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(Original Article)

Releasing Phosphorus in Calcareous Sandy Soil as a Function of Adding Modified Bone Char and Bone Char with Sulfur and Humic Acid: An Incubation Study

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Abstract

The objective of this study was to examine the effect of adding modified bone char, bone char with sulfur and humic acid on some chemical properties and phosphorus availability in calcareous sandy soil. This experiment consisted of twelve treatments, viz, control (CK), bone + sulfur (B+S), bone + humic acid (B+HA), bone char + sulfur (BC+S), bone char + humic acid (BC+HA), modified bone char (MBC), modified bone char + humic acid (MBC+HA), bone char acidified with 0.1 N sulfuric acid (0.1ABC), bone char acidified with 1 N sulfuric acid (1ABC), phosphate rock (RP), phosphate rock + sulfur (RP+S), phosphate rock + humic acid (RP+HA). This experiment was incubated for 0, 7, 15, 30, 60, and 90 days under laboratory conditions. At the end of the incubation period, adding B+S, B+HA, BC+S, BC+HA, MBC, MBC+HA, 0.1ABC, 1ABC, RP+S, and RP+ led to a significant increase in available phosphorus compared to the control. The results obtained from this study revealed that the highest contents of phosphorus released from bone char were observed in BC+S, MBC+HA, and MBC treatments. In addition, available P in the soil increased with increasing incubation time. Soil pH significantly decreased with increasing incubation periods under adding B+S, B+HA, BC+S, BC+HA, MBC, MBC+HA, RP, RP+S, and RP+HA compared to the control. Accordingly, we can suggest applying bone char with sulfur as an alternative and safe source of phosphate fertilizers in sustainable agriculture

Keywords: *Bone char، Humic acid، Modified bone char،Phosphorus availability، Sulfur*

Introduction

Phosphorus (P) is an essential nutrient required for optimal plant growth. Large areas of the world suffer from phosphorus deficiency (Blake *et al*., 2000). Phosphorus deficiency in soil cannot be compensated for by any other element. Therefore, plant growth and production are severely affected by P deficiency (Grant *et al*., 2005). The increasing expansion of agricultural production to meet human food needs has led to an increasing global demand for phosphate rock, as phosphate fertilizers are mainly based on phosphate rock (Gupta *et al*., 2014). Crop production consumes a high proportion of the total P source (Leitem and Mikkelsen, 2005). Due to the highly wasteful nature of phosphorus rock, it is considered a finite and non-renewable resource (Carpenter and Bennett, 2011). It is likely to be depleted within 50 to 100 years (Cordell *et al.,* 2009).

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Moreover, phosphate fertilizers are a source of natural radioactive contamination of agricultural lands (El-Taher and Althoyaib, 2012; Qamouche *et al.*, 2020). Chemical phosphate fertilizers also contain some heavy elements such as cadmium and lead, which also cause ecosystem deterioration (Khan *et al.*, 2018; Attallah *et al.*, 2019). Thus, human health is affected by phosphate fertilizers that are found in different sources such as soil, water, and agricultural plants (Yasmin *et al.*, 2018).

Globally, nutrient recycling strategies in agriculture are promising approaches to address the challenges of depleting non-renewable resources as well as reducing environmental pollution levels from mining and manufacturing operations (Robles *et al.,* 2020). Recycling slaughterhouse waste, especially bones, into bone char is one of the processes used to preserve soil fertility, especially phosphorus. Bone char is produced through the pyrolysis process of animal bones under limited oxygen or anoxic conditions at temperatures of 300–1050 °C. Bone char product that mostly contains hydroxyapatite (Amin, 2024). Bone char is a clean and renewable alternative to chemical phosphate fertilizers and helps protect society and the environment through safe waste disposal (Glæsner *et al.,* 2019; Amin, 2020)**.** Bone char is a highly efficient phosphate fertilizer obtained from dried bones, possibly due to mineral changes during the pyrolysis process (Glæsner *et al*., 2019). The release of phosphorus from bone char into the soil is greatly affected by the chemical properties of the soil (Warren *et al*., 2009; Amin, 2021). Therefore, this study hypothesizes that producing bone char rich in sulfur, as well as mixing bone char with sulfur or humic acid, will lead to an increase in the release of phosphorus from bone char in calcareous sandy soil. So, the objectives of this study were to examine the influence of applying modified bone char as well as co-applying bone char with sulfur or humic acid on soil chemical properties and phosphorus availability in calcareous sandy soil.

Materials and Methods

Bone char production

Cow bones were collected from a butcher shop in Assiut city, Assiut, Egypt. The fat and gelatin were removed from collected bones (B) which were then broken into pieces 10 to 20 cm long. The bones were crushed into powder by a stainless-steel grinder then sieved with a 1 mm diameter sieve. The bone powder alone, or the combination of bone powder with 10% elemental sulfur were pyrolyzed at 380±10 °C for four hours in an outdoor pyrolysis reactor in a stainless-steel cylindrical container with a radius of 30 cm and a length of 60 cm to produce bone char (BC) and modified bone char by sulfur (MBC), respectively. After the pyrolysis process, it turns into bone char. Acidified bone char was produced from treated bone char powder with 0.1 and 1 N H_2SO_4, where sulfuric acid was added to the bone char at saturation. Then, the acidified bone char was dried at 105-110 C for 24h. The chemical properties of bone, bone char, modified bone char, and acidified bone char are shown in (Table 1)

Table 1. Some important properties of amendments used in this experiment. Data were average ± standard error (SE).

Amendments	pH $_{(1:5)}$	EC $_{(1:5)}$ (ds m^{-1})	Olsen-P (mg kg^{-1})	Total P (g kg^{-1})
B	6.7±0.14	0.87±0.01	446.29±19.70	69.64±0.51
BC	8.15±0.21	0.91±0.00	627.85±20.48	105.13±3.56
MBC	6.95±0.07	0.77±0.02	676.24 ± 5.43	106.63±1.44
0.1ABC	6.85±0.07	0.88±0.02	708.95±15.06	127.51±1.67
1ABC	5.5±0.28	1.41±0.09	770.93±6.05	153.11±7.79
RP	8.2±0.28	2.30±0.04	22.35±1.49	87.47±0.03
HA	8.85±0.35	4.54±0.05	-	0.82±0.03
S	4.55±0.21	0.64±0.01	7.40±0.60	-

B: bone; BC: bone char; MBC: modified bone char; 0.1ABC: acidified BC with 0.1 N H_2SO_4; 1ABC: acidified BC with 1 N H_2SO_4; RP: rock phosphate; HA: humic acid; S: sulfur. EC: electrical conductivity.

Incubation experiment

The current incubation experiment was conducted to examine the effect of applying modified bone char as well as co-applying bone char with sulfur and humic acid on soil chemical properties and phosphorus availability in calcareous sandy soil. Soil samples for the cultivated layer (0 − 30 cm) were collected from Al-Gharib Farm, Faculty of Agriculture, Assiut University, Assiut, Egypt. Soil samples were air-dried and ground so that it pass through a 2 mm sieve. The physical and chemical properties of the soil under study are presented in (Table 2). This prepared soil is used in all experiments under study. This experiment consists of twelve treatments: control (unamended soil, CK), bone + sulfur (B + S), bone + humic acid (B + HA), bone char + sulfur (BC + S), bone char + humic acid (BC + HA), modified bone char (MBC), modified bone char + humic acid (MBC+HA), bone char acidified with 0.1 N H_2SO_4 (0.1ABC), bone char acidified with 1 N H_2SO_4 (1ABC) phosphate rock (RP), phosphate rock + sulfur (RP+S), phosphate rock + humic acid (RP+HA). Each treatment consists of 100 grams of air-dried soil placed in airtight plastic jars (330 ml). B, BC, MBC, 0.1ABC, 1ABC, and RP were added to the soil in doses of 0.43, 0.29, 0.31, 0.29, 0.29, and 0.34 g per jar, respectively, and mixed well with the soil in all treatments on straight. The doses of these amendments were added based on the total phosphorus at 300 mg P kg^{-1} soil (equivalent to 300 kg P $feddan^{-1}$ or 714 kg P $hectare^{-1}$). However, HA and S were added to the soil in doses of 0.02 and 0.06 grams per jar, respectively. All treatments were moistened to 40% from saturation capacity by distilled water and incubated for 0, 7, 15, 30, 60, and 90 days under laboratory conditions at approximately 19 to 22°C in the dark and were performed in a completely randomized design. Typically, jar lids are opened to maintain aerobic conditions and moisture content by weighing the jars, compensating for moisture loss using distilled water. After each incubation period, soil samples were air-dried, crushed, and prepared for analysis of soil chemical properties.

This incubation experiment and all soil analysis were conducted at the Department of Soils and Water, Faculty of Agriculture, Assiut University, Assiut, Egypt.

Table 2. Some chemical and physical properties of the soil under study. Data were average ± standard error (SE).

Property	Unit	Value
Sand	g kg $^{-1}$	922 ± 8.48
Silt	g kg $^{-1}$	62 ± 2.82
Clay	g kg $^{-1}$	16 ± 5.65
Texture		Sand
O.M	g kg $^{-1}$	3.57±0.00
$CaCO_3$	g kg $^{-1}$	195 ± 4.24
pH (1:1)		7.83 ± 0.35
EC (1:1)	dS m $^{-1}$	0.70 ± 0.05
Available P	mg kg $^{-1}$	13.90 ± 0.99
Available K	mg kg $^{-1}$	148.04 ± 8.60

EC: electrical conductivity, O.M: Organic matter

Analysis of amendments

For all amendments, pH was measured in suspensions (1:5) and electrical conductivity (EC) was measured in the soil extracts (1:5). Available phosphorus (Olsen-P) in bone char was extracted using 0.5 M $NaHCO_3$ at pH 8.5 (Olsen et al., 1954). Phosphorus in the extracts was measured by colorimetric analysis using the chlorostannous phosphomolybdic acid method (Jackson, 1973). Total phosphorus in B, BC, MBC, 0.1ABC, 1ABC, RP, and HA was digested using concentrated H_2SO_4, HNO_3, and $HClO_4$ (Grimshaw, 1989). Phosphorus in the extracts was measured by colorimetric method using the chlorostannous phosphomolybdic acid method (Jackson, 1973).

Soil analysis

The particle size distribution of the soil under study was determined using the pipette method (Kroetsch and Wang, 2008). The calcium carbonate content of the soil was determined before the experiment using a calcium meter (Pansu and Gautheyrou, 2006*)*. The organic matter in the soil was determined by the dichromate oxidation procedure (Skjemstad and Baldock, 2008). The pH of the soil was measured in a suspension (1:1) via a glass electrode (Jackson, 1973). The electrical conductivity (EC) of the soil extracts at 1:1 extraction was measured using an electrical conductivity meter (Jackson, 1973). The soluble calcium in all soil extracts was determined by titration with Na_2EDTA solution (Jackson, 1973). The soluble sulfate was determined by the turbidity method (Baruah and Barthakur, 1997). Available phosphorus (Olsen-P) in soil samples was extracted with 0.5 M $NaHCO_3$ at pH 8.5 (Olsen *et al*., 1954). Phosphorus in the extracts was measured by colorimetric analysis using the chlorostane phosphomolybdic acid method (Jackson, 1973).

Statistical analysis

The collected data was statistically analyzed using SAS program version 9.00 (2002). The analysis of variance (ANOVA) was performed on the data. Significant differences among treatments were carried out by Tukey's honestly significant difference test (Tukey's HSD) at the 0.01 levels of probability (p).

Results and Discussions

Effect of amendments on soil pH

At the beginning of the incubation, the addition of B+S, B+HA, BC+S, MBC, MBC+HA, 0.1ABC, and 1ABC to calcareous sandy soil resulted in a significant decrease in pH value compared to the control (non-fertilized soil), while applying rest of the treatments in the soil did not show any statistically significant differences in the decrease in the soil pH (Figure 1). The pH value decreased from 7.83 (control) to 7.77, 7.78, 7.78,7.82, 7.77, 7.76, 7.51, 7.22, 7.83, 7.78, and 7.82 respectively. Moreover, applying B+S, B+HA, BC+S, BC+HA, MBC, MBC+HA, 0.1ABC, 1ABC, and RP+S to calcareous sandy soil resulted in a significant decrease in pH value compared to the control at one day of the incubation, the pH value decreased from 7.80 (control) to 7.70, 7.71, 7.70, 7.71, 7.68, 7.64, 7.47, 7.17, 7.78, 7.75, and 7.78 for B+S, B+HA, BC+S, BC+HA, MBC, MBC+HA, 0.1ABC, 1ABC, RP, RP+S, and RP+HA, respectively (Figure 1) Compared to the control treatment, the applications of B+S, B+HA, BC+S, BC+HA, MBC, MBC+HA, 0.1ABC, 1ABC, and RP+S resulted in a significant decrease in soil pH at fifteen days of the incubation, soil pH value decreased from 7.72 for control treatment to 7.61, 7.61, 7.59, 7.54, 7.51, 7.41, 7.43, 7.36, 7.71, 7.64, and 7.68 for B+S, B+HA, BC+S, BC+HA, MBC, MBC+HA, 0.1ABC, RP, RP+S, and RP+HA, respectively. At the end of the incubation (i.e., after 90 days), the applications of the B+S, B+HA, BC+S, BC+HA, MBC, MBC+HA, RP, RP+S, and RP+HA to decreased significantly soil pH in calcareous sandy soil compared to the control treatment, pH value decreased from 7.73 (control) to 7.53, 7.58, 7.52, 7.57, 7.50, 7.36, 7.49, 7.43, 7.65, 7.58, and 7.58 for B+S, B+HA, BC+S, BC+HA, MBC, MBC+HA, 0.1ABC, 1ABC, RP, RP+S, and RP+HA, respectively. Soil pH significantly decreased with increasing incubation periods under application of B+S, B+HA, BC+S, BC+HA, MBC, MBC+HA, RP, RP+S, and RP+HA to calcareous sandy soil compared to the control treatment. The soil pH values at the beginning of incubation (zero) were 7.84, 7.77, 7.78, 7.78, 7.82, 7.77, 7.76, 7.51, 7.83, 7.78 and 7.82 for control, B+S, B+HA, BC+S, BC+HA, MBC, MBC+HA, 0.1ABC, RP, RP+S, and RP+HA, respectively. However, these values of soil pH decreased to 7.73, 7.53, 7.58, 7.52, 7.57, 7.50, 7.36, 7.49, 7.65, 7.58, and 7.58 at the end of incubation time (i.e., after 90 days) for control, B+S, B+HA, BC+S, BC+HA, MBC, MBC+HA, 0.1ABC, RP, RP+S, and RP+HA, respectively (Figure 1). On the other hand, the applications of 1ABC to calcareous sandy soil decreased significantly at one day of the incubation, then soil pH increased significantly with increasing incubation periods. The lowest value of soil pH was observed in the 1ABC treatment after one day of incubation. While the highest value of soil pH was observed in the control treatment at the beginning of the experiment (zero day). Several studies have found that adding sulfur to alkaline soils resulted in a decrease in soil pH (Amin, 2018; Soaud *et al*., 2011). It was also found that treating bone char with sulfur resulted in a significant and significant decrease in soil pH at all stages of the incubation periods (Amin, 2020). This is due to the production of H_2SO_4 by sulfur-oxidizing microorganisms (Jaggi *et al*., 2005; Soaud *et al*., 2011). Also, adding elemental sulfur to some alkaline soils increased soil pH by 0.08 to 0.8 units compared to the control treatment (Soaud *et al*., 2011). Adding humic acid to alkaline soils caused a significant decrease in soil pH in addition to a significant increase in the availability of phosphorus and potassium (Li *et al*., 2019). These slight changes in soil pH in this study can be explained by the high buffering capacity of this soil that resists changes in soil pH. In general, soil pH is one of the soil chemical properties that plays an important role in nutrient availability and microbial activity (Amin, 2020).

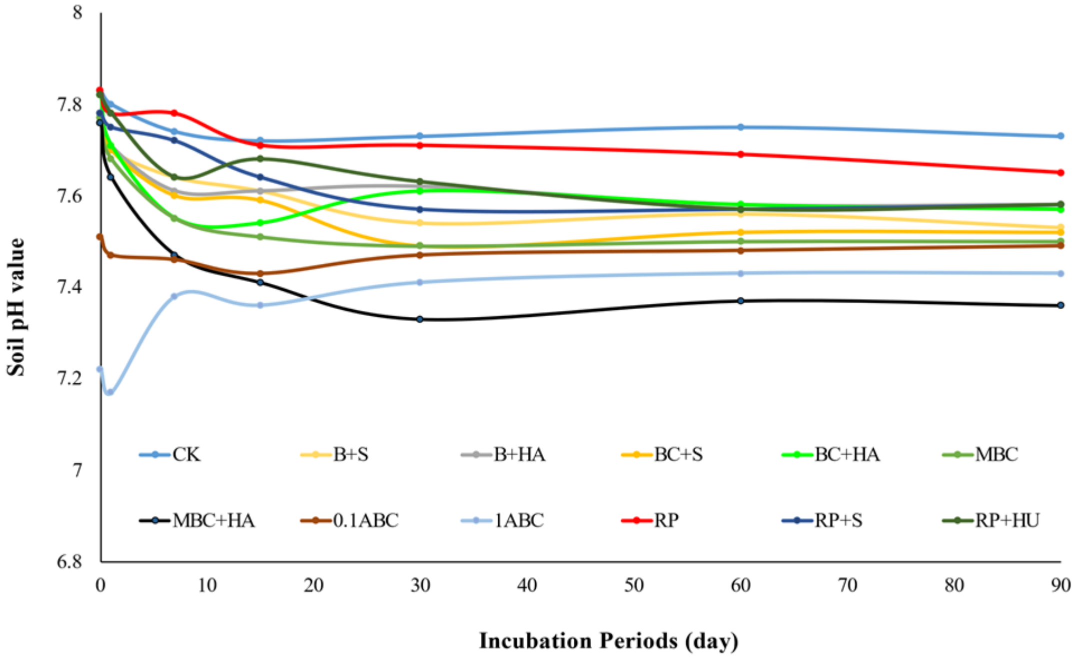

Figure 1. Changes in soil pH under different treatments during incubation periods. Each value represents the average of three replicates. CK: control (unamended soil); B+S: bone + sulfur; B+HA: bone + humic acid; BC+S: bone char + sulfur; BC+HA: bone char + humic acid; MBC: modified bone char; MBC+HA: modified bone char + humic acid; 0.1ABC: acidified bone char with 0.1 N H_2SO_4; 1ABC: acidified bone char with 1 N H_2SO_4; RP: rock phosphate; RP+S: rock phosphate + sulfur; RP+HA: rock phosphate + humic acid

Effect of amendments on soil electrical conductivity

At seven days of incubation, adding 1ABC treatment led to a significant increase in electrical conductivity (EC) compared to the control treatment, while adding rest of the treatments in the soil did not show any statistically significant differences in the EC value. The EC value in the soil was increased from 0.46 dS m^{-1} (control) to 0.60 dS m^{-1} (Table 3). Moreover, the EC increased significantly in calcareous sandy soil under the applications of B+S, BC + S, MBC + HA, 0.1ABC, 1ABC, and RP + S at 30th day of the incubation compared to the control, the EC value increased from 0.50 dS m^{-1} in the control treatment to 0.76, 0.63, 0.83, 0.63, 0.66, 0.83, 0.76, 0.90, 0.76 and 0.63 dS m^{-1} for B+S, B+HA, BC + S, BC + HA, MBC, MBC + HA, 0.1ABC, 1ABC, RP + S, and RP + HA, respectively (Table 4). At the end of the incubation, the applications of B+S, B +HA, BC+S, BC+HA, MBC, MBC+HA, 0.1ABC, 1ABC, RP+S and RP+HA to calcareous sandy led to significantly increased EC values compared to the control treatment, EC value increased from 0.53 dS m^{-1} for control treatment to 1.06, 0.73, 1.06, 0.66, 1.00, 1.06, 0.96, 1.06, 0.96, and 0.83 dS m^{-1} for B+S, B +HA, BC+S, BC+HA, MBC, MBC+HA, 0.1ABC, 1ABC, RP+S and RP+HA, respectively. The values of EC in the soil under study significantly increased with increasing incubation period. The EC values in the soil at the beginning of incubation (zero) were 0.43, 0.40, 0.40, 0.40, 0.46, 0.40, 0.43, 0.43, 0.36, 0.46, 0.46 and 0.53 for control, B+S, B+HA, BC+S, BC+HA, MBC, MBC+HA, 0.1ABC, RP, RP+S, and RP+HA, respectively. However, these values of EC increased to 0.53, 1.06, 0.73, 1.06, 0.66, 1.00, 1.06, 0.96, 1.06, 0.53, 0.96, and 0.83 dS m^{-1} for B+S, B +HA, BC+S, BC+HA, MBC, MBC+HA, 0.1ABC, 1ABC, RP, RP+S and RP+HA, respectively at the end of incubation time (i.e., after 90 days) for control, B+S, B+HA, BC+S, BC+HA, MBC, MBC+HA, 0.1ABC, 1ABC, RP, RP+S, and RP+HA, respectively (Table 3). The highest EC values were noticed at the applications of MBC+HA and 1ABC treatments at the end of incubation time (Table 3). The combined application of sulfur and bone char to calcareous sandy soil significantly increased the EC value with increasing incubation periods compared to the control treatment (Amin, 2020), which is due to the oxidation of elemental sulfur to produce H^{+} and SO_4^{-2} ions, which increases the salt content in the soil (Zhi-Hui *et al*., 2010).

Table 3. Electrical conductivity changes in sandy calcareous soil under different treatments during incubation periods. Each value represents the mean of three replicates. Different lowercase letters indicate significant differences between treatments using Tukey's Honestly Significant Difference test at P < 0.01.

Treatment	Electrical Conductivity (dS m^{-1})						
	Incubation Periods (day)						
	Zero	1	7	15	30	60	90
CK	0.43±0.03 nop	0.43±0.03 nop	0.46±0.03 mnop	0.46±0.03 mnop	0.50±0.00 imno	0.53±0.03 kimn	0.53±0.03 kimn
B+S	0.40±0.06 op	0.46±0.03 mnop	0.56±0.03 jklm	0.56±0.03 jkim	0.76±0.03 fgh	0.86±0.03 def	1.06±0.03 a
B+HA	0.40±0.06 op	0.40±0.00 op	0.43±0.03 nop	0.53±0.03 kimn	0.63±0.03 ijk	0.66±0.03 hij	0.73±0.03 ghi
BC+S	0.40±0.00 op	0.46±0.03 mnop	0.56±0.03 jklm	0.60±0.00 jki	0.83±0.03 efg	0.96±0.03 abcd	1.06±0.03 a
BC+HA	0.46±0.07 mnop	0.43±0.03 nop	0.50±0.00 imno	0.56±0.03 jkim	0.63±0.03 ijk	0.66±0.03 hij	0.66±0.03 hij
MBC	0.40±0.06 op	0.36±0.03 p	0.43±0.03 nop	0.46±0.03 mnop	0.66±0.03 hij	0.93±0.03 bcde	1.00±0.00 abc
MBC+HA	0.43±0.03 nop	0.46±0.03 mnop	0.53±0.03 kimn	0.56±0.03 jklm	0.83±0.03 efg	0.96±0.03 abcd	1.06±0.03 a
0.1ABC	0.43±0.03 nop	0.46±0.03 mnop	0.53±0.03 kimn	0.50±0.06 imno	0.76±0.03 fgh	0.90±0.00 cde	0.96±0.03 abcd
1ABC	0.36±0.03 p	0.43±0.03 nop	0.60±0.03 jki	0.63±0.03 ijk	0.90±0.00 cde	1.03±0.03 ab	1.06±0.03 a
RP	0.46±0.03 mnop	0.50±0.06 imno	0.56±0.03 jkim	0.53±0.03 kimn	0.50±0.00 imno	0.53±0.03 kimn	0.53±0.03 kimn
RP+S	0.46±0.07 mnop	0.46±0.03 mnop	0.56±0.03 jkim	0.56±0.03 jkim	0.76±0.03 fgh	0.90±0.00 cde	0.96±0.03 abcd
RP+HA	0.53±0.03 kimn	0.53±0.03 kimn	0.56±0.03 jkim	0.560.03 jkim	0.63±0.07 ijk	0.76±0.03 fgh	0.83±0.03 efg

CK: control (unamended soil); B+S: bone + sulfur; B+HA: bone + humic acid; BC+S: bone char + sulfur; BC+HA: bone char + humic acid; MBC: modified bone char; MBC+HA: modified bone char + humic acid; 0.1ABC: acidified bone char with 0.1 N H_2SO_4; 1ABC: acidified bone char with 1 N H_2SO_4; RP: rock phosphate; RP+S: rock phosphate + sulfur; RP+HA: rock phosphate + humic acid.

Effect of amendments on soluble calcium

Adding MBC, MBC + HA, and 1ABC applications led to an increase in the concentrations of soluble calcium in the soil compared to the control treatment (unfertilized soil) at the beginning of the study, while adding the rest of the treatments in the soil did not show any significant effect. Soil-dissolved calcium content was increased from 1.23 mmol kg^{-1} (control) to 1.33, 1.26, 1.36, 1.30, 1.40, 1.40, 1.33, 1.43, 1.33, and 1.26 mmol kg^{-1} for B+S, B +HA, BC + S, BC + HA, MBC, MBC+HA, 0.1ABC, 1ABC, RP+S, and RP+HA, respectively (Figure 2). At one day of the incubation, applying BC+S, MBC, MBC+HA, 0.1ABC, and 1ABC to calcareous sandy soil resulted in a significant increase in soluble calcium compared to the control, the soluble calcium concentration increased from 1.26 mmol kg^{-1} (control treatment) to 1.40, 1.30, 1.43, 1.36, 1.50, 1.46, 1.46, 1.66, and 1.30 for B+S, B+HA, BC+S, and BC+HA, MBC, MBC+HA, 0.1ABC, 1ABC, and RP+S, respectively. Significant increase in soluble calcium in soil solution under adding B+S, B+HA, BC+S, BC+HA, MBC, MBC+HA, 0.1ABC, 1ABC, RP+S, and RP+HA treatments to the soil at fifteen days of the incubation, dissolved calcium increased from 1.30 mmol kg^{-1} to 1.66, 1.46, 1.76, 1.56, 1.83, 2.16, 1.76, 2.43, 1.33, 1.53, and 1.53 mmol kg^{-1} for B+S, B+HA, BC+S, BC+HA, MBC, MBC+HA, 0.1ABC, 1ABC, RP, RP+S, and RP+HA, respectively (Figure 2). At the end of the incubation (i.e. after 90 days), All applications of amendments to the soil led to a significant increase in the concentration of dissolved calcium compared to the control treatment, the dissolved calcium concentration increased from 1.36 mmol kg^{-1} to 2.06, 1.83, 2.46, 1.83, 2.46, 2.56, 1.53, 1.90, 1.90, 1.63 and 1.73 mmol kg^{-1} for B+S, B+HA, BC+S, BC+HA, MBC, MBC+HA, 0.1ABC, 1ABC, RP, RP+S, and RP+HA, respectively. All applications, except the control treatment significantly increased the soluble calcium with increasing incubation periods until it reached its maximum at 60 days and then decreased with increasing incubation periods. The concentration of soluble calcium in the soil at the beginning of incubation (zero) were 1.23, 1.33, 1.26, 1.36, 1.30, 1.40, 1.40, 1.33, 1.43, 1.23, 1.33, and 1.26 for control, B+S, B+HA, BC+S, BC+HA, MBC, MBC+HA, 0.1ABC, RP, RP+S, and RP+HA, respectively. However, these concentrations of soluble calcium increased to 1.36, 2.06, 1.83, 2.46, 1.83, 2.46, 2.56, 1.53, 1.90, 1.90, 1.63, and 1.73 for B+S, B +HA, BC+S, BC+HA, MBC, MBC+HA, 0.1ABC, 1ABC, RP, RP+S and RP+HA, respectively at 90 days of the incubation time. The highest concentration of dissolved calcium was observed in the MBC+HA treatment after 60 days of incubation (Figure 2). The lowest concentration of dissolved calcium was observed in the control treatment before the beginning of incubation (day zero). The increase in Ca concentration in soil solution with the addition of elemental sulfur in sandy calcareous soil is due to the partial biodegradation of apatite from bovine bone char and calcium carbonate from soil to provide Ca (Amin, 2020). Elemental sulfur in alkaline calcareous soil is thus chemically oxidized to produce H_2SO_4, which lowers soil pH and dissolves $CaCO_3$, which in turn improves the availability of soil nutrients such as phosphorus and some micronutrients (Soaud *et al*., 2011).

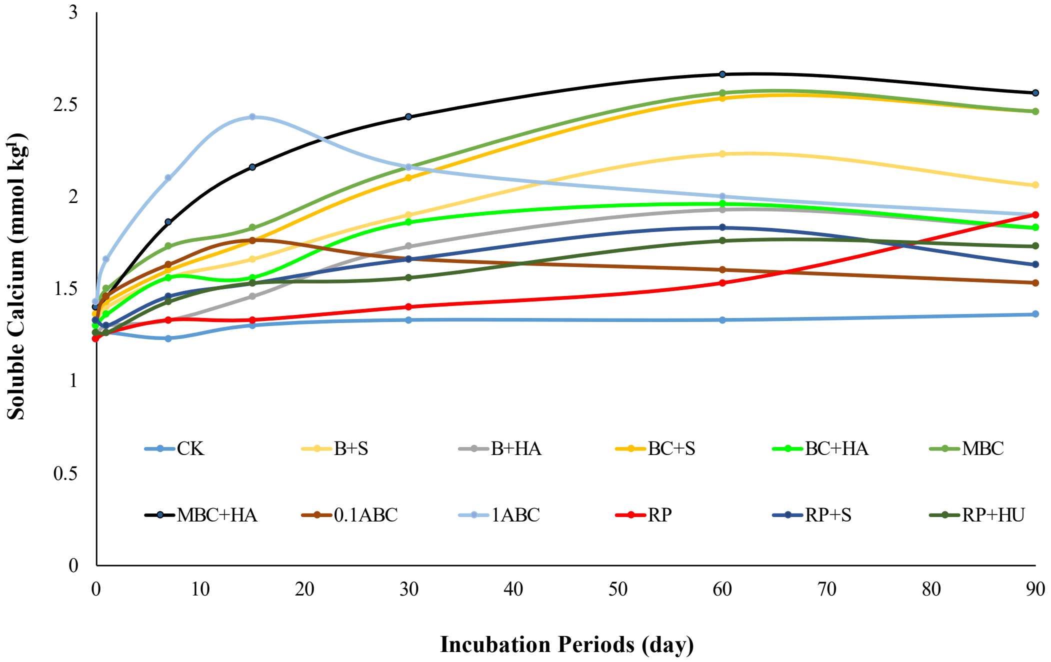

Figure 2. Variability of soluble calcium in calcareous sandy soil under different treatments during incubation periods. Each value represents the average of three replicates. CK: control (unamended soil); B+S: bone + sulfur; B+HA: bone + humic acid; BC+S: bone char + sulfur; BC+HA: bone char + humic acid; MBC: modified bone char; MBC+HA: modified bone char + humic acid; 0.1ABC: acidified bone char with 0.1 N H_2SO_4; 1ABC: acidified bone char with 1 N H_2SO_4; RP: rock phosphate; RP+S: rock phosphate + sulfur; RP+HA: rock phosphate + humic acid.

Effect of amendments on soluble sulfate

Adding 0.1ABC and 1ABC treatments led to an increase in soluble sulfate concentrations in the soil compared to the control treatment (unfertilized soil) at the beginning of the incubation, while adding the rest of the treatments in the soil did not show any significant effect (Table 4). The soluble sulfate content in the soil solution was increased from 0.71 mmol kg-1 (control) to 0.78, 0.72, 0.72, 0.72, 0.72, 0.83, 1.08, 0.73, 0.72, and 0.73 mmol kg-1 for B+S, B+HA, BC+HA, MBC, MBC+HA, 0.1ABC, 1ABC, RP, RP+S, and RP+HA, respectively. At one day of the incubation, the application of B+S, 0.1ABC, and 1ABC to calcareous sandy soil significantly increased soluble sulfate compared to the control, the soluble sulfate increased from 0.69 mmol kg-1 (control treatment) to 0.84, 0.74. 0.82, 0.72, 0.80, 0.81, 0.93, 1.17, and 0.76 mmol kg-1 for B+S, B+HA, BC+S, BC+HA, MBC, MBC+HA, 0.1ABC, 1ABC, and RP+S, respectively (Table 4). After fifteen days of incubation, applying B+S, BC+S, MBC, MBC+HA, 0.1ABC, 1ABC, and RP+S significantly increased soluble sulfate in the soil solution. soluble sulfate increased from 0.68 mmol kg-1 to 1.12, 0.73, 1.16, 0.75, 1.26, 1.35, 1.33, 1.83, 0.71, 1.04, and 0.71 mmol kg-1 for B+S, B+HA, BC+S, BC+HA, MBC, MBC+HA, 0.1ABC, 1ABC, RP, RP+S, and RP+HA, respectively. At the end of the incubation period (i.e., after 90 days), applications of B+S, BC+S, MBC, MBC+HA, 0.1ABC, 1ABC, and RP+S resulted in a significant increase in soluble sulfate compared to the control treatment. The soluble sulfate increased from 0.70 mmol kg-1 to 2.09, 0.73, 2.13, 0.82, 2.18, 2.53, 1.18, 1.63, 0.74, 1.90, and 0.77 mmol kg-1 for B+S, B+HA,

BC+S, BC+HA, MBC, MBC+HA, 0.1ABC, 1ABC, RP, RP+S, and RP+HA, respectively (Table 4). Applications of B+S, BC+S, MBC, MBC+HA, 0.1ABC, 1ABC, and RP+S significantly increased the soluble sulfate with increasing incubation periods. The concentrations of soluble sulfate in the soil at the beginning of incubation (zero) were 0.78, 0.72, 0.69, 0.72, 0.72, 0.72, 0.83, 1.08, 0.73, 0.72, and 0.73 mmol kg-1 for B+S, B+HA, BC+S, BC+HA, MBC, MBC+HA, 0.1ABC, 1ABC, RP, RP+S, and RP+HA, respectively. However, the soluble sulfate concentrations increased to 2.09, 0.73, 2.13, 0.82, 2.18, 2.53, 1.18, 1.63, 0.74, 1.90, and 0.77 mmol kg-1 for B+S, B+HA, BC+S, BC+HA., MBC, MBC+HA, 0.1ABC, 1ABC, RP, RP+S and RP+HA, respectively at 90 days of incubation time (Table 4). The highest soluble sulfate concentration was observed in the MBC+HA treatment after 90 days of incubation. The lowest concentration of soluble sulfate was observed in the RP treatment after seven days of incubation.

Table 4. Changes of soluble sulfur under different treatments during incubation periods Each value represents the average of three replicates. Different lowercase letters indicate the significant differences among treatments by using Tukey's Honestly Significant Difference test at P < 0.01.

Treatment	Soluble Sulfate (mmol kg $^{-1}$)						
	Incubation Periods (day)						
	Zero	1	7	15	30	60	90
CK	0.71±0.03 uvwxy	0.69±0.03 uvwxy	0.63±0.01 xy	0.68±0.04 uvwxy	0.70±0.03 uvwxy	0.75±0.05 uvwxy	0.70±0.03 uvwxy
B+S	0.78±0.02 tuvwxy	0.84±0.01 stu	1.00±0.01 opqr	1.12±0.04 lmnop	1.35±0.01 hi	1.80±0.05 ef	2.09±0.02 bc
B+HA	0.72±0.04 uvwxy	0.74±0.03 uvwxy	0.65±0.01 wxy	0.73±0.04 uvwxy	0.78±0.04 tuvwxy	0.77±0.04 tuvwxy	0.73±0.05 uvwxy
BC+S	0.69±0.04 uvwxy	0.82±0.01 stuvw	1.03±0.02 nopq	1.16±0.04 lmno	1.35±0.04 hi	1.91±0.02 de	2.13±0.03 b
BC+HA	0.72±0.04 uvwxy	0.72±0.03 uvwxy	0.78±0.11 tuvwxy	0.75±0.01 uvwxy	0.75±0.03 uvwxy	0.83±0.04 stuv	0.82±0.02 stuvw
MBC	0.72±0.05 uvwxy	0.80±0.02 tuvw	1.05±0.02 nopq	1.26±0.03 hijkl	1.37±0.02 hi	1.97±0.04 cd	2.18±0.04 b
MBC+HA	0.72±0.05 uvwxy	0.81±0.01 tuvw	1.09±0.02 mnop	1.35±0.06 hi	1.56±0.06 g	2.06±0.06 bc	2.53±0.04 a
0.1ABC	0.83±0.02 stu	0.93±0.04 qrst	1.17±0.01 klmn	1.33±0.02 hij	1.32±0.04 hijk	1.22±0.06 ijklm	1.18±0.03 jklmn
1ABC	1.08±0.02 mnopq	1.17±0.03 klmn	1.41±0.05 h	1.83±0.12 def	1.69±0.05 fg	1.55±0.04 g	1.63±0.15 g
RP	0.73±0.04 uvwxy	0.69±0.03 uvwxy	0.62±0.02 y	0.71±0.04 uvwxy	0.69±0.04 uvwxy	0.77±0.06 tuvwxy	0.74±0.04 uvwxy
RP+S	0.72±0.05 uvwxy	0.76±0.01 uvwxy	0.85±0.11 rstu	1.04±0.02 nopq	1.26±0.05 hijkl	1.65±0.05 g	1.90±0.11 de
RP+HA	0.73±0.04 uvwxy	0.68±0.05 uvwxy	0.65±0.02 vwxy	0.71±0.01 uvwxy	0.97±0.17 pqrs	0.80±0.04 tuvwx	0.77±0.04 tuvwxy

CK: control (unamended soil); B+S: bone + sulfur; B+HA: bone + humic acid; BC+S: bone char + sulfur; BC+HA: bone char + humic acid; MBC: modified bone char; MBC+HA: modified bone char + humic acid; 0.1ABC: acidified bone char with 0.1 N H_2SO_4; 1ABC: acidified bone char with 1 N H_2SO_4; RP: rock phosphate; RP+S: rock phosphate + sulfur; RP+HA: rock phosphate + humic acid.

Addition of elemental sulfur to some sandy calcareous soils significantly increased sulfate concentration (Soaud *et al*., 2011). This is due to the oxidation of sulfur by soil sulfur-oxidizing microorganisms. Moreover, each gram of soil contains about one billion bacterial cells, in addition to 200 including sulfur-oxidizing bacteria and one million fungal hyphae (Zhang *et al*., 2014). In treatments where sulfur was not added, an increase in sulfate concentration in the soil solution was observed. This is due to the decomposition of insoluble sulfur minerals in these soils (Soaud *et al*., 2011).

Effect of amendments on soil available phosphorus

At the beginning of the incubation, applying 1ABC to calcareous sandy soil led to a significant increase ($P \leq 0.01$) in the phosphorus availability (Olsen-P) compared to the control treatment (unfertilized soil), while adding the rest of the treatments did not cause any significant effect (Figure 3). The concentration of available phosphorus in the soil was increased from 13.06 mg kg^{-1} (control) to 13.78, 13.23, 14.44, 13.77, 14.71, 14.46, 14.36, 15.63, 13.56, and 14.10 mg kg^{-1} for B+S, B+HA, BC+S, BC+HA, MBC, MBC+HA, 0.1ABC, 1ABC, RP+S, and RP+HA, respectively. At one day of incubation, the application of BC+S, MBC, MBC + HA, 0.1ABC, and 1ABC to calcareous sandy soil significantly increased available phosphorus compared to the control treatment, where available phosphorus concentration increased from 12.60 mg kg^{-1} (control) to 14.18, 13.33, 14.82, 13.71, 14.79, 14.73, 14.91, 17.88, 13.71, and 13.45 mg kg^{-1} for B+S, B+HA, BC+S, BC+HA, MBC, MBC+ HA, 0.1ABC, 1ABC, RP+S, and RP+HA, respectively (Figure 3). After fifteen days of incubation, the applications of B+S, B+HA, BC+S, BC+HA, MBC, MBC+HA, 0.1ABC, 1ABC, RP+S, and RP+HA caused a significant increase in phosphorus availability, where available phosphorus increased from 12.54 mg kg^{-1} to 19.78, 15.95, 22.10, 15.85, 23.84, 21.71, 19.30, 26.83, 14.08, 16.66, and 15.64 mg kg^{-1} for B+S, B+HA, BC+S, BC+HA, MBC, MBC+HA, 0.1ABC, 1ABC, RP, RP+S, and RP+HA, respectively (Table 7). At the end of the incubation period (i.e. after 90 days), adding B+S, B+HA, BC+S, BC+HA, MBC, MBC+HA, 0.1ABC, 1ABC, RP+S, and RP+ led to a significant increase in available phosphorus compared to the control treatment. The concentration of available phosphorus was increased from 13.30 mg kg^{-1} to 20.70, 17.52, 29.54, 17.47, 31.20, 32.43, 17.49, 22.82, 13.91, 19.87, and 16.70 mg kg^{-1} for B+S, B+HA, BC+S, BC+HA, MBC, MBC+HA, 0.1ABC, 1ABC, RP, RP+S, and RP+HA, respectively. Applying B+S, B+HA, BC+S, BC+HA, MBC, MBC+HA, 0.1ABC, 1ABC, RP+S, and RP+HA caused an increase significant in phosphorus availability with increasing incubation periods. The concentration of available phosphorus in the soil at the beginning of incubation (zero) was 13.06, 13.78, 13.23, 14.44, 13.77, 14.71, 14.46, 14.36, 15.63, 12.41, 13.56, and 14.10 mg kg^{-1} for control, B+S, B+HA, BC+S, BC+HA, MBC, MBC+HA, 0.1ABC, 1ABC, RP, RP+S, and RP+HA, respectively (Figure 3). However, these available phosphorus concentrations increased to 13.30, 20.70, 17.52, 29.54, 17.47, 31.20, 32.43, 17.49, 22.82, 13.91, 19.87, and 16.70 mg kg^{-1} for B+S, B+HA, BC+S, BC+HA, MBC, MBC+ HA, 0.1ABC, 1ABC, RP, RP+S and RP+HA, respectively at 90 days of incubation time (Figure 3). The highest concentration of available phosphorus was observed in the MBC+HA treatment at 60 days of incubation. The lowest concentration of available phosphorus was observed in the RP treatment at one day of incubation. In this study, a gradual increase in the available P amounts with incubation periods was observed, which could be attributed to the release of P from these P-rich amendments. Three main factors play an important role in controlling and balancing the concentration of P in soil solution: first, the pH value of the soil, second, the concentration of calcium which precipitates with P ions, and third, the concentration of sulfate and bicarbonate which in turn compete with P for exchange sites (Hinsinger, 2001). Thus, the efficiency of calcium phosphate precipitation is highly influenced by the ratio of calcium to phosphorus at a constant pH (Mekmene *et al*., 2009). The combined application of bovine bone char and elemental sulfur may be beneficial for increasing the availability of P in sandy soils poor in P. The application of sulfur increased the release of P from bovine bone char due to the increased solubility of calcium phosphate resulting from the oxidation of elemental sulfur to produce H^+ and SO_2^{-4} ions, which significantly decreased the soil pH (Zhi-Hui *et al*., 2010). Enrichment of sulfur-rich bone char with phosphorus, calcium and sulfur enhances direct biological activity due to the oxidation of elemental sulfur by bacteria, thus improving the availability of nutrients released from bone char (Zimmer *et al*., 2018). The release of phosphorus from fixation sites was due to adsorption from exchange sites by sulfate ions with the application of sulfur in calcareous soils (Jaggi *et al*., 2005). The availability of phosphorus in calcareous and non-calcareous soils increased after the addition of organic matter and humic acid with phosphate fertilizers. This increase is due to the high capacity of organic matter and humic acid to chelate calcium in phosphorus-fixing soils (Toor, 2009).

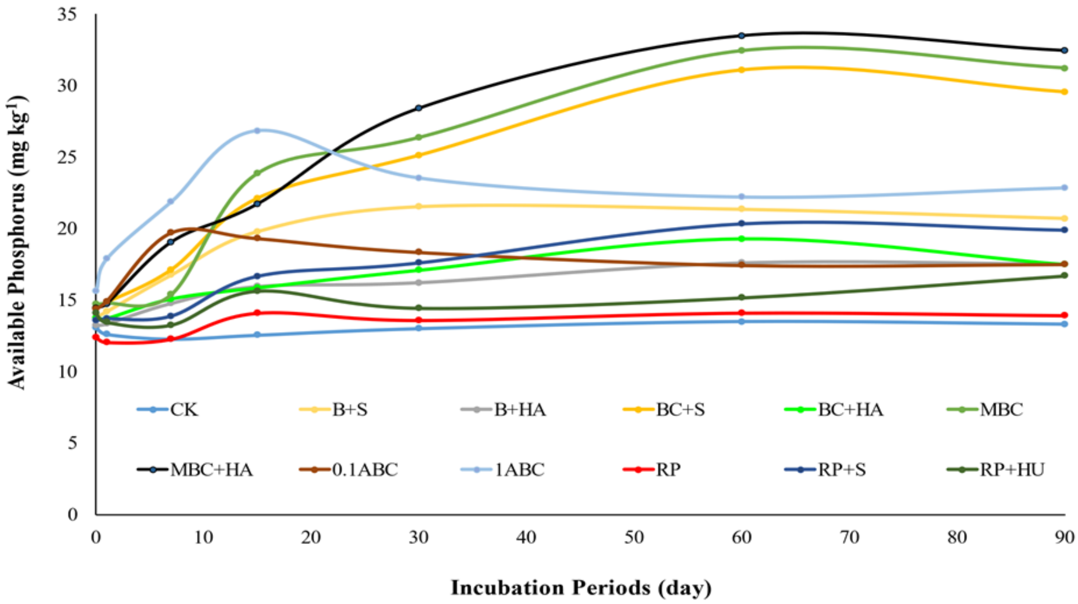

Figure 3. Available phosphorus as influenced by different treatments during the incubation periods. Each value indicates the average of three replicates with the standard error shown by the vertical bars. CK: control (unamended soil); B+S: bone + sulfur; B+HA: bone + humic acid; BC+S: bone char + sulfur; BC+HA: bone char + humic acid; MBC: modified bone char; MBC+HA: modified bone char + humic acid; 0.1ABC: acidified bone char with 0.1 N H_2SO_4; 1ABC: acidified bone char with 1 N H_2SO_4; RP: rock phosphate; RP+S: rock phosphate + sulfur; RP+HA: rock phosphate + humic acid.

Conclusion

In developing countries, slaughterhouse waste as bone is a serious problem, so it is recycled by pyrolyzing and converting it into bone char that is used as an alternative to chemical phosphate fertilizers in the soil and has become one of the most used solutions. Bone char is considered one potential source of renewable, clean, and low-cost phosphate fertilizer. The results of the present study revealed that the application of elemental sulfur and humic acid with bone char represents a promising and sustainable strategy to enhance phosphorus availability in calcareous sandy soil.

Therefore, we recommend applying bone char with sulfur in the calcareous sandy soil as a slow-release fertilizer as a source of phosphorus because of cheap sulfur. Generally, utilizing bone char as a slow-release fertilizer is potentially beneficial because it reduces the hazard of excessive fertilizing and nutrient leaching which have negative impacts on the ecosystem.

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تحرر الفوســـفور في تربة رملية جيرية كدالة لإضـــافة فحم العظام المعدل وفحم العظام مع الكبريت وحمض الهيوميك: دراسة حضانة

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الملخص

تهدف هذه الدراســـة إلى تقييم تأثير إضـــافة فحم العظام المعدل وفحم العظام مع الكبريت وحمض الهيوميك على بعض الخصائص الكيميائية وتوفر الفسفور في تربة رملية جيرية. تتكون هذه التجربة من اثني عشـــر معاملة: الكنترول (CK)، العظام + الكبريت (B+S)، العظام + حمض الهيوميك (B+HA)، فحم العظام + الكبريت (BC+S)، فحم العظام + حمض الهيوميك (BC+HA)، فحم العظام المعدل (MBC)، فحم العظام المعدل + حمض الهيوميك (MBC+HA)، فحم العظام المحمض بحمض الكبريتيك 0.1 ع (0.1ABC)، فحم العظام المحمض بحمض الكبريتيك 1ع(1ABC) ، صخور الفوسفات (RP)، صخر الفوسفات + الكبريت (RP+S)، صخر الفوسفات + حمض الهيوميك (RP+HA). تم تحضـــين هذه التجربة لمدة صـــفر، 1، 7، 15، 30، 60 و90 يومًا تحت ظروف المعمل. في نهاية فترة التحضين، أدى إضافة B+S، B+HA، BC+S، BC+HA، MBC، MBC+HA، 0.1ABC، 1ABC، RP+S و RP+HA إلى زيادة كبيرة في الفوسفور المتاح مقارنة بالمعاملة الكنترول. وكشفت النتائج التي تم الحصـــول عليها من هذه الدراســـة أن أعلى محتوى من الفوســـفور المنطلق من فحم العظام ظهر في معاملات BC+S ، MBC+HA و MBC.كما زاد الفوسفور المتاح في التربة مع زيادة فترة التحضين. انخفض الرقم الهيدروجيني للتربة بشكل ملحوظ مع زيادة فترات التحضين عند إضافة B+S، B+HA، BC+S، BC+HA، MBC، MBC+HA، RP، RP+S ، RP+HA مقارنة بمعاملة الكنترول. بناءاً عليه، يمكننا اقتراح تطبيق فحم العظام مع الكبريت كمصدر بديل وآمن للأسمدة الفوسفاتية في الزراعة المستدامة لأنه صديق للبيئة واقتصادي. ويمكن أن يحل فحم العظام محل الأسمدة الكيماوية المصنعة من صخور الفوسفات.

الكلمات المفتاحية: *الكبريت، توافر الفوسفور، حمض الهيوميك، فحم العظام، فحم العظام المعدل.*