

Evaluation of Poultry Manure and Acidified Water for Improving Phosphorus Utilization from Bone Char: A Comparative Study

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ANIMAL bone char (BC) is, recently, evaluated as one of potentially sustainable sources of phosphate fertilizer to face the accelerated depletion of mined phosphate rock (PR). Further explorations are needed to investigate its behavior when applied to soils in comparison with direct application of PR. Column leaching and greenhouse experiments were carried out to evaluate and compare the behavior of both BC and PR. Results of column leaching experiments showed that water soluble phosphorus (WSP) released from BC-filled columns represented 5 to 476 folds of that released from PR columns throughout the experiment period. Mixing poultry manure (PL) with BC or PR enhanced rate of columns leaching of WSP particularly with increasing the rate of application. Leaching by acidified water significantly increased the solubility of BC-P and PR-P compared with those released in water leachates. Similar increases were only obtained in PL:BC mixtures whereas the increases in acidified WSP were observed only in PR columns and did not occur in PL:PR mixtures. Besides the occurrence of organic fractions of P in BC, poultry litter degradation derivatives are, probably, serve as potential complexing legends with calcium and cause a further release of P from BC in water leachate. While the huge amounts of protons (H^+) in acidified water play a crucial role in apatite dissolution in both sources (BC and PR). Results of P availability after leaching showed that Olsen-P was greatly higher in all columns of BC than PR. In BC columns, concentrations of formic acid-P and citric acid-P resulted from water leached columns were higher than those resulted from acidified water leached ones while; there was no significant changes in PR columns. The higher solubility and availability of BC-P than PR-P were translated in higher biomass and grain yield of maize treated by BC-P in comparison with those treated with PR-P and their mixtures with PL.

Keywords: Animal waste, Sustainable phosphate source, Poultry litter, Phosphate rock, P availability.

Introduction

Phosphorus (P) is an essential macronutrient that is critically needed for the normal functioning of ecosystems and has no substitute in food production. The availability of soil native P to plant growth and organisms' propagation is seldom covering their requirements and thus application of P fertilizers is recommended to increase soil productivity. According to the P fertilizer industry records, phosphate rock as a non-renewable raw material of P, which is going towards depletion (Koppelaar & Weikard, 2013). It is estimated that demand would outstrip supply of rock phosphate

in the next 50–100 years (Smil, 2000; Cordell et al., 2009; Gilbert, 2009). The phosphorus scarcity challenge means that other sources of P has to be recovered for reuse as fertilizers to substitute phosphate rock (Cordell et al., 2011). Phosphorus recovery from waste materials is considered as one of the major solutions to overcome the current depletion in mined-P resources all over the world. Economically and technically, the recovery of P from animal wastes is the most suitable and tractable solution. (Rittmann et al., 2011).

Animal bones belong to the group of phosphorus-rich wastes. Calcium phosphates are

the primary constituents of bones. Bone mineral structure is commonly classified as biogenic apatite (LeGeros, 1994; Deydier *et al.*, 2005, Wopenka & Pasteris, 2005; Zwetsloot *et al.*, 2014). The incorporation of animal bones in fertilizers is the only rational way of managing it, especially after the EU prohibited the use of meat and bone meal in animal feed after crisis of the bovine spongiform encephalopathy (BSE). High-temperature of pyrolysis during charring animal bones may overcome this problem through incineration and heat sterilization (European Union, 2002; Chaala & Roy, 2003; Jeng *et al.* 2006; Cascarosa *et al.*, 2012). Warren *et al.* (2009), Weber *et al.* (2014), El-Refaey *et al.* (2015), Zwetsloot *et al.*, (2014 and 2016); Buss *et al.* (2016) have shown that P in bone char is as effective as mineral P fertilizers besides being more economic alternative. X-ray diffraction patterns of bone char and phosphate rock displayed evident similarities and pointed out to the analogy between the structures of PR-apatite and bone char -apatite mineral. Moreover, water soluble-P, Olsen-P, formic acid-P and citric acid-P extracted from bone char showed higher concentrations than those obtained from PR (El-Refaey *et al.*, 2015). Moreover, nearly null or low concentrations of heavy metal contents, such as Pb, Ni, Cr and Cd could be found in bone char compared with phosphate rock and/or triple superphosphate (Deydier *et al.*, 2005; Siebers & Leinweber, 2013; El-Refaey *et al.*, 2015). Warren *et al.* (2009) reported that the facts of the soils and circumstances when PR is useful as a fertilizer can be applied also to bone char. So, the soil factors that control P dissolution for phosphate rock appears to the same for bone char. This soil properties were those associated to soil acidity and the more exchangeable acidity and lower the pH, the greater the dissolution of bone char. To be utilized by plants, phosphorus should first be dissolved. Several attempts were used to increase the efficiency of PR-P solubilization including using the acidifier microbes (Bianco & Defez, 2010; Vassilev *et al.*, 2013; Sharma *et al.*, 2013; Mendes *et al.*, 2014), organic manure and organic acids (Al-Oud, 2011) or even through plant exudates (Hinsinger, 2001; Hinsinger *et al.*, 2003; Rengel & Marschner, 2005). P-recovery by acid leaching is one of the traditional methods and sulphuric acid is considered the economic most common leaching agent used for solubilising P from phosphate rock (Franz, 2008; Petzet *et al.*, 2012; Atienza-Martínez *et al.*, 2014). Poultry litter, mixture of poultry manure and bedding materials, is valuable amendments acidic conditions in the

soil (Brown *et al.* 2005; Xie *et al.*, 2015) and can be effectively contribute in increasing P solubilization.

In the present investigation, the dissolution and bioavailability of P in bone char produced by thermo-chemical conversion was evaluated its suitability to substitute rock phosphate to overcome limited P resources and also assess the ability of bone char as a P-amendment to improve plant growth and P availability in soil. P recovery from BC and PR was studied using leaching column experiment by distilled water and acidified water amended with different ratios of poultry litter. In addition, P-uptake by maize from amended soil with BC was considered in comparison with PR.

Materials and Methods

Materials

The animal (cattle) bone was collected from the local market (Alexandria, Egypt), leached with diluted acid solution (0.005 M HNO₃), then leached several times with deionized water for discard meat residues and oven-dried for 24 hours at 70 °C. Dried animal bones were then converted into bone char (BC) through thermo-chemical conversion at 650°C for 2 hours according to El-Refaey *et al.* (2015). Phosphate rock (PR) was purchased from El-Nasr Mining Company, Aswan, Egypt. Both BC and PR were crushed by hand in a porcelain mill, passed through 0.5-mm polypropylene sieve and stored in plastic bottles. Table (1) summarized elemental analysis and P fractions of BC and PR (El-Refaey *et al.*, 2015).

Poultry litter (PL) was obtained from the Poultry Farm of Agricultural Research Station, Faculty of Agriculture, Alexandria University. PL was oven-dried for 48 h at 60 °C and the contents of carbon, phosphorus and calcium were analyzed as outlined by Crompton (2000) then the Ca/P ratio was calculated. PL contained 25.2% C, 3.43% P₂O₅ and 3.5% CaO and calculated Ca/P ratio was 1.44.

Sand samples used in the experiments were treated by diluted hydrochloric acid, for two days, to decompose carbonates (Tributh and Lagaly, 1986), citrate-dithionite solution to dissolve oxides and hydroxides (Stul and van Leemput, 1982) and hydrogen peroxide to oxidize organic matter (Anderson, 1961). Samples were then leached several times by distilled water then dried at 105 °C for 24 h and stored in plastic jars.

TABLE 1: Elemental analysis and P fractions of bone char (BC) and phosphate rock (PR).

Measured parameters	BC	PR		
Total content (%)				
P ₂ O ₅	36.07	33.21		
CaO	38.08	35.24		
Ca/P ratio	1.73	1.74		
C	5.86	0.28		
N	1.95	0.18		
Total carbonate	6.21	10.45		
Heavy metals (mg kg⁻¹)				
Cd	2.50	7.20		
Cr	6.50	91.00		
Pb	nd*	nd		
Cu	nd	7.75		
Fe	17.00	12.50		
Mn	8.00	13.00		
Ni	nd	nd		
P fractions				
	mg g ⁻¹	% of total P	mg g ⁻¹	% of total P
WS- P ₂ O ₅	0.44	0.12	0.05	0.02
Olsen- P ₂ O ₅	3.44	0.95	0.12	0.04
FA- P ₂ O ₅	312.95	86.77	170.38	51.31
CA- P ₂ O ₅	139.30	38.62	105.34	31.72

• nd: not detected.

Column Leaching Experiment

A leaching column experiment was carried out in Pyrex glass column (2.0-cm in diameter and 14.0-cm in height). Glass wool plugs (2-cm distance) at the base of the columns was used to maintain and facilitate continuous flow of leaching solutions. Columns were then packed with washed sand (equivalent to 25 grams dry weight) together with one of the following treatments i.e. 25.0 grams of BC, 25.0 g PR or with ratios of BC (or PR): PL equal 25:0(CR0), 25:2.5 (CR1), 25:5 (CR2) or 25:12.5 (CR3). Columns were initially flushed with distilled water for 2 h and the volume of flushed water was collected to represent zero time sampling. Afterwards, columns were splitted into two groups; the first ones were leached with distilled water and the others were leached with acidified water (0.005M H₂SO₄). A peristaltic pump (ISMATEC ISM 931, Switzerland) was used to flow the influents of the column to maintain a constant upward flow rate in the column equals 0.25 mL min⁻¹. Leachates were collected daily for 30 days and analyzed for their pH and P-contents. At the end of leaching experiment, the columns were evacuated and their contents were oven-dried at 105 °C for 24 hours. Then, the oven-dried contents were crushed and sieved using 0.5-cm polypropylene sieve. Various extractable fractions: water soluble (WS-P), Olsen (O-P), 2% formic acid(FA-P) and 2% citric acid (CA-P), were determined in each column.

Water soluble P (WS-P):

One gram of the sample was transferred into 250-mL conical flasks then 50 mL distilled water was added and the flask was shaken for one hour. The suspensions were filtered using filter paper Whatman No. 42 and P concentration was determined in the supernatants.

Olsen P fraction:

According to Murphy & Riley (1962), 2.0g sample was shaken for 30 minutes with 40.0 mL 0.5 M sodium bicarbonate (NaHCO₃) solution adjusted to pH 8.5 with 0.01 mol L⁻¹ NaOH or 0.01 mol L⁻¹ HCl. Suspensions were filtered through filter papers Whatmann No. 42 and P was then measured in supernatants.

Formic acid-P fraction (FA-P)

A 0.4g sample was extracted with 40 mL 2% formic acid at 23 °C for 1 hour (Hoffman & Mager, 1953). The samples were then centrifuged at 10,000 rpm for 5 min. and filtered using 0.45µm Millipore filter.

Citric acid-P fraction (CA-P)

0.4g soils were shaken with 40 mL 2% citric acid at 23 °C for 1 hour. The samples were then centrifuged at 10,000 rpm for 5 min, filtered using 0.45µm Millipore filter and the residue was extracted again with another 40 mL of 2% fresh citric acid.

Concentration of phosphorus was measured colorimetrically using ascorbic acid-molybdate blue method (Olsen & Sommers, 1982) at wavelength 882 nm or using vanado-molybdate yellow method at wavelength 445 nm (Chapmann & Pratt, 1961) depending on the concentration ranges of P as mentioned above using UV/VIS double beam JENWAY spectrophotometer model 6850. All P concentrations and pH measurements were carried out in duplicate.

Greenhouse experiment

The phytoavailability of P in bone char was examined through evaluating P- uptake by maize plants versus phosphate rock. The experiment was laid out in a randomized complete block design with four replicates of plots (120 cm x 70 cm) containing clay soils. Soil properties are listed in Table 2 and located according to global positioning system (GPS) at “26.56’29°56 east and “02.11’31°13 north. The plot received a fixed rate of applied P equivalent to 46 kg P per hectare based on P-content in the different P-sources. The experimental treatments were as follows: PR or BC applied either solely or in mixtures with PL i.e. 10%PL+90%PR (BC), 20%PL+80%PR(BC), 50%PL+50%PR(BC). Seeds of maize cultivar (Giza176, monohybrid) were then sown, in all

plots, in two rows, four plants per row and all plots received the recommended dose of N and K as recommended by the Ministry of Agriculture. Soil properties are listed in Table (2). BC and PR and their ratios with PL which represented the P fertilizer were applied to soil with N and K. The applied rate of P was 105 kg P₂O₅ per hectare based on the content of P₂O₅ in BC and PR. This rate of applied P was fixed and PL was added by 10, 20 and 50% of applied phosphate (the treatments were 105 kg P₂O₅ (R0), 105 kg P₂O₅+10.5 kg PL (R1), 105 kg P₂O₅+21 kg PL (R2) and 105 kg P₂O₅+52.5 kg PL (R3)). Potassium was applied at the rate of 50 kg K per hectare in the form of potassium sulfate and Nitrogen application was divided to three dose 60 kg N per hectare). All additives were thoroughly mixed in the 15-cm soil surface before sowing. Irrigation schedule was conducted according to maintain the moisture content in soil at approximately field capacity. Leaf samples were taken at tasseling growth stage for detecting the P and Ca uptake. At the physiological maturity growth stage (harvest), plant height, total biomass and grain yield were estimated.

The obtained results were statistically analyzed and analysis of variance was conducted using Costat software (Costat, 1985).

TABLE 2. Some soil physicochemical properties.

Sand	% _____		pH	EC dS m ⁻¹	CaCO ₃ %	O.M %	Olsen-P mg kg ⁻¹
	Silt	Clay					
6.21	18.20	75.59	7.42	3.37	3.98	1.03	4.21

Results

Effect of Poultry litter mixing on P leaching

Leaching by water

Daily phosphorus released in the leachates of columns filled with either BC or PR with/without the poultry litter (PL) versus time is presented in Fig 1. Water soluble phosphorus (WSP) recorded about 400 mg kg⁻¹ at the beginning of the incubation period. These concentrations decreased with time in BC columns until the 10th day then slightly increased afterwards. With respect to PR, concentrations of WSP were relatively lower and almost no changes occurred in the WSP amount in leachate along with the studied period (see Table 3). The amounts of daily phosphorus in the leachate from BC columns by water were about 5 to 476 fold higher than the corresponding ones leached out from the PR columns.

Mixing of PL with either BC or PR increased generally the concentration of WSP in the leachate. Increasing the rate of applied PL resulted in further increases in the rates of leaching P from BC or PR columns (Table 3). Addition of PL to BC columns increased the rate of the leached P and the concentration of released P increased proportionally with increasing the rate of applied. Likewise, the released P from PR columns increased significantly when amended with PL especially at its high rates (Table 3). Values of pH of leachate markedly decreased with time in the leachate of BC columns (9.28 – 7.29) while did not vary significantly (7.5 – 8.1) in the PR columns (Table 4). Further reductions in pH were detected with mixing of PL with BC and vice versa with PR. Also, these trends in pH changes were observed with the increase of PL:BC (or PR) ratios (Table 4).

TABLE 3. PH changes and daily P released from bone char (BC) and phosphate rock (PR) treated by different ratios of poultry litter (PL) and leached by fixed rate of water in column experiment under laboratory conditions.

Time days	Bone Char (BC)										Phosphate Rock (PR)									
	pH					Daily released P, mg/g BC					pH					Daily released P, mg/g PR				
	R0#	R1	R2	R3	R0	R1	R2	R3	R0	R1	R2	R3	R0	R1	R2	R3	R0	R1	R2	R3
1	9.28	8.61	8.26	5.86	0.3750	0.3438	0.2700	0.2856	7.5	7.02	5.35	4.84	0.00136	0.00052	0.11040	0.02294	0.00052	0.00052	0.11040	0.02294
2	8.89	8.43	8.55	8.69	0.3240	0.0450	0.0056	0.0045	7.5	7.02	5.35	4.84	0.00136	0.00052	0.11040	0.02294	0.00052	0.00052	0.11040	0.02294
3	8.63	7.36	7.86	8.4	0.2574	0.0099	0.0180	0.0105	7.5	7.02	5.35	4.84	0.00136	0.00052	0.11040	0.02294	0.00052	0.00052	0.11040	0.02294
4	8.23	7.67	7.41	7.77	0.0747	0.0245	0.0511	0.0150	7.63	7.94	8.25	8.04	0.00255	0.00320	0.00044	0.00092	0.00320	0.00320	0.00044	0.00092
5	7.91	7.62	7.26	6.75	0.0257	0.0238	0.0943	0.0291	7.56	7.82	7.06	8.03	0.00251	0.00015	0.04050	0.00090	0.00015	0.00015	0.04050	0.00090
6	7.65	7.5	7.23	6.55	0.0197	0.0310	0.1405	0.0843	7.56	7.82	7.06	8.03	0.00251	0.00015	0.04050	0.00090	0.00015	0.00015	0.04050	0.00090
7	7.61	7.39	7.18	6.52	0.0074	0.0376	0.1489	0.1104	7.83	8.02	7.64	8.1	0.00260	0.00004	0.00052	0.00010	0.00004	0.00004	0.00052	0.00010
8	7.53	7.41	7.07	6.72	0.0051	0.0406	0.3333	0.7440	7.83	8.02	7.64	8.1	0.00260	0.00004	0.00052	0.00010	0.00004	0.00004	0.00052	0.00010
9	7.7	7.38	6.97	6.67	0.0203	0.0646	0.3333	0.6510	7.83	8.11	7.79	7.87	0.00255	0.00096	0.00017	0.00083	0.00096	0.00096	0.00017	0.00083
10	7.6	7.7	7.25	6.72	0.0100	0.0533	0.2025	0.8160	7.83	8.11	7.79	7.87	0.00255	0.00096	0.00017	0.00083	0.00096	0.00096	0.00017	0.00083
11	7.52	7.48	7.08	6.71	0.0086	0.0583	0.3980	0.8640	7.83	7.98	7.21	7.12	0.00227	0.00046	0.00726	0.02493	0.00227	0.00046	0.00726	0.02493
12	7.39	7.52	7.01	6.74	0.0110	0.0658	0.3864	0.8160	7.83	7.98	7.21	7.12	0.00227	0.00046	0.00726	0.02493	0.00227	0.00046	0.00726	0.02493
13	7.44	7.83	7.43	6.76	0.0057	0.0401	0.2025	0.4000	7.88	8.22	7.98	6.88	0.00226	0.00013	0.00009	0.00266	0.00226	0.00013	0.00009	0.00266
14	7.43	7.35	7.12	6.7	0.0129	0.0820	0.3828	1.0920	7.88	8.22	7.98	6.88	0.00226	0.00013	0.00009	0.00266	0.00226	0.00013	0.00009	0.00266
15	7.44	7.41	7.15	6.9	0.0111	0.0620	0.3440	0.6080	8.1	8.32	8.05	7.03	0.00234	0.00025	0.00013	0.01971	0.00234	0.00025	0.00013	0.01971
16	7.42	7.42	7.26	6.85	0.0125	0.1188	0.2898	0.6300	8.1	8.32	8.05	7.03	0.00234	0.00025	0.00013	0.01971	0.00234	0.00025	0.00013	0.01971
17	7.4	7.35	7.17	6.77	0.0113	0.1316	0.2312	0.4624	8.02	8.3	8.03	6.95	0.00231	0.00037	0.00013	0.05112	0.00231	0.00037	0.00013	0.05112
18	7.4	7.25	7.1	6.82	0.0116	0.1014	0.2227	0.4080	8.02	8.3	8.03	6.95	0.00231	0.00037	0.00013	0.05112	0.00231	0.00037	0.00013	0.05112
20	7.51	7.31	7.16	7.01	0.0238	0.2168	0.2580	0.8260	7.85	8.26	8.04	6.9	0.00228	0.00003	0.00014	0.04816	0.00228	0.00003	0.00014	0.04816
22	7.53	7.3	7.5	7.03	0.0230	0.2075	0.2160	2.0640	7.91	8.32	7.51	6.99	0.00233	0.00003	0.00014	0.02028	0.00233	0.00003	0.00014	0.02028
24	7.55	7.3	7.52	7	0.0330	0.2078	0.2079	0.2700	7.91	8.32	7.51	6.99	0.00233	0.00003	0.00014	0.02028	0.00233	0.00003	0.00014	0.02028
27	7.29	7.05	7.65	7.22	0.0243	0.2884	0.1700	0.1741	7.91	8.32	7.51	6.99	0.00233	0.00003	0.00014	0.02028	0.00233	0.00003	0.00014	0.02028
30	7.36	7.1	7.54	7.05	0.0250	0.2675	0.2509	0.7525	7.91	8.32	7.51	6.99	0.00233	0.00003	0.00014	0.02028	0.00233	0.00003	0.00014	0.02028

R represents the ratio of PL to BC (or PR) and the numbers 0, 1, 2 and 3 represent the 0:10, 1:10, 2:10 and 5:10 PL: BC (or PR), respectively.

TABLE 4. pH changes and daily P released from bone char and phosphate rock treated by different ratios of poultry manure and leached by fixed rate of 0.01 M H₂SO₄-acidified water in column experiment under laboratory conditions.

Time, days	Bone char										Phosphate Rock													
	pH					Daily released P, mg/g BC					pH					Daily released P, mg/g PR								
	R0#	R1	R2	R3	R0	R1	R2	R3	R0	R1	R2	R3	R0	R1	R2	R3	R0	R1	R2	R3				
1	9.11	8.85	7.82	7.75	0.35	0.12	0.08	0.11	8.13	7.31	6.01	5.52	0.00093	1.28E-06	1.90E-06	8.60E-07	8.13	7.31	6.01	5.52	0.00093	1.28E-06	1.90E-06	8.60E-07
2	8.16	8.23	7.92	7.58	0.04	0.00	0.02	0.02	8.13	7.31	6.01	5.52	0.00093	1.28E-06	1.90E-06	8.60E-07	8.13	7.31	6.01	5.52	0.00093	1.28E-06	1.90E-06	8.60E-07
3	7.55	7.55	7.11	7.5	0.01	0.03	0.11	0.10	8.13	7.31	6.01	5.52	0.00093	1.28E-06	1.90E-06	8.60E-07	8.13	7.31	6.01	5.52	0.00093	1.28E-06	1.90E-06	8.60E-07
4	7.79	7.22	7.02	6.18	0.01	0.05	0.13	0.31	8.1	7.88	7.49	8.3	0.0085	2.60E-07	3.60E-08	1.08E-06	8.1	7.88	7.49	8.3	0.0085	2.60E-07	3.60E-08	1.08E-06
5	6.77	6.95	6.63	5.22	0.34	0.23	0.47	1.07	8.1	7.88	7.49	8.3	0.0085	2.60E-07	3.60E-08	1.08E-06	8.1	7.88	7.49	8.3	0.0085	2.60E-07	3.60E-08	1.08E-06
6	5.45	6.1	5.64	5.3	3.23	1.70	2.32	3.34	8.06	7.39	7.91	7.91	5.40E-06	3.60E-08	2.20E-07	8.06	7.39	7.91	7.91	5.40E-06	3.60E-08	2.20E-07	2.20E-07	
7	5.3	5.62	5.52	4.54	3.04	2.77	3.11	4.57	8.06	7.39	7.91	7.91	5.40E-06	3.60E-08	2.20E-07	8.06	7.39	7.91	7.91	5.40E-06	3.60E-08	2.20E-07	2.20E-07	
8	5.33	5.66	5.61	5.05	3.57	2.75	4.11	4.31	7.92	8.15	7.33	7.15	0.0058	1.12E-07	2.00E-09	3.40E-07	7.92	8.15	7.33	7.15	0.0058	1.12E-07	2.00E-09	3.40E-07
9	5.57	5.6	5.51	5.15	1.68	2.72	4.25	3.28	7.77	8.19	8.15	6.89	0.00224	1.92E-07	2.24E-06	7.00E-06	7.77	8.19	8.15	6.89	0.00224	1.92E-07	2.24E-06	7.00E-06
10	5.96	5.56	5.53	4.85	0.59	1.96	3.25	2.58	7.77	8.19	8.15	6.89	0.00224	1.92E-07	2.24E-06	7.00E-06	7.77	8.19	8.15	6.89	0.00224	1.92E-07	2.24E-06	7.00E-06
11	5.83	5.43	5.47	4.01	0.55	4.34	3.60	3.50	7.77	8.19	8.15	6.89	0.00224	1.92E-07	2.24E-06	7.00E-06	7.77	8.19	8.15	6.89	0.00224	1.92E-07	2.24E-06	7.00E-06
12	5.68	5.49	5.69	5.17	2.17	4.70	3.59	5.23	7.12	8.1	7.32	7.3	0.2464	8.80E-07	6.70E-08	2.09E-06	7.12	8.1	7.32	7.3	0.2464	8.80E-07	6.70E-08	2.09E-06
13	5.49	5.55	5.72	5.74	1.87	2.70	2.16	3.10	7.12	8.1	7.32	7.3	0.2464	8.80E-07	6.70E-08	2.09E-06	7.12	8.1	7.32	7.3	0.2464	8.80E-07	6.70E-08	2.09E-06
14	5.44	5.51	5.6	3.17	4.07	5.26	4.95	5.25	5.98	8.21	7.98	6.67	2.2188	1.80E-07	1.30E-07	3.62E-06	5.98	8.21	7.98	6.67	2.2188	1.80E-07	1.30E-07	3.62E-06
15	5.37	5.46	5.47	3.01	4.51	5.06	5.62	5.54	5.98	8.21	7.98	6.67	2.2188	1.80E-07	1.30E-07	3.62E-06	5.98	8.21	7.98	6.67	2.2188	1.80E-07	1.30E-07	3.62E-06
16	5.37	5.46	5.26	4.8	4.23	5.10	5.06	5.61	5.6	8.24	7.94	7.12	6.68	1.93E-07	5.20E-07	3.30E-05	5.6	8.24	7.94	7.12	6.68	1.93E-07	5.20E-07	3.30E-05
17	5.35	5.45	4.42	3.65	4.02	4.57	4.41	5.55	5.6	8.24	7.94	7.12	6.68	1.93E-07	5.20E-07	3.30E-05	5.6	8.24	7.94	7.12	6.68	1.93E-07	5.20E-07	3.30E-05
18	5.36	5.44	3.92	5.22	3.88	4.40	4.39	6.28	5.52	8.16	8.1	7.88	6.46	2.56E-07	3.80E-08	9.00E-07	5.52	8.16	8.1	7.88	6.46	2.56E-07	3.80E-08	9.00E-07
20	5.37	5.42	3.57	3.86	8.96	10.27	7.93	13.46	5.11	8.05	8.04	8.19	3.492	5.52E-08	2.40E-08	5.81E-06	5.11	8.05	8.04	8.19	3.492	5.52E-08	2.40E-08	5.81E-06
22	5.35	5.07	3.23	5.14	9.08	8.59	10.16	11.93	4.82	8.19	8.01	7.98	7.392	1.40E-08	5.80E-08	2.24E-05	4.82	8.19	8.01	7.98	7.392	1.40E-08	5.80E-08	2.24E-05
24	5.36	3.97	3.02	3.77	7.39	7.54	7.01	7.99	4.82	8.19	8.01	7.98	7.392	1.40E-08	5.80E-08	2.24E-05	4.82	8.19	8.01	7.98	7.392	1.40E-08	5.80E-08	2.24E-05
27	5.38	3.59	2.86	3.14	9.36	8.92	7.11	9.66	4.82	8.19	8.01	7.98	7.392	1.40E-08	5.80E-08	2.24E-05	4.82	8.19	8.01	7.98	7.392	1.40E-08	5.80E-08	2.24E-05
30	5.38	3.42		2.75	5.90	7.65	7.58		4.82	8.19	8.01	7.98	7.392	1.40E-08	5.80E-08	2.24E-05	4.82	8.19	8.01	7.98	7.392	1.40E-08	5.80E-08	2.24E-05

R represents the ratio of PL to BC (or PR) and the numbers 0, 1, 2 and 3 represent the 0:10, 1:10, 2:10 and 5:10 PL: BC (or PR), respectively.

Leaching of P by acidified water

Table 4 presents the leachate of P from the different investigated sources of P i.e. BC and PR using acidified-water. Such dilute acid probably resembles the effect of root exudates thus increasing the release of P and its solubility from both sources (Table 4). With time, the released amount of P from the leachates of P from BC columns remained relatively unchanged until the 4th or 5th day and increased afterwards while in PR columns no P was detected in the leachate up to 12 days of incubation and increased thereafter. Increasing the rate of applied PL in the PL-BC mixtures led to further increases in the released amounts of P whereas the vice versa was observed in PL-PR mixtures (Table 4).

The pH values of acidified-water leachates significantly decreased with the progress of leaching days and also decreased with increasing PL incorporation with BC while, mixing of PL with PR rendered the decrease in pH (Table 4).

*Phosphorus availability after leaching**Water Extracted-P (WEP)*

Results of water extracted-P are presented in Table 5. The amount of WEP obtained after leaching of BC by water was less than that obtained after leaching by acidified-water. Mixing of PL with BC increased the WEP in both kinds of leaching and WSP increased with increasing the PL:BC ratios. Similar trends were observed in the columns packed with PR and PL-PR mixtures (Table 5).

Olsen Extracted-P (OEP)

The extracted P by 0.5 M NaHCO₃ solution

(adjusted at pH 8.5) reflects the phyto-availability of soil phosphorus regardless of the source of P amendment. After 30 day-leaching of BC and BC+PL columns (or 22day-leaching in case of PR +/- PL), sodium bicarbonate-extracted P (Olsen P) was determined to get the residual available P concentration. Generally, OEP extracted from columns leached with acidified water was higher (1.5 and 2.6 times, respectively) than those obtained after columns leached with none acidified deionized water (Table 5). Also, application of PL to either BC or PR increased noticeably the amounts of OEP especially at its higher rates of application.

Formic acid-extracted P (FAP)

The extracted P by 2% formic acid or 2% citric acid solutions reflects the agronomic effectiveness of BC and PR as P amendments for the soil. In general, the amounts of FAP extracted with water leaching were higher than those extracted with acidified-water leaching except in the treatment PL:PR 5:10 (Table 5).

Citric acid-extracted P (CAP)

According to the results presented in Table 5, the concentration of CAP remained after water leaching of BC columns was higher than the remained after acidified-water leaching in all treatments of BC. In acidified-water leached columns of BC, the CAP decreased as PL:BC increased (Table 5). The concentration of CAP in PR columns leached by water was higher than that leached by acidified-water. On the other hand, CAP of PL-PR mixtures were higher in acidified-water than in water leached ones (Table 5).

TABLE 5. Bioavailability of residual P (mg g⁻¹) extracted by water, Olsen extract, formic acid and citric acid after 30 and 22 days leaching of bone char (BC) and phosphate rock, respectively, by water or acidified water.

Extracted form of P	After water leaching				After acidified water leaching			
	Bone Char (BC)							
	BC	PL:BC 1:10	PL:BC 2:10	PL:BC 5:10	BC	PL:BC 1:10	PL:BC 2:10	PL:BC 5:10
WSP	0.048	0.209	0.343	0.490	0.124	0.142	0.148	0.213
OP	1.230	2.320	1.550	0.780	1.870	1.460	1.560	0.640
FAP	68.500	67.000	68.250	43.250	32.188	28.625	23.500	7.250
CAP	47.350	61.375	70.750	54.250	37.500	33.000	25.500	17.125
	Phosphate Rock (PR)							
	PR	PL:PR 1:10	PL:PR 2:10	PL:PR 5:10	PR	PL:PR 1:10	PL:PR 2:10	PL:PR 5:10
WSP	0.007	0.074	0.053	0.149	0.308	0.050	0.058	0.100
OP	0.089	0.008	0.041	0.340	0.232	0.153	0.140	0.219
FAP	39.500	44.500	38.250	29.375	29.625	34.875	36.250	36.250
CAP	44.350	35.938	36.813	30.688	31.125	36.413	38.250	33.600

Response of maize Yield to applied BC and PR

Results of biomass of maize showed that application of P in the form of BC increased the yield by 36.8 and 56.5% over control and PR application treatments, respectively (Table 6). Further increases in biomass yield were obtained as a result of PL mixing with BC and PR. With respect to the PR applications, the biomass yield increased only in the PL: PR mixtures and the increases were proportional to the increase in applied PL in soil.

Similar trends were observed on the maize grain yield which significantly increased with application of BC compared with PR (Table 6). About 31.8% of grain yield increased as a result of only BC application whereas about 11.2% decreased in yield as a result of PR application comparing with control treatment. Incorporation of poultry litter in soil with either bone char or phosphate rock led to significant increases in grain yield. Grain yield increased about 33.2, 66.9 and 92.7% when PL mixed with BC at rates of 10, 20 and 50%, respectively, whereas such increases were 16.7, 25.5 and 38.2% when PL mixed with RP at corresponded percentages. Previous studies showed relatively similar results related to mixing and/or composting of PL with PR and its influence on crop yield such as maize and cowpea grown in acid soils (Akande *et al.*, 2005) and wheat crop grown in a basic soil (Sharif *et al.*, 2013).

Uptake of P and Ca by Maize

Uptake of P and Ca by maize plants grown under the greenhouse conditions as affected by the source of P-amendment (BC or PR) in presence or absence of PL were shown in Table 6. Both phosphorus and calcium contents in ear-leaf were higher in the plants fertilized by BC than those

fertilized by PR. Application of PL to both BC-P and PR-P lead to noticeable significant increases in P and Ca uptake particularly for PL:BC (or PR) 5:10. Therefore, the values of P:Ca ratio showed the beneficiary of fertilization by BC and PL co-application with BC and PR for increasing P and Ca uptake by maize plants (Table 6).

Discussions

Application of phosphate rock (PR) is one of the regular methods worldwide to enrich acid soils with P (Duarte *et al.*, 2015). However, in soils, PR application seemed to be less effective as dissolution and availability decreased noticeably under these alkaline conditions. Thus, introducing bone char (BC) as a competitor to PR might be the challenge to attain a potential sustainable and, environmentally safe source of P (El-Refaey *et al.*, 2015 and Warren *et al.*, 2009). Leaching experiments of BC-P and PR-P by water and acidified water showed that the amounts of released BC-P were higher than those released from BC-PL mixtures within the first four days of leaching by water. The highest concentration of released P was associated with the highest pH values of leachate. For leaching by water experiments, the relationship was polynomial as follow:

$$\text{BC-P (mg P/gm BC)} = 0.092 (\text{pH})^2 - 1.328 \text{ pH} + 4.765 \quad (R^2 = 0.956^{***}, n = 23) \dots (1)$$

And for leaching by acidified water experiments, the relationship was exponential as follow:

$$\text{BC-P (mg P/gm BC)} = 19679e^{-1.56\text{pH}} \quad (R^2 = 0.65^{***}, n = 23) \dots (2)$$

which reflects a strong dependence of water soluble BC-P, along with the period of study, on

TABLE 6. Two-way ANOVA for the effect of phosphorus (P sources and poultry litter (PL) ratio on biomass, grain yield of maize and its P and Ca uptake at tasseling growth stage.

Factor	Treatment	Biomass g/m ²	Grain yield g/m ²	Ca %	P %	P/Ca
P sources	BC	2814.00a	853.44a	1.43a	0.245a	0.173a
	PR	1990.00b	641.00b	1.24b	0.159b	0.131b
	LSD (0.05) [#]	167.95	40.28	0.09	0.012	0.013
PL Ratio	0:10	1983.75c	602.63d	1.32ab	0.186bc	0.143b
	1:10	2334.50b	682.88c	1.23b	0.1828c	0.149b
	2:10	2393.50b	799.13b	1.37a	0.201b	0.146b
	5:10	2896.38a	602.63a	1.41a	0.240a	0.168a
	LSD (0.05)	237.52	56.96	0.129	0.016	0.018
Significance						
	P sources	***	***	***	***	***
	PL Ratio	**	**	*	***	*
	Interaction	ns	**	ns	ns	ns

[#] LSD values were calculated using Duncan's multiple range test (MRT),

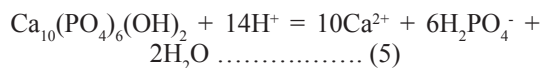
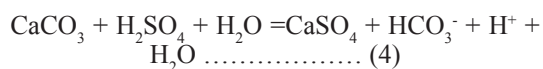
P* < 0.05; *P* < 0.01; ****P* < 0.001; ns: not significant

pH and the released P in the first four days may be related to mineralization of organic P compounds (P-O-C) as a result of pyrolysis process. Novotny et al. (2012) stated that, using ^{13}C -NMR characterization, the carbonization caused a mineralization of the bones and also a probably destruction of protein phosphoryl compounds. On the other hand, association of PL with BC had positive influences on P solubilization which probably related to organic derivatives (OD) released during the decomposition of PL. Therefore, the amounts of released P in water leachates increased with increasing PL to BC ratio (Table 3). The OD can play as precipitation reaction inhibitor of Ca-P and this might extend the time of remaining soluble P in soil (Saleh et al., 2015; Guppy et al., 2005; Genza et al., 2004).

In contrast to the results of BC, P leached out by water from PR columns was not significantly correlated with changes in pH ($r^2=0.234$, $n=15$). Further increases in the released PR-P were pronounced by leaching with acidified water as the following relationship:

$$\text{PR-P (mg P/gm PR)} = 45016e^{-2.27\text{pH}} \quad (R^2 = 0.884^{***}, n = 23) \dots \dots \dots (3)$$

Application of PL at rates of 10, 20 and 50% of PR recorded no significant effect on P leached out the column by the acidified water throughout the experimental period. In absence of PL, acidified water did not increase leached P until the 14th day, afterwards it increases progressively. The characteristics of both PR and PL may affect the dissolution of P in the column experiment. The high content of calcium carbonate (CaCO_3) accompanied the structure of PR (10.45%) competes with Hydroxyapatite $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ probably reacted with the acidified water, and resulted in releasing protons (H^+) to the surrounding environment according to the following reaction:



Further dissolution of PR occurred through continuous acidification and leaching out continuous high supply of protons and removing of the dissolution products (Mahimairaja et al., 1995). Therefore, leaching with H_2SO_4 -acidified water facilitates a faster and higher amounts of hydrogen ions which reflected in more the release of P from both sources (PR and BC).

The residual P in both BC and PR and their mixtures with PL was further influenced by leaching effluent (Table 4). Concentrations of P

fractions extracted by formic acid (FAP) and citric acid (CAP) were higher in water-leached columns comparing with those obtained from acidified-water leached ones.

It seems that these results are mirror to the amounts of P released by water and acidified water, respectively. Therefore, leaching BC (or PR) by acidified water liberates significantly the more easily mobile fractions of P than the water did; consequently, P fractions extracted by formic acid (FAP) and citric acid (CAP) were lower in columns leached out with acidified water than the corresponding ones in columns leached with non acidified water. These results are presented in the cumulative P-curves (Figures 1 and 2).

Leaching of BC-P by water or acidified water might be an implement for interpreting what will happen in the field amended with BC as source of P and irrigate with acidified water to increase the solubility of P released to the grown plants. The highest rate of PL mixed with BC or PR gave relatively similar results for residual P fractions to those attained with acidified water beside of the cumulative P. These results highlight the importance of amending soils with animal manures to liberate P from the different P-sources (Agyin-Birikorang et al., 2007; Akande et al., 2005).

The results of agronomic effectiveness of BC versus PR on maize yield and its uptake of P and Ca indicated the importance of BC as an alternative sustainable source of P amendment for crops grown in calcareous soils. The beneficial effect of PL addition to BC and PR applied to soil reflected in more bioavailable P for maize plant (Figure 4 and Table 4, respectively). Organic matter might also may retard adsorption of P by soil components and this could improve P-solubility in soil for maize plant (Guppy et al., 2005). On the other hand, the previous works on the agronomic effectiveness of partially acidified PR (Agyin-Birikorang et al., 2007; Kumar et al., 1993; Rajan and Watkinson, 1992; Chien and Hammond, 1989) indicated the importance of and applicability of using acidified water to facilitate and release P from BC and partially from PR and its bioavailability to maize plants. The higher P and Ca uptake by BC-fertilized plants comparing with PR in the single and PL-BC (or-PR) mixtures reflects the beneficial role of PL co-application in P availability to grown plants in calcareous soil (Fig. 6) which also reflected on higher biomass and grain yields (Fig. 5). Irrigation with acidified water can play significant roles in controlling P release and availability from nontraditional P-sources e.g. BC .

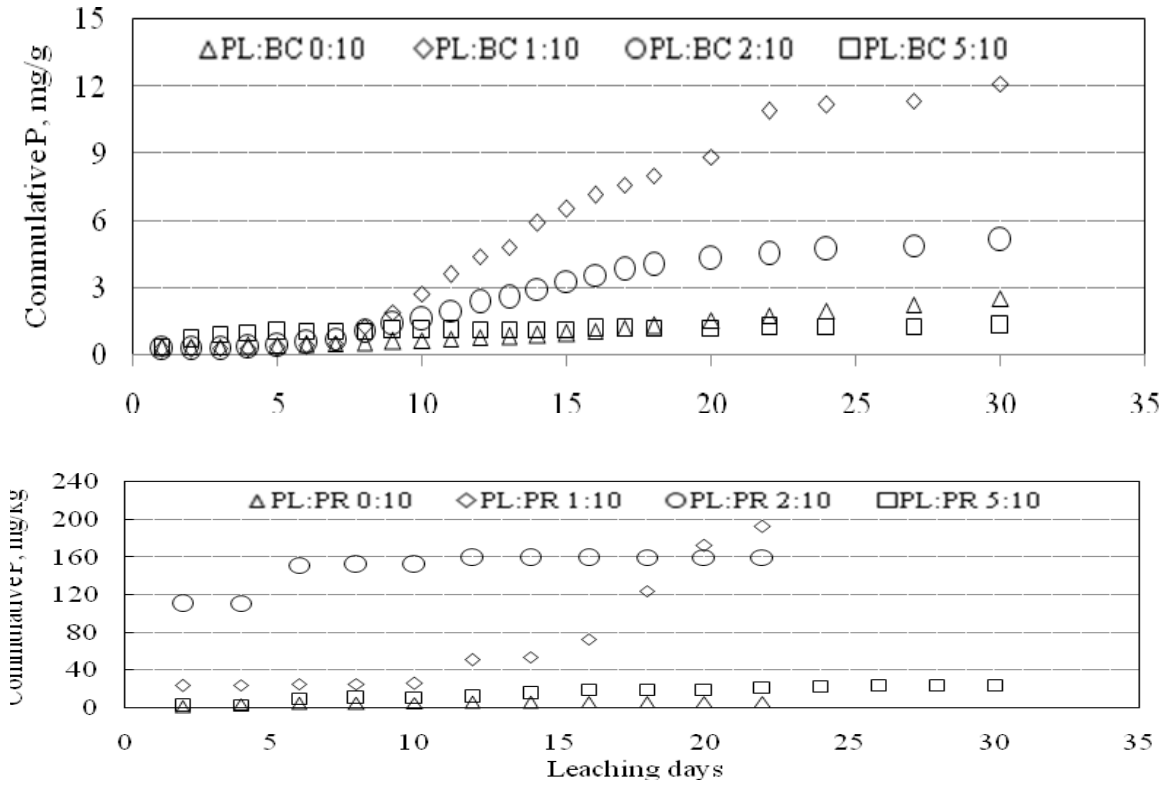


Fig. 1. Cumulative dissolved P by water from BC and PR as a function of the rate of PL addition and leaching time.

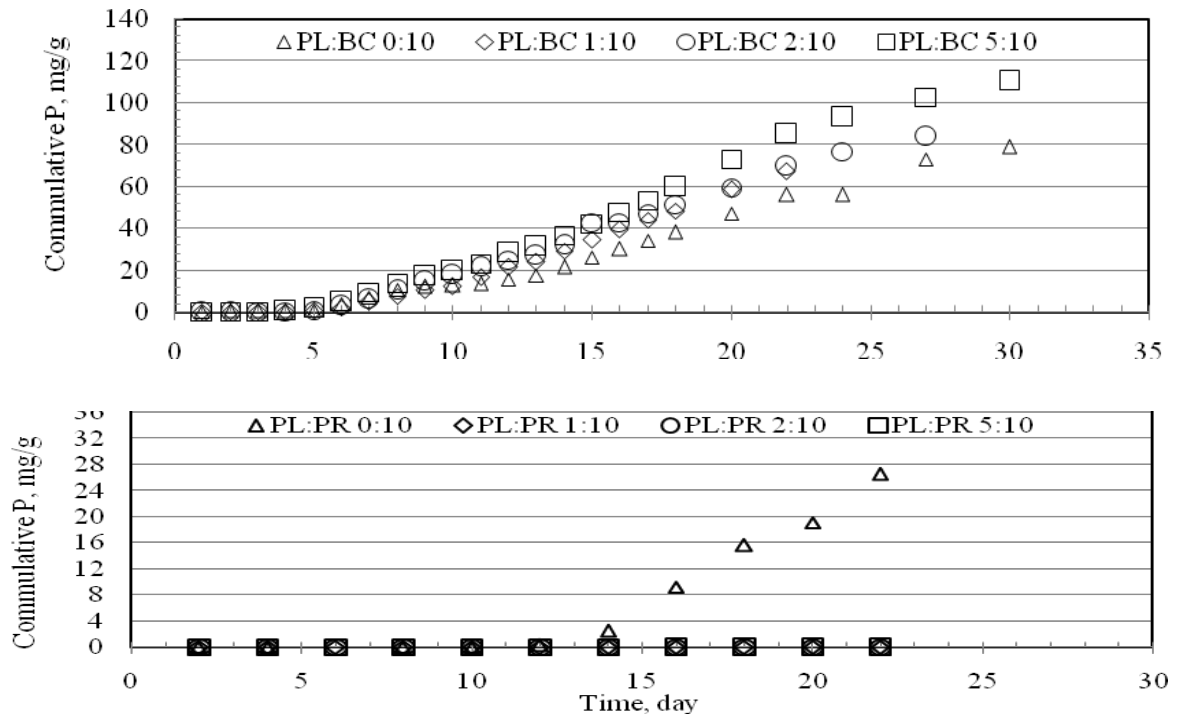


Fig. 2. Cumulative dissolved P by acidified water released from BC and PR as a function of the rate of PL addition and leaching time.

Conclusion

The importance of current study concentrates on confirming the feasibility of using bone char as a sustainable resource for P to satisfy plant requirements. BC contained higher concentrations of soluble-P than PR. Leaching (e.g., irrigation) with acidified water can play further significant role in increasing P release from BC than from PR. Mixing poultry manure with studied BC increased P solubility and P uptake by maize plants for all tested ratios whereas mixing PL with PR by 50% was effective in P solubilization. Further studies on chemistry of BC-P in calcareous soils and its behavior with soil organic and mineral solids surfaces are needed to set answers on various suspended questions.

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