











Bone char: characterization and agronomic application as an alternative source of phosphorus

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ABSTRACT: Alternative materials can be used to reduce reliance on mining for P-based fertilizers. In this sense, the pyrolysis process of bovine bones produces the “bone char”, which can be used as a source of P. This study aimed to characterize bone char and conduct a comparative analysis with both soluble (triple superphosphate) and non-soluble (Bayóvar phosphate rock) phosphate fertilizers, specifically examining its behavior in soil and uptake by plants. Bone char characterization was performed by X-ray diffraction (XRD), attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR), and scanning electron microscopy coupled with energy-dispersive (SEM-EDS). The XRD analyses have shown the presence of hydroxyapatite in the bone char, bands assigned to P-O stretching from phosphate have been observed in ATR-FTIR, and Ca, P, C, and O elements were identified in the materials by EDS analyses. Solubility from fertilizer extractants was higher for bone char compared to Bayóvar, and both sources showed lower solubility compared to triple superphosphate. Cumulative amount of P released from bone char was higher than Bayóvar and lower than triple superphosphate. Amount of total dry matter, total shoot P uptake, and total shoot Ca uptake were higher for triple superphosphate compared to bone char and Bayóvar. Release profile of P from bone char strongly suggests this material can be used as a slow-release P source, with intermediate solubility between soluble and non-soluble commercialized sources.

Keywords: bovine bone, pyrolysis, phosphate fertilizer, slow release.

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INTRODUCTION

Phosphorus (P) is one of the most limiting elements to plant growth and development under tropical conditions. The high P demand required by Brazilian soils to maintain satisfactory agricultural productivity requires the application of phosphate fertilizers at high rates, which increases production costs. Phosphate rocks in their “*in natura*” state or acidified in wet processes using sulfuric and phosphoric acids have been widely used in agriculture as P fertilizers. However, high-quality phosphate rocks are limited in some parts of the world (Scholz and Wellmer, 2013; Santos et al., 2019). This problem accompanies inquiring about the high cost of applying phosphate fertilizers to maintain agricultural production in countries with predominantly tropical soils, such as Brazil, compared to countries with temperate soils, which require lower rates of P (Roy et al., 2016).

Reuse of residues or other alternative sources of P are options to solve the future problem of phosphate fertilizer supply. Bovine and swine bones, often discarded from the production chain, can be a potential source of P for the fertilizer industry. In 2023, 33.9 million cattle were slaughtered in Brazil, with an average productivity of 262.97 kg per animal (IBGE, 2023). Considering bones represent 17.9 % of the animal weight (Ribeiro et al., 2008; Pascoal et al., 2009), approximately 1.6 million tons of bones would be available for bone char production in the last year.

Mineral composition of bone consists basically of hydroxyapatite - $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ (Zimmer et al., 2018), which is also part of the composition of natural phosphate rocks. Chemical composition has stimulated researchers to explore the use of bone char as an alternative source of P and as a soil amendment (Siebers and Leinweber 2013; Gulyás et al., 2014; Siebers et al., 2014; Morshedizad et al., 2016; Robinson et al., 2018; Leinweber et al., 2019; Pinheiro and Nair, 2021; Wakweya et al., 2022; Mielke et al., 2023).

Bones have hydroxyapatite of low solubility, as well as phosphate rocks, so their use as a P-fertilizer provides, in most cases, less P than required by most crops with an annual cycle. In addition, there is concern about the use of bovine bones, which can lead to contamination and transmission of diseases such as bovine spongiform encephalopathy, also known as mad cow disease. The application of heat treatment to bovine bones, employing temperatures above 400 °C, has demonstrated its effectiveness in eradicating infectious agents within the bones (Warren et al., 2009). Investigations have been conducted to explore the technical aspects of the pyrolysis process and the characterization and solubilization of carbonized or pyrolyzed bone materials (Piccolla et al., 2021). Recycling bovine bones, for example, through pyrolysis, can generate a product called “bone char,” which can be studied as a safe and renewable alternative phosphate fertilizer source.

Although it is an alternative to natural rocks, bone char has a lower solubility than commercially available soluble phosphate sources (Warren et al., 2009). A mixture produced from the interaction between bone char pyrolyzed at 750 °C and root arbuscular mycorrhizal fungi displayed an efficient fertilizer for maize (*Zea mays*) plants (Zwetsloot et al., 2016). When it was applied in roots inoculated with arbuscular mycorrhizal fungi, maize plants accumulated similar amounts of P compared to triple superphosphate (TSP), a soluble P source. However, without the roots inoculation, the corn plants in the pot trial accumulated more P from TSP than bone char (Zwetsloot et al., 2016). Bone chars with different solubilities were obtained by carbonization in different temperatures (400 to 800 °C) without oxygen gas flow and in a controlled environment with/without nitrogen gas - N_2 (Piccolla et al., 2021). The authors highlighted the agronomic efficiency of bone char can change over environmental conditions (soil pH value, biological activity, texture, etc), and pyrolysis conditions can produce materials with different solubilities, allowing its application as a more soluble and slow-release source of P.

There are substantial references in bone char performance focused on soils from temperate regions not very weathered, with a texture and mineralogy distinct from well-weathered

tropical soils (Panten and Leinweber, 2020; Kruse et al., 2022; Jia et al., 2023). These highly weathered soils are naturally acidic and usually cultivated at a pH between 5.5 and 6.5, which can contribute to the solubilization of bone char and enable its use in these environments. The study of bone char as an alternative source of P is still a challenge, especially in evaluating its agronomic efficiency in highly weathered soils with successive crop cultivation and in comparing it with sources of different solubilities. Therefore, this study aimed to: (i) characterize bone char produced by a technical pyrolysis process; (ii) evaluate the solubility of bone char using different P extractors, comparing the results with Bayóvar phosphate rock and TSP; (iii) study the “*in vitro*” release of P from bone char, Bayóvar and TSP; and (iv) evaluate the potential of bone char as a P source through sequential greenhouse plant cultivation in a highly weathered soil and compare the results with commercial P sources such as Bayóvar and TSP.

MATERIALS AND METHODS

Synthesis and characterization of bone char

Bovine bones used in the synthesis of bone char were collected from a butcher shop. The bones were then cut into smaller pieces and subjected to pre-pyrolysis to remove tissue, fat, and blood. This procedure was carried out in a muffle furnace for 2 to 5 h at 100-300 °C. Subsequently, the produced material was crushed in a mill, obtaining a particle size of 2-4 mm. Bone char was obtained by pyrolysis of granular bone in a tube furnace under limited oxygen conditions at a heating rate of 30 °C min⁻¹ for 2-4 h at 600 °C. The synthesized bone char was sieved using a 150-mesh sieve.

Bone char was characterized by X-ray diffraction (XRD), attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR), and scanning electron microscopy (SEM) coupled with energy dispersive spectroscopy (EDS). The XRD analyses were performed using Shimadzu XRD-6000 equipment, with a graphite crystal monochromator to select Cu-K α_1 radiation with $\lambda = 1.5406 \text{ \AA}$, and a step rate from 0.02° s⁻¹. The 2 θ scan range was 4° and 70°. ATR-FTIR spectra were obtained using a Jasco spectrometer, model FT/IR-4100. The wavelength range used was 4000 to 400 cm⁻¹, at 4 cm⁻¹ resolution, and 256 scanning. The materials were deposited over a conductive double-sided adhesive tape to perform SEM analyses and recovered with a thin gold film using a Quorum Q150RS. The JEOL JSM-6010LA analytical scanning electron microscope was used to analyze the morphologies of the materials. Energy dispersive spectroscopy (EDS) was performed for the micro compositional analysis of materials. The conventional P sources (Bayóvar and TSP) used in the agronomic studies were also characterized by XRD and ATR-FTIR.

The Manual of Official Analytical Methods for Fertilizers and Correctives was used for the P and Ca solubility of bone char, Bayóvar, and TSP, which is available from the Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA, 2017). The method consists of extracting total Ca, P in water (P_{H₂O}), neutral ammonium citrate (P_{NAC}), 2 % citric acid (P_{CA}), and total P (P_{total}). The P content in sources was calculated as a percentage of the mass and presented as phosphorus pentoxide (P₂O₅), which is required by current Brazilian fertilizer legislation (MAPA, 2018).

Phosphorus and calcium release test

Phosphorus and calcium release test was performed in a beaker containing 50 mL of deionized water, in which 15 mg of P was added using Bayóvar, TSP, and bone char sources. Aliquot's of 0.2 mL were collected from 0.25 to 96 h. The study was carried out at a temperature of 25 °C, in a randomized complete block design with three replications. Phosphorus present in the solution was determined by molecular absorption spectrometry, in the SP-22 Spectrophotometer, according to the method described by Braga and Defelipo (1974). Calcium concentrations were also analyzed using molecular absorption spectrometry.

Greenhouse experiment

Soil sample was collected from the 0.00-0.20 m layer of an Oxisol (Soil Survey Staff, 1999) in Sete Lagoas, Minas Gerais State, Brazil. According to Soil Brazilian Classification System, the soil used in this study is classified as *Latossolo Vermelho Distrófico*. Soil sample was homogenized, sieved (2 mm), and air-dried. The soil had the following chemical and physical properties: pH (H₂O) 4.67; cation exchange capacity (CEC) 8.60 cmol_c dm⁻³; P (Mehlich-1) 1.0 mg dm⁻³; Ca²⁺ (KCl 1.0 mol L⁻¹) 0.52 cmol_c dm⁻³; total organic carbon 3.56 %; clay 810 g kg⁻¹; silt 80 g kg⁻¹; fine sand 30 g kg⁻¹; coarse sand 80 g kg⁻¹; soil bulk density 1.03 Mg m⁻³.

Lime at the rate of 4.8 Mg ha⁻¹ was applied to the soil using CaCO₃ and MgCO₃ in a Ca:Mg ratio of 4:1. After 30 days of incubation with soil moisture at the field capacity, the soil samples were air-dried and sieved (2 mm). Before the first cultivation, the soil pH was determined according to the methodology described by Teixeira et al. (2017), in which the pH value reached was 6.1. This pH value is common in soils used for agriculture in Brazil.

A factorial 3 × 5 was established with three sources of P (bone char, TSP, and Bayóvar) and five rates of total P (0, 25, 50, 100, and 200 mg dm⁻³). These rates for the soil with the bulk density of 1.03 Mg m⁻³ and collected from the 0.00-0.20 m layer represent respectively 0, 50, 100, 200, and 400 kg ha⁻¹ of total P. The bone char, TSP, and Bayóvar presented 14.9, 17.9, and 12.13 % of P, respectively. A complete randomized block design with four replicates was used in this experiment. Phosphorus sources were homogenized, and plastic pots were filled with 2.0 kg of soil. Additionally, basal fertilization was added to all treatments, giving a total dose per dm⁻³ of 70 mg of potassium (K), 40 mg of sulfur (S), and 100 mg of nitrogen (N). The sources used were KCl, (NH₄)₂SO₄, and urea.

Immediately afterward, ten seeds of corn (*Zea mays* L.) were sown per pot. After four and six days, seedlings were thinned to the four most uniform ones in each pot. Additional liquid basal nutrients were added three times, in 10 mL of solution per pot, resulting in a total basal nutrient dose per dm⁻³ of 100 mg of N as urea, 70 mg of K as KCl, 40 mg of S as (NH₄)₂SO₄, 1.32 mg of copper (Cu) as CuSO₄·5H₂O, 1.55 mg of iron (Fe) as FeSO₄·7H₂O, 3.66 mg of manganese (Mn) as MnSO₄·H₂O, 0.15 mg of molybdenum (Mo) as (NH₄)₆Mo₇O₂₄, 4 mg of Zn as ZnSO₄·7H₂O and 1 mg of boron (B) as H₃BO₃. Pots were watered with distilled water to maintain 80 % of the soil field capacity. After 30 days of cultivation, corn plants were harvested by cutting the stems at the soil surface. Corn roots were not removed from the pots. Plant biomass was dried at 65 °C for 72 h and weighed to obtain dry matter and ground. Phosphorus and Ca contents of plant tissues were determined by nitric-perchloric acid digestion performed according to Carmo et al. (2000). The soil was homogenized to carry out chemical analyses and samples of 100 g from each experimental unit were collected after the first cultivation to determine the availability of P (Mehlich-1), Ca (KCl 1.0 M), and soil pH (H₂O) according to the methodology described by Teixeira et al. (2017).

After the corn harvest, the residue of the stem and the roots were kept in the soil, and the basal fertilization was added to all treatments, giving a dose of 70 mg dm⁻³ of K, 40 mg dm⁻³ of S, and 100 mg dm⁻³ of N, using KCl, (NH₄)₂SO₄, and urea respectively. No P fertilization was applied, requiring the plants to use the soil residual P. Twenty seeds of black oat (*Avena strigosa* L.) were sown per pot 40 days after corn harvest. Five days after emergence, each pot was thinned to ten plants. The other nutrients were applied at the same rates and sources as in the first cultivation. Pots were watered with distilled water to maintain 80 % of the soil field capacity. At 45 days after seeding, the shoots plants were harvested, and the plant biomass was dried at 65 °C for 72 h and weighed. This sequential cultivation gave results at 30 and 115 days after the P application. The plant biomass was ground, and the P and Ca contents in the plant tissues were analyzed according to the method described by Carmo et al. (2000). After the second cultivation, the soil was homogenized, and 100 g were collected from each experimental unit to determine the availability of P, Ca, and soil pH value (Teixeira et al., 2017).

Agronomic efficiency (AE; Equation 1) and recovery rate (RR; Equation 2) for the sum of the total cultivation of corn and black oat were calculated. The equations were obtained from Doberman (2005).

$$AE = \frac{Pp - P_0}{RAp} \quad \text{Eq. 1}$$

$$RR = \left(\frac{PU - PU_0}{RAp} \right) \times 100 \quad \text{Eq. 2}$$

in which: Pp is a measurement of productivity (dry matter) for each P rate applied; RAp is the rate of P applied as fertilizer; P₀ is the productivity (dry matter) of the treatment without fertilizer; PU is the shoot P uptake for the treatment with P application; and PU₀ is the shoot P uptake of the treatment without fertilizer.

Statistical analysis

Data were subjected to analysis of variance. Regression analyses were adjusted between plant dry matter, soil available P, soil available Ca, shoot P uptake, and shoot Ca uptake as a function of P rates applied to each soil. In addition, the analysis of variance and regression were adjusted for the sum of the total cultivation of maize and black oat. In this analysis, plant dry matter, soil available P, soil available Ca, shoot P uptake, and shoot Ca uptake were added considering maize and black oat cultivations. The R environment was used for statistical analysis (R Development Core Team, 2016).

RESULTS

The XRD patterns revealed three sharp peaks at $2\theta = 7.47, 22.90,$ and $24.04,$ which are characteristic of the crystal structure of calcium hydrogen phosphate hydrate $\text{Ca}(\text{H}_2\text{PO}_4)_2$ (JCDPS 70-1381) (Figure 1). The presence of hydroxyapatite ($\text{Ca}_5(\text{PO}_4)_3\text{OH}$ (JCDPS 09-0432)) with different crystallinity can be observed in the XRD patterns of Bayóvar and bone char, with the main peaks at $2\theta = 25.91, 31.78, 32.27,$ and $32.90.$ The Miller indices (hkl) of a crystallographic plane were also identified for the main peaks (Figure 1).

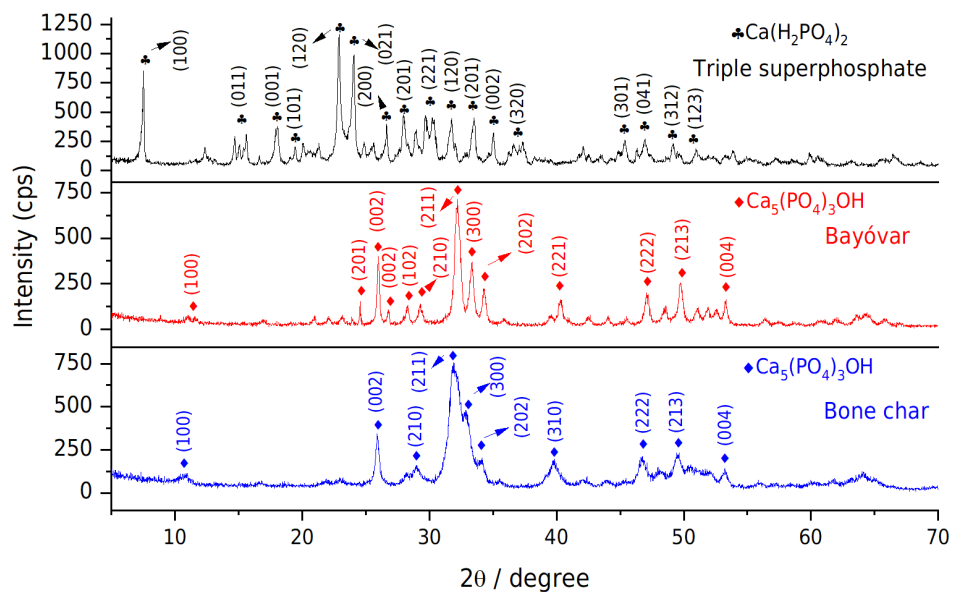


Figure 1. Diffractograms of bone char, Bayóvar, and triple superphosphate. The graphite crystal monochromator to select Cu-K α_1 radiation with $\lambda = 1.5406 \text{ \AA}$ was used. Step rate: $0.02^\circ \text{ s}^{-1}$; 2θ scan range: $4^\circ - 70^\circ$.

The ATR-FTIR spectra showed the presence of broad bands at 3472-3233 cm^{-1} due to the O-H stretching mode of H_2O (Figure 2). The couple bands at 1238 and 868 cm^{-1} are assigned to the in-plane P-O-H bending (A_2), and the out-of-plane bending (A_1) vibrations, respectively. The O-H bending mode appeared as weak broad bands at 1640-1643 cm^{-1} . The absorption band at 1460-1470 cm^{-1} corresponds to the asymmetric stretching mode of the C-O bond. The bands at 1093, 1034, 960, 608-680, and 560 cm^{-1} are correlated with P-O stretching of phosphate. The bands at 2600 cm^{-1} are related to the asymmetric stretching of CO_2 . The band at 1411 cm^{-1} is related to the presence of the CO_3^{2-} vibrational mode, and the bands present in all spectra at 2353 cm^{-1} are attributed to the CO_2 vibrational mode from the air.

The extractable P or Ca from the bone char (Table 1) shows higher values for the $\text{P}_{\text{H}_2\text{O}}$, P_{NAC} , P_{CA} , and P_{total} than Bayóvar, and as expected, both sources showed lower results compared to TSP. Additionally, the total Ca was higher for Bayóvar, followed by bone char and TSP (Table 1).

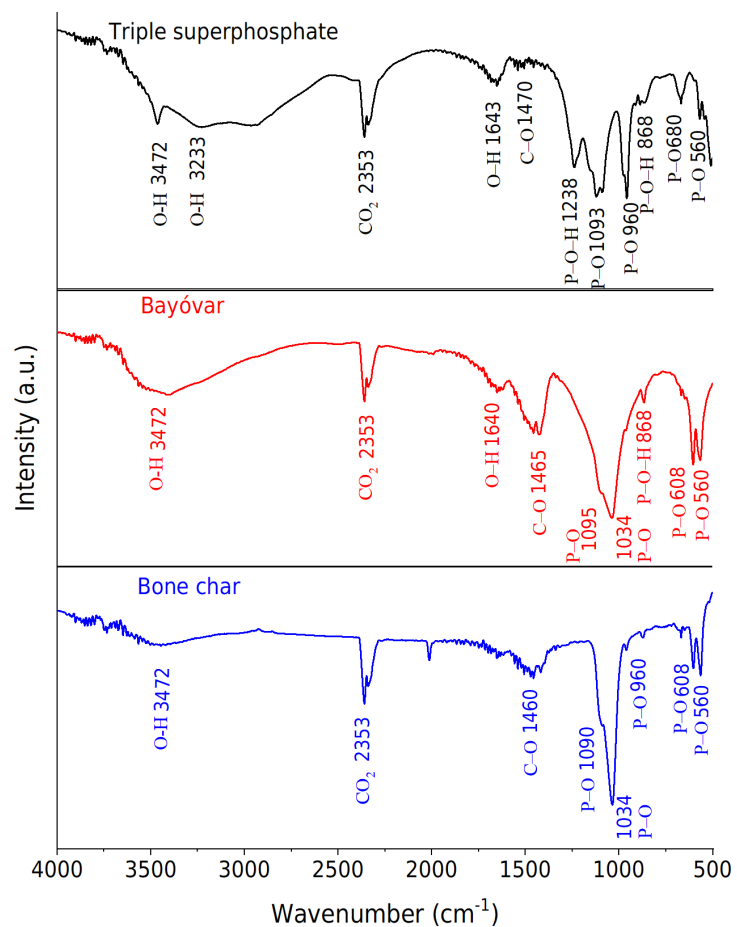


Figure 2. Attenuated total reflectance Fourier transform infrared spectroscopy spectra of bone char, Bayóvar, and triple superphosphate.

Table 1. Percentage (w/w) of dissolved phosphate (P_2O_5 basis) by water-soluble ($\text{P}_{\text{H}_2\text{O}}$), neutral ammonium citrate (P_{NAC}), 2 % citric acid solution (P_{CA}), total P_2O_5 (P_{total}), and total Ca

Source	$\text{P}_{\text{H}_2\text{O}}$	P_{NAC}	P_{CA}	P_{total}	Total Ca
Bone char	0.264	19.111	21.002	34.127	22.273
Bayóvar	0.128	4.402	2.357	27.839	28.470
Triple superphosphate	34.959	43.875	37.928	47.720	13.460

Cumulative P release analysis results indicated, across all collection times, TSP exhibited the highest phosphorus release, followed by bone char. Statistical analysis using the Tukey test revealed phosphorus release from bone char was significantly higher than Bayóvar phosphate rock, with a significance level of 5 % (Figure 3). After 72 h, 100 % of P was released with the application of TSP. After 96 h of bone char and Bayóvar application, 1.17 and 0.21 % of the P were released, respectively.

After corn and black oat cultivation, the residual amount of P in the soil was determined using the Mehlich-1 extractant. There was a linear increase of soil available P to the increasing P rates regardless of the P sources, in which a similar linear response has been observed for TSP and bone char, and a higher response was detected for Bayóvar (Figures 4a and 4d). The coefficients of the equations describing the dry matter and shoot P uptake were higher for TSP, followed by bone char and Bayóvar (Figures 4b and 4c). For dry matter and shoot P uptake of black oat, the coefficients of the equations describing each P source were similar (Figures 4e and 4f).

Since one of the objectives during this trial was to evaluate the residual effect of P sources in the soil, the total soil available P and Ca, total dry matter, and total shoot P and Ca uptake were summed for both cultivations. Total soil available P increased linearly with increasing P rates regardless of P sources, in which the coefficients of the linear equation have been higher for Bayóvar, and similar for TSP and bone char (Figure 5a).

There was a linear increase with the increasing rates of P for total dry matter, total shoot P uptake, and total shoot Ca uptake (Figures 5b, 5c, and 5d), and the results showed a higher coefficient of the equations for TSP source, followed by bone char and Bayóvar.

The AE responded quadratically to the increasing P rates of TSP, bone char, and Bayóvar. The highest P rates related to greater AE were calculated from the quadratic equations, presenting the values of 123.73, 119.09, and 112.99 mg dm⁻³ of P for TSP, bone char, and Bayóvar, respectively (Figure 6). The AE values calculated for these respective P rates were 11.28 g g⁻¹ for TSP, 10.08 for bone char, and 8.94 g g⁻¹ for Bayóvar. Considering the TSP as the source of greatest AE (100 %), the bone char and Bayóvar presented respectively the AE of 85.4 and 79.2 %, compared to TSP.

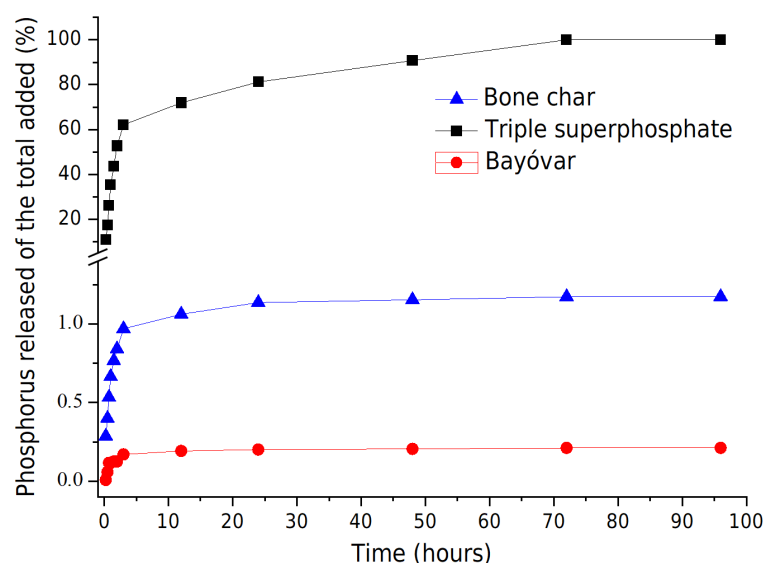


Figure 3. Cumulative release of phosphorus from bone char, Bayóvar, and triple superphosphate. Temperature: 25 °C. Rate of P applied: 15 mg. Solution: 50mL.

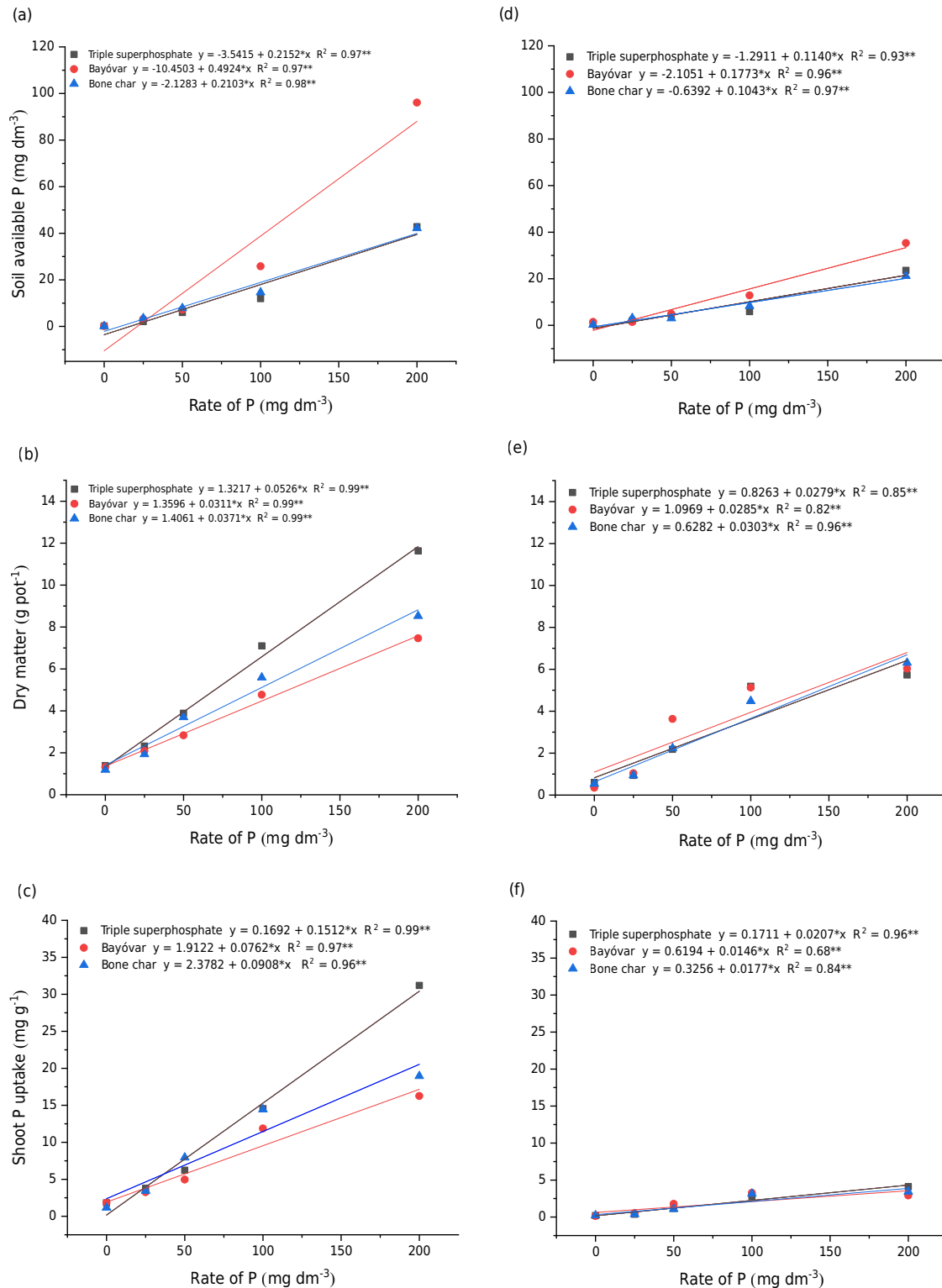


Figure 4. Soil available P, dry matter, and shoot P uptake as a function of P rates as triple superphosphate, bone char, and Bayóvar. Corn cultivation: a, b, and c. Black oat cultivation: d, e, and f. *, and **: significant at $p < 0.05$, and $p < 0.01$ by t-test, respectively.

For the RR, the models that best fit the data were quadratic, as observed for AE (Figure 7). The values calculated from equations for TSP, bone char, and Bayóvar were 140.89, 111.44, and 114.73 mg dm⁻³ of P, respectively. At these P rates, the RR according to the quadratic regressions were 19.46, 17.10, and 13.77 % for TSP, bone char, and Bayóvar, respectively, meaning TSP is considered as the source with the highest RR followed by bone char and Bayóvar.

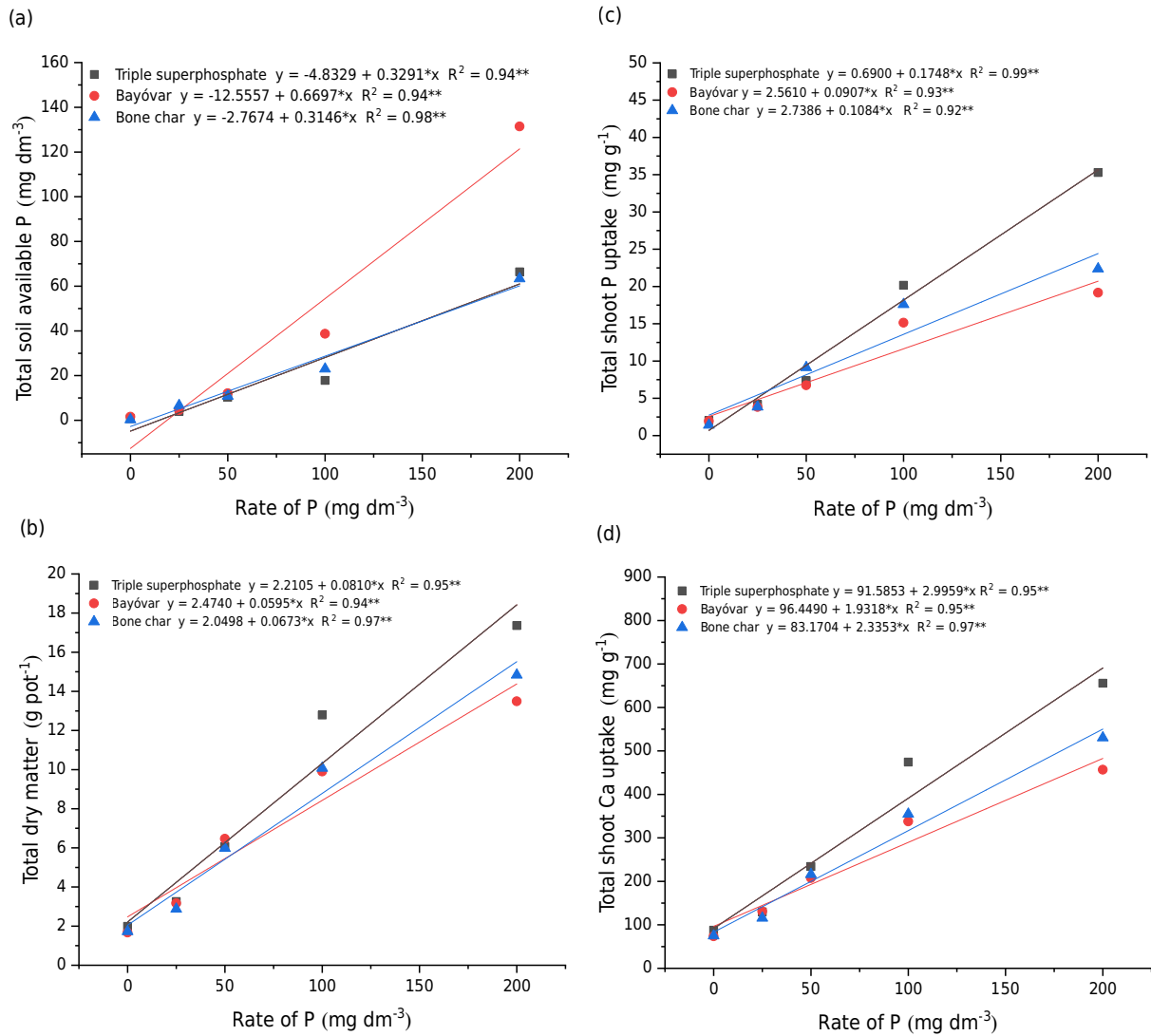


Figure 5. Total soil available P, dry matter, shoot P uptake, and shoot Ca uptake of corn and black oat calculated from the sum of first and second cultivations as a function of the P rates as triple superphosphate, bone char, and Bayóvar. *, and **: significant at $p < 0.05$, and $p < 0.01$ by t-test, respectively.

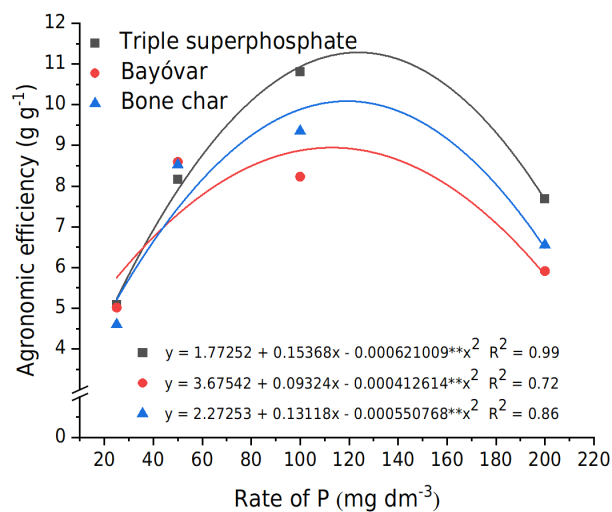


Figure 6. Agronomic efficiency of corn and black oat calculated from the sum of first and second cultivations as a function of the P rates as triple superphosphate, bone char, and Bayóvar. **: significant at $p < 0.05$ by t-test.

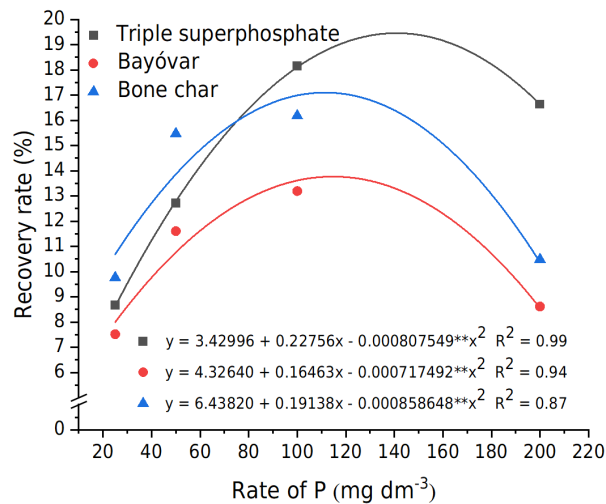


Figure 7. Recovery rate of corn and black oat calculated from the sum of first and second cultivations as a function of the P rates as triple superphosphate, bone char, and Bayóvar. **: significant at $p < 0.05$ by t-test.

DISCUSSION

The TSP fertilizer is originally derived from phosphates rocks, which contain hydroxyapatite. The wet acid treatment of this mineral can lead to the crystalline phase $\text{Ca}(\text{H}_2\text{PO}_4)_2$, as showed in figure 1 (Seesanong et al., 2021). Although Bayóvar and bone char are both composed of hydroxyapatite (Medellin-Castillo et al., 2014; Rojas-Mayorga et al., 2015; Flores-Cano et al., 2016; Klaic et al., 2018; Robinson et al., 2018; Nigri et al., 2019; Zimmer et al., 2019; Schütze et al., 2020), Bayóvar showed a higher crystallinity than bone char, as indicated by the higher intensity and narrower width at mid-height (Figure 1). This difference can be attributed to the composite nature of bone char, consisting of polymer fibrils and hydroxyapatite, where the temperature of 600 °C was insufficient to eliminate all organic polymers (collagen), as shown on thermogravimetry analysis performed by Figueiredo et al. (2010). Furthermore, between 600 °C and 1000 °C, hydroxyapatite partial dehydroxylation and carbonates decomposition also occur. In a study performed by Pramanik et al. (2013), bone treatment at 500 °C resulted in the gradual splitting of the broad peak at 32.5° into three distinct crystalline peaks: 32°, 32.4°, and 33°, as shown in figure 1.

ATR-FTIR spectra showed vibrational bands related to the presence of O-H groups from water and P-O from phosphate at 1238 and 868 cm^{-1} assigned, respectively, to P-O-H bending and out-of-plane P-O-H bending, as observed by Laohavisuti et al. (2021). The presence of CO_2 and CO_3^{2-} vibrations at 460 cm^{-1} was also verified in the materials. The absorption band at 1460 cm^{-1} is related to the asymmetric stretching mode of the C-O bond (Laohavisuti et al., 2021). In fact, TSP presents a small band of carbonate at 1460 cm^{-1} compared to the other materials, once CO_2 was released when the phosphate was acidified. In addition, bone char spectra show the band at 3472 cm^{-1} is less intense, and the one at 1640 cm^{-1} is not present. This behavior is explained by water release after thermal treatment at 600 °C.

Fertilizer solubility is evaluated using conventional extraction solutions, which can extract the available P in the soil, and can be absorbed by plant roots (Binh and Zapata, 2002; Santos et al., 2019). Phosphorus in water and neutral ammonium citrate solutions have been used mainly as acidulants or water-soluble commercial P fertilizers, such as TSP, ammonium phosphates, and partially acidified phosphate rocks, while P in 2 % citric acid has been used for non-soluble sources such as phosphate rocks (Chien et al., 2011; Santos et al., 2019; Piccola et al., 2021). The P_{total} and total Ca from bone char were

34.1 % of P_2O_5 , and 22.3 % of Ca (Table 1). The conversion of P_{total} represented by P_2O_5 (% m/m) to P is exactly 149.1 g kg^{-1} of P or 14.91% of P. These results are in agreement with the previous studies from Flores-Cano et al. (2016) (239 g kg^{-1} of Ca and 157 g kg^{-1} of P), Iriarte-velasco et al. (2016) (242 g kg^{-1} of Ca and 115 g kg^{-1} of P), Robinson et al. (2018) (238 g kg^{-1} of Ca and 130 g kg^{-1} of P), and Zwetsloot et al. (2016) (271 g kg^{-1} of Ca and 127 g kg^{-1} of P).

Bone char solubility in water was lower than TSP, but it was approximately twice compared to Bayóvar (0.264 and 0.128 % of P_2O_5 , respectively) (Table 1). Also, the P_{NAC} and P_{CA} from bone char were almost five times higher than Bayóvar. Similar results were demonstrated by Warren et al. (2009); although the P_{total} from bone char and Gafsa phosphate rock were similar, the P_{H_2O} and P_{NAC} were higher for bone char compared to Gafsa phosphate rock. The P_{H_2O} and P_{NAC} were 244 mg kg^{-1} and 11.5 % for bone char, while for Gafsa phosphate rock, these values were 42 mg kg^{-1} and 4.1 % (Warren et al., 2009). Notably, the extractants used in this study represent the potential solubility of the P sources. However, studies with controlled release “*in vitro*” conditions, and with plant cultivation are needed to validate soil available P and plant uptake of P.

Cumulative release of P was higher for the TSP, while bone char released more P compared to Bayóvar (Figure 3). These results corroborate with the previous ones presented on the solubility of fertilizers by extractants (Table 1), and suggest the release pattern of these fertilizers will follow the same trend when applied to the soil for plant cultivation. After 145 days of bone char and TSP incubation in 11 soils, Siebers and Leinweber (2013) reported lower solubility for bone char (19.3 %) than TSP (24 %). Warren et al. (2009) studied the solubility of bone char, Gafsa phosphate rock, and TSP using the Olsen extractant. After application in twelve soils, the authors showed solubility from bone char was higher than Gafsa phosphate rock and lower than TSP, and concluded bone char has an intermediate solubility between the P commercial sources with high and low solubility (Warren et al., 2009). These results can be explained and correlated with XRD patterns, in which the Bayóvar presented a more crystalline structure than bone char (Figure 1). The bone char has a higher carbonate concentration and lower crystalline structure compared to the hydroxyapatite mineral from Bayóvar, resulting in higher solubility (Wopenka and Pasteris, 2005; Pan and Darvell, 2010; Leinweber et al., 2019). Unlike bone char and Bayóvar, the synthesis of TSP is carried out through acidification with phosphoric acid, which explains its higher solubility.

Soil available P after the first and second cultivation (Figures 4a and 4d), and total soil available P (Figure 5a) were higher for Bayóvar, while a similar result was obtained for TSP and bone char. Phosphorus availability was determined using Mehlich-1 extractant, which is the most widely used in Brazil, and many studies have confirmed it as a suitable extractant of available P in soils (Steiner et al., 2012; Novais et al., 2015; Valadares et al., 2017; Reis et al., 2020; Castro et al., 2023). The acid dissolution (solution pH = 1.2) of Mehlich-1 can lead to the extraction of P-Ca when phosphate rocks are applied in the soils, overestimating the amount of P extracted (Alcântara et al., 2008; Freitas et al., 2013; Camêlo et al., 2015; Reis et al., 2020). This result suggests, when bone char is applied in the soil, there will be no problem in overestimating P-Ca forms, as it usually happens in soils that received application of phosphate rocks, therefore, it could be an advantage compared to Bayóvar.

In general, the soil available Ca was not influenced by the sources and the P rates applied. This can be explained by the fact the soils were previously incubated with Ca and Mg carbonate before first cultivation, with the aim of increasing the soil pH value and supplying Ca and Mg for plant cultivation (Greenhouse experiment Section). For soil pH value, several studies have been reporting carbonized materials such as biochar can increase soil pH value (Major et al., 2010; Van Zwieten et al., 2010; Kumari et al., 2014; Bakar et al., 2015; Mendes et al., 2021). This increase can be reached when higher

rates of biochar are applied to the soil (at tons per hectare) and when the material has a longer contact time with the soil. Thus, in the present study, the applied rates of bone char for P supply were not higher, since the highest dose was 200 mg dm^{-3} of P, which represents 400 kg ha^{-1} of P in the 0.00-0.20 m layer of soil, or 2.600 kg ha^{-1} of bone char.

Total dry matter, total shoot P uptake, and total shoot Ca uptake obtained by the sum of maize and black oat cultivation were higher for TSP, and were slightly higher for bone char compared to Bayóvar (Figures 5b, 5c, and 5d). The TSP is a more soluble source than bone char and Bayóvar, therefore these results are expected because in a shorter period of cultivation, the soluble source will release more P and Ca to the soil, supporting a higher accumulation of dry matter and absorption of P and Ca by the plants. Bone char presented slightly superior to Bayóvar, which supports the theory that, under field conditions and a longer contact time with soil, the release pattern of P and Ca from bone char can be different from Bayóvar. Zwetsloot et al. (2016) evaluated the absorption of P after the application of bone char and TSP in a highly P-fixing soil with 50 % clay. The authors found corn plants in soils fertilized with bone char had significantly lower plant biomass, P uptake, and Ca uptake than soils that received TSP, which corroborates those found in the present study. However, when the authors inoculated corn with arbuscular mycorrhizae, the absorption of P and Ca by the plants was the same when fertilized with bone char or TSP. These results confirm the solubility of pure bone char is inferior than the soluble commercial sources.

The AE and RR shows the increase in dry matter and P uptake due to each unit of P applied up to a maximum rate, later, these values decrease with the increase in P application for both P sources. This effect is expected, according to Mitscherlich law, the law of diminishing returns (Ernani and Barber, 1991), in which the efficiency of plants using the nutrients decreases as the availability of those nutrient increases. The AE depends on the type of fertilizer, the fertilizer management, and soil properties, such as pH, texture, etc., (Chien and Hammond, 1989; Chien and Menon, 1995; Li et al., 2011). Considering the only treatment studied in the present research was the different fertilizers and rates applied, it can be observed the TSP quickly increased soil available P, which led plants to accumulate more dry matter and shoot P uptake per unit of P applied compared to other sources.

The RR depends on the interaction between the nutrient release in the soil and plant demand (Doberman, 2005; Benício et al., 2017). In general, RR provides the information that any increase in RR lead to a reduction of P rate applied, without any production loss. The TSP was superior to the other sources. Also, there were a higher AE and RR when the bone char was applied compared to Bayóvar, showing a greater accumulation of dry matter and P uptake per unit of P applied. These results demonstrate the behavior of bone char application in weathered soils, showing this alternative source can help the efficiency of P use compared to Bayóvar.

Analyzing the results obtained from release and greenhouse experiments, the release pattern of P from bone char suggests it is a slower source of P than soluble commercial sources, but it is noteworthy that their characteristics are intermediate between soluble (TSP) and non-soluble (Bayóvar) sources. In addition, the bone char can be synthesized at different temperatures and controlled conditions, which can result in materials with different chemical, physical, and structural properties (Piccola et al., 2021). These characteristics show bone char can be used as a phosphate fertilizer with higher or lower solubility performance.

Published research on the application of bone char in soil and its subsequent uptake by plants, particularly in weathered and acid soils, is still relatively limited. Furthermore, the available studies primarily consist of short-term investigations. Studies with field application in the long term were carried out in Dystric Cambisol and Haplic Luvisol soil

with 360 g kg⁻¹ of sand, 570 g kg⁻¹ of silt, and 70 g kg⁻¹ of clay (Panten and Leinweber, 2020; Kruse et al., 2022; Jia et al., 2023). In these studies, the application rate of phosphate fertilizers was found to be 45 kg ha⁻¹ of P per year (Panten and Leinweber, 2020; Kruse et al., 2022; Jia et al., 2023). This application rate is considerably lower compared to the typical P rates employed in Brazil's P-deficient weathered soils. For instance, in such soils, the P application rates can reach as high as 1600 kg ha⁻¹ of P₂O₅ (equivalent to 699.2 kg ha⁻¹ of P) when broadcasting with incorporation, or 550 kg ha⁻¹ of P₂O₅ (equivalent to 240.3 kg ha⁻¹ of P) when employing band application specifically for carrot cultivation (Gonçalves et al., 2019). The strong P fixation by iron and aluminum oxyhydroxides reduces its availability to plants, requiring high rates of P application to increase its concentration in the soil solution. However, such high rates pose challenges when comparing studies conducted on Brazilian soils with those in temperate climate regions with less clay content. There is a need for future studies that not only demonstrate the results of consecutive plant cultivations, as shown in this study, but also explore the application of bone char in diverse field conditions and various types of weathered soils. These studies would contribute significantly to our understanding of the effectiveness and behavior of bone char in different agricultural settings.

CONCLUSIONS

Bone char was successfully produced using the technical pyrolysis process and characterized using XRD, ATR-FTIR, and SEM-EDS techniques. The chemical characterization confirmed the presence of hydroxyapatite in the bone char, as well as the elements P, Ca, C, and O. The solubility tests using fertilizer extractants and the P release test demonstrated bone char exhibits intermediate solubility as a P source, positioned between TSP and Bayóvar (commercial sources known for their higher and lower P release, respectively). These results were supported by the successive cultivation of corn and black oat, in which the dry matter and P uptake by plants were higher for the TSP, followed by the bone char and Bayóvar. It is noteworthy that bone char can be used as a safe, alternative, and renewable source of phosphate fertilizer with slow solubility characteristics compared to conventional soluble sources. Our study provides valuable insights into the characterization and potential agronomic benefits of bone char, supporting its use as a sustainable resource in agriculture for weathered and acid soils.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://www.rbcjournal.org/wp-content/uploads/articles_xml/1806-9657-rbcs-48-e0230165/1806-9657-rbcs-48-e0230165-suppl01.pdf.




DATA AVAILABILITY




All data was generated or analyzed in this study.






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