

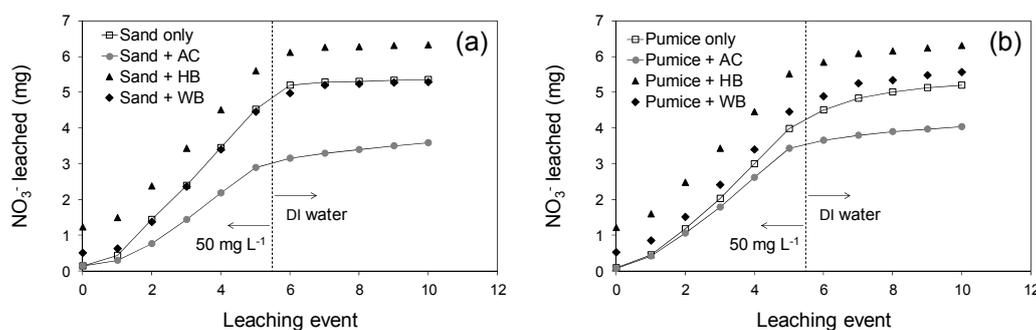


Figure 5. Cumulative amount of nitrate (NO_3^-) leached in column effluents: (a) sand and (b) pumice.

4. Conclusions

Results in this study suggested that sand and pumice geomeedia amended with biochars (5% by weight) were not as effective as those amended with AC. The presence of Fe and Al in the AC was likely to promote nitrate adsorption possibly through surface complexation to some extent. There was no substantial difference between sand and pumice columns in all carbon amendment types. Our study indicated that net negatively charged surfaces of the biochars may be attributed to the poor adsorption of nitrate anion due to electrostatic repulsion. To improve its efficacy for aqueous nitrate removal, an additional process during biochar production is desirable to create acid functional groups (which have a positive charge) that can protonate surface $-\text{OH}$ group in biochars [23,24]. It is important to note that while biochar may not be effective in reducing nitrate leaching, it can provide other benefits such as increasing the N use efficiency by plants and decrease nitrous oxide (N_2O) emission from N-rich soils. Our study included nitrate only under controlled leaching conditions and future study should consider evaluating multiple anions and/or cations for the field applications of geomeedia amended with pyrogenic carbon materials. In addition, future study investigating the synergistic effect of the carbon amendment in geomeedia combined with redox condition is needed to advance our understanding of biotic and abiotic processes of nitrate retention in stormwater control measures.

Acknowledgments: We gratefully acknowledge UTRGV graduate assistantship from the School of Earth, Environmental and Marine Sciences, UTRGV work study program, and US EPA Border 2020 Program for the partial funding of this project.

Author Contributions: Kang supervised the overall project and wrote the manuscript. Davila and Mireles collected column and batch adsorption data, respectively. Ho contributed to manuscript preparation and data analyses.

Conflicts of Interest: The authors declare no conflict of interest. The use of trade names in this publication does not imply endorsement of the products named or criticism of similar ones not mentioned by these organizations.

References

1. US Environmental Protection Agency. *National Water Quality Inventory: Report to Congress: 2004 Reporting Cycle*; USEPA, Office of Water: Washington, DC, USA, 2009.
2. Li, L.; Davis, A.P. Urban stormwater runoff nitrogen composition and fate in bioretention systems. *Environ. Sci. Technol.* **2004**, *48*, 3403–3410. [[CrossRef](#)] [[PubMed](#)]

3. Camargo, J.A.; Alonso, Á. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. *Environ. Int.* **2006**, *32*, 831–849. [[CrossRef](#)] [[PubMed](#)]
4. Chang, N.B.; Hossain, F.; Wanielista, M. Filter media for nutrient removal in natural systems and built environments: I—Previous trends and perspectives. *Environ. Eng. Sci.* **2010**, *27*, 689–706. [[CrossRef](#)]
5. Demiral, H.; Gündüzoğlu, G. Removal of nitrate from aqueous solutions by activated carbon prepared from sugar beet bagasse. *Bioresour. Technol.* **2010**, *101*, 1675–1680. [[CrossRef](#)] [[PubMed](#)]
6. Ahmedna, M.; Marshall, W.E.; Husseiny, A.A.; Rao, R.M.; Goktepe, I. The use of nutshell carbons in drinking water filters for removal of trace metals. *Water Res.* **2004**, *38*, 1062–1068. [[CrossRef](#)] [[PubMed](#)]
7. Kazemipour, M.; Ansari, M.; Tajrobehkar, S.; Majdzadeh, M.; Kermani, H.R. Removal of lead, cadmium, zinc, and copper from industrial wastewater by carbon developed from walnut, hazelnut, almond, pistachio shell, and apricot stone. *J. Hazard. Mater.* **2008**, *150*, 322–327. [[CrossRef](#)] [[PubMed](#)]
8. Ippolito, J.A.; Laird, D.A.; Busscher, W.J. Environmental benefits of biochar. *J. Environ. Qual.* **2012**, *41*, 967–972. [[CrossRef](#)] [[PubMed](#)]
9. Ahmad, M.; Rajapaksha, A.U.; Lim, J.E.; Zhang, M.; Bolan, N.; Mohan, D.; Ok, Y.S. Biochar as a sorbent for contaminant management in soil and water: A review. *Chemosphere* **2013**, *99*, 19–33. [[CrossRef](#)] [[PubMed](#)]
10. Liang, B.; Lehmann, J.; Solomon, D.; Sohi, S.; Thies, J.E.; Skjemstad, J.O.; Wirrick, S. Stability of biomass-derived black carbon in soils. *Geochim. Cosmochim. Acta* **2008**, *72*, 6069–6078. [[CrossRef](#)]
11. Ahiablame, L.M.; Engel, B.A.; Chaubey, I. Effectiveness of low impact development practices: Literature review and suggestions for future research. *Water Air Soil Pollut.* **2012**, *223*, 4253–4273. [[CrossRef](#)]
12. Ho, J.; Kang, J. Hydrologic impacts of bioswale porous media on parking lot drainage. *Int. J. Civ. Struct. Eng. Res.* **2017**, *5*, 23–30.
13. Gee, G.W.; Bauder, J.W. Particle-size analysis. In *Methods of Soil Analysis*, 2nd ed.; Klute, A., Ed.; Agronomy Monograph No. 9; ASA and SSSA: Madison, WI, USA, 1986; Part 1, pp. 404–408.
14. Venezia, A.M.; Floriano, M.A.; Deganello, G.; Rossi, A. The structure of pumice: An XPS and 27Al MAS NMR study. *Surf. Interface Anal.* **1992**, *18*, 532–538. [[CrossRef](#)]
15. Mireles, S.; Ok, Y.; Cheng, C.; Kang, J. Adsorptive and kinetic characterization of aqueous zinc removal by biochars. *SDRP J. Earth Sci. Environ. Stud.* **2016**, *1*, 1–7.
16. Beesley, L.; Moreno-Jiménez, E.; Gomez-Eyles, J.L.; Harris, E.; Robinson, B.; Sizmur, T. A review of biochars' potential role in the remediation, revegetation and restoration of contaminated soils. *Environ. Pollut.* **2011**, *159*, 3269–3282. [[CrossRef](#)] [[PubMed](#)]
17. Lehmann, J.; Rillig, M.C.; Thies, J.; Masiello, C.A.; Hockaday, W.C.; Crowley, D. Biochar effects on soil biota—A review. *Soil Biol. Biochem.* **2011**, *43*, 1812–1836. [[CrossRef](#)]
18. Yao, Y.; Gao, B.; Zhang, M.; Inyang, M.; Zimmerman, A.R. Effect of biochar amendment on sorption and leaching of nitrate, ammonium, and phosphate in a sandy soil. *Chemosphere* **2012**, *89*, 1467–1471. [[CrossRef](#)] [[PubMed](#)]
19. Azargohar, R.; Dalai, A.K. Biochar as a precursor of activated carbon. In *Twenty-Seventh Symposium on Biotechnology for Fuels and Chemicals*; Humana Press: New York, NY, USA, 2006; pp. 762–773.
20. Hu, X.; Ding, Z.; Zimmerman, A.R.; Wang, S.; Gao, B. Batch and column sorption of arsenic onto iron-impregnated biochar synthesized through hydrolysis. *Water Res.* **2015**, *68*, 206–216. [[CrossRef](#)] [[PubMed](#)]
21. Cantu, J.; Gonzalez, L.E.; Goodship, J.; Contreras, M.; Joseph, M.; Garza, C.; Eubanks, T.M.; Parsons, J.G. Removal of arsenic from water using synthetic Fe₇S₈ nanoparticles. *Chem. Eng. J.* **2016**, *290*, 428–437. [[CrossRef](#)] [[PubMed](#)]
22. Mukome, F.N.; Parikh, S.J. Chemical, Physical, and Surface characterization of Biochar. In *Biochar: Production, Characterization, and Applications*; Ok, Y.S., Ed.; CRC Press: Boca Raton, FL, USA, 2015; pp. 68–96.
23. Gai, X.; Wang, H.; Liu, J.; Zhai, L.; Liu, S.; Ren, T.; Liu, H. Effects of feedstock and pyrolysis temperature on biochar adsorption of ammonium and nitrate. *PLoS ONE* **2014**, *9*, e113888. [[CrossRef](#)] [[PubMed](#)]
24. Yang, J.; Li, H.; Zhang, D.; Wu, M.; Pan, B. Limited role of biochars in nitrogen fixation through nitrate adsorption. *Sci. Total Environ.* **2017**, *592*, 758–765. [[CrossRef](#)] [[PubMed](#)]

