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Effect of aging on soil phosphorus fractions treated with poultry manure and its derived biochar

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ABSTRACT- Nowadays, organic fertilizers such as biochar and manure in organic agriculture are widely used due to their considerable benefits. A new method should be provided to supply enough nutrients without polluting the environment. Converting animal waste to biochar was suggested as a sustainable agriculture practice to supply enough nutrients and improve soil quality. This study aims to assess the effect of poultry manure (PM) and its derived biochar (PM-BC), as well as time on distribution of soil phosphorous (P) fractions and release, along with phosphatase activity. Soil samples were treated with PM and PM-BC at three levels including 0.5%, 1%, and, 1.5% (w/w). Then, it was incubated at 25 °C with constant moisture equal to the field capacity (FC) for 140 days. In the next step, the soil samples were taken at time intervals of three hours for 1, 3, 7, 14, 28, 42, 63, 84, 112, and 140 days to evaluate the P fractions and phosphatase activity. In the next procedure, kinetic equations were fitted to the data. The results revealed a significant difference between both treatments. However, different levels of treatments did not affect the P concentration in exchangeable (EXC), carbonate (CAB), oxide (OX), and organically bound fractions significantly. On average, the highest contents in the EXC fraction were found in 1.5% of BC treatment (PM-BC) (23 mg kg^{-1}). In addition, the lowest P concentration in EXC fraction was observed in the control soil (8.6 mg kg^{-1}). The P content in the OX fraction increased up to the 28th day of the incubation and continued to decrease until its end. The highest P concentration in the OX fraction was found in 1.5% PM, which was about 71% more than the control soil. An increase in time decreased the organically bound fraction. The first order (R^2 = 0.97 and 0.98; S.E. = 0.14 and 0.11 for PM and PM-BC, respectively) and power equation (R^2 = 0.96 and 0.98; S.E. = 0.15 and 0.21 for PM and PM-BC, respectively) described the P release from different fractions appropriately. Unlike acid phosphatase activity, PM and PM-BC increased alkaline phosphatase activity. Based on the results, the manure was more effective than biochar. No significant difference was reported among various levels of treatments (0.5%, 1%, and 1.5%), indicating the cost-effectiveness of low treatment levels.

INTRODUCTION

Utilizing eco-friendly compounds such as biochar and manure as fertilizer has attracted a lot of attention during the last decades (Chan et al., 2008). High levels of pollutants and pathogens affect soil health negatively although manure has been traditionally applied as a nutrient source and natural fertilizer for soil-plant health improvement. A new method should be employed to supply enough nutrients without polluting the environment (Vakal et al., 2021). Converting animal waste to biochar has been proposed as a sustainable agriculture practice to provide enough nutrients and improve soil quality (Akdeniz, 2019).

Biochar is considered as a rich carbon generated from pyrolysis of biomass under limited oxygen conditions (Ravindiran et al., 2024). Based on some studies, biochar affects soil properties both directly and indirectly (Idbella et al., 2024), resulting in improving nutrient content and bioavailability (Ziadi et al., 2020). The aforementioned materials help improve soil fertility, absorb potential toxic element, and mitigate greenhouse gas (GHG) emissions through carbon sequestration. Biochar can be derived from various biomass sources such as plants and animal wastes, which results in generating biochar with various chemical and physical properties. Biochar reclaims soil productivity by enhancing cation exchange capacity (CEC) and surface area, as well as decreasing bulk density (Li et al., 2019). Sun et al. (2018) focused on recovering and reusing phosphorous (P) through pyrolysis processes and biochar production, arguing that such processes create enriched P because this element is conserved while CO_2 and NH_3 loss. The P content in biochar is two to three times higher than that in raw materials due to the fast release of carbon compound compared to P during pyrolysis processes. Based on some reports, P content varies between 0.13-42.92 g kg^{-1} (Glaser and Lehr, 2019).

The P fractions are influenced by biochar from altering P chemical forms and soil P sorption, along with desorption capacities, enzyme activity, and metal chelating production by soil microorganisms (Hemati

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Matin et al., 2020). In addition, Toor et al. (2006) reported that biochar addition increased the Olsen P fraction. Further, Xu et al. (2014) studied the effect of biochar on the P distribution in soils, claiming that adding biochar leads to a significant increase in the Ca–P fraction and a slight rise in the Fe–P fraction. Furthermore, Cao et al. (2021) found that biochar increased Al–P, Fe–P, and Ca–P content in the soil. P can hardly be accessed, especially in acid and alkaline soils due to the presence of Fe and Al hydroxide and calcium carbonate, respectively. P bioavailability increases in treated soils with biochar since having a high negative charge after utilizing biochar results in adsorbing cations, as well as decreasing P sorption and precipitation. The change of pH in the presence of biochar is considered as another reason to increase P availability due to the stronger anionic repulsion, resulting in declining P fixation by iron oxide in low pH, carbonate calcium in alkaline pH, and organic ligand production (Li et al., 2019). P is released from biochar both instantly and slowly (Li et al., 2019). In another study, Qian et al. (2013) asserted that P is released as orthophosphate and pyrophosphate within the first eight hours. However, the rate of P release from manure is higher than manure-derived biochar (PM-BC) (Liang et al., 2014). According to Zolfi-Bavariani et al. (2016), adding PM-BC at 200, 300, and 400 °C increased the bioavailability in nutritional ingredients such as phosphorous (P), potassium (K), iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu). Biochar prepared at 300 °C affected the availability of nutrients and their durability in the soil significantly. Saleem et al. (2017) declared that composting reduced the P bioavailability from poultry litter, resulting in optimizing P fertility in the soil and minimizing losses to the environment.

The phosphatase enzyme controls the P release. Soil enzymes are involved in critical biochemical processes in the soil such as decomposition of organic matter, formation of humus, and cycle of nutrients (Li et al., 2019; Yuan et al., 2011). Soil phosphatase including acidic and alkaline breaks down organic P into inorganic forms that are easily absorbed by the plant. Adding biochar to soils can alter the physicochemical and biological properties of the soil indirectly, resulting in affecting its phosphatase activity. Based on some studies, biochar affects soil phosphatase activity both positively and negatively. For instance, Jin et al. (2016) indicated that applying 0.5% and 1.5% pig manure biochar increased the activity of alkaline phosphatase enzyme in the soil by 28.5% and 95.1%, while decreasing that of acid phosphatase by 18.6% and 34.0%, respectively. In addition, Foster et al. (2016) reported that employing 30 t ha^{-1} from virgin pine biochar led to a 40% decrease in phosphatase enzyme activity in loamy soil. Using corn stalk biochar increased the alkaline phosphatase activity by 615% (Lehmann et al., 2011). Biochar can alter the soil pH and affect its phosphatase activity. High pH values can increase soil alkaline phosphatase activity (Li et al., 2019). Further, Du et al. (2014) claimed that utilizing 9 t ha^{-1} y^{-1} of corncob biochar increased soil alkaline phosphatase activity two-to three-fold in 0-5 cm depth of sandy loam soil. The mechanisms by which biochar affects soil P forms have been examined during the recent years. Biochar includes different types of P, among which soluble one may be released to soils after biochar application, leading to a significant increase in the amount of available P (Fei et al., 2019). Based on some reports, employing biochar facilitates the growth of mycorrhizal fungi and survival rate of soil P-dissolving bacteria, which affects the solubility of soil P (Rafique et al., 2020). The reduction of soil P after biochar addition stems from the high surface absorption of phosphate by biochar (Eduah et al., 2019; Matin et al., 2020). A coherent, detailed and extensive knowledge of biochar performance in the field of abundance, accessibility, and leaching ability of soil P has not yet been formed due to the above-mentioned contradictory results. Few studies have been conducted on the effect of biochars and their raw materials, as well as different forms of an element and its availability. Some researchers investigated the usable amount of elements and plant growth. The present study seeks to review the effect of poultry manure (PM) and poultry manure-derived biochar (PM-BC) on P fractions during incubation, as well as discussing the generation and alteration of enzymes involved in P cycling at time intervals.

MATERIALS AND METHODS

A composite soil sample was collected from a 20 cm depth from a potato field located in Hamedan, west of Iran. Soil samples were air-dried, grinded, and sieved through a 2 mm sieve and saved for future analysis.

Chemical and physical analysis

Soil texture was determined by hydrometer method (Gee and Or, 2002). In addition, electrical conductivity and pH were measured using soil:water ratio suspension of 1:5 and solid:solvent ratio of 1:20 for PM-BC and PM (Sun et al., 2014). Further, field capacity (FC) was determined by gravimetric method (Michael et al., 2017). Furthermore, calcium carbonate equivalent (CCE) was determined by reverse titration method. Bower and Walkely-Black methods were utilized for CEC and organic matter determination, respectively (Nelson and Sommers, 1996). Finally, total nitrogen in samples was measured applying Kjeldahl method.

Treatment preparation

To select the manure with high P content, different PM wastes were supplied from various companies in Bijar, Kurdistan province, west of Iran. Then, the samples were compared together and one company was selected. The manure was aired-dried, grinded, and sieved through a 0.5 mm sieve. In the next step, the manure sample was divided into two subsamples. Next, one sample was employed as soil amendment and another one was transformed into biochar through pyrolysis process. The manure was heated at 400 °C in limited oxygen condition for two hours. Then, it was maintained at 70 °C for 24 hours and screened from a 0.5 mm sieve.

Experiment medium was prepared by mixing 200 kg ha^{-1} urea and 200 kg ha^{-1} superphosphate (recommended dose for crop cultivation) before treating the soils. Then, 200 g of soil sample treated with PM and PM-BC at

0.5%, 1%, and 1.5% was incubated for 4.5 months at 25 °C in the incubator. The percentages of PM and biochar were selected based on the amount of poultry used by farmers and that utilized by Jin et al. (2015), respectively. A control soil sample was run, along with treated soils. The moisture of soil samples were maintained in FC during incubation. Soil samples were removed from incubated soil at three hours, as well as 1, 3, 7, 14, 28, 42, 63, 84, 112, and 140 days for future analysis.

Measuring P fraction

P distribution was analyzed by sequential extraction methods (Ann et al., 1999; Paludan and Morris, 1999; Poach and Faulkner, 1998). Murphy and Riley (1962) measured P concentration in different fractions applying spectrophotometer at 820 nm. Total P in PM, PM-BC, and soil samples were determined by vanadomolybdophosphoric yellow color method after dry ashing method for organic material and wet digestion employing HNO_3:$HClO_4$ ratio of 3:1 (v/v) for soil sample (Pierzynski, 2000; Rao et al., 2011). Orthophosphate reacts with ammonium and vanadate molybdate in HNO_3 medium, resulting in forming a yellow color complex. The color intensity was assessed using a spectrophotometer at 470 nm (Jackson, 2005).

Determining PM and PM-BC characteristic

Electrical conductivity and pH were measured utilizing solid:solvent ratio of 1:20 (Sun et al., 2014). The biochar morphology and surface were studied by scanning electron microscopy (SEM) and energy dispersive EDX (X-ray spectrometry). Surface functional groups on biochar surface were determined by FT-IR, Bomem MB-series.

Kinetic equations

Changes in P concentration during incubation were evaluated employing the kinetic method. Several kinetic models including first and second order, Elovich, and exponential were employed to examine P adsorption kinetics. The following equations were applied to describe four kinetic models:

First order equation

$$\ln (q_0 - q_t) = a - bt \qquad \text{Eq. (1)}$$

Parabolic partial differential equation

$$q = a + bt^{1/2} \qquad \text{Eq. (2)}$$

Exponential equation

$$lnq_t = lna - blnt \qquad \text{Eq. (3)}$$

Elovich equation

$$q = a + blnt \qquad \text{Eq. (4)}$$

where

q_t: Cumulative amount of desorbed P at time t (mg kg^{-1})
q_0: Maximum amount of desorbed cumulative P (mg kg^{-1})
a: Constant equation (mg kg^{-1})
b: Rate constant (mg kg^{-1} h^{-1})
t: Time (h)

The best model to describe P adsorption was selected based on a higher and lower value of coefficients of determination (R^2) and the standard errors of estimate (SE), respectively.

Assaying phosphatase enzyme activity

A colorimetric method was used for assaying soil phosphatase activity. To this aim, 1 g of soil sample was taken in centrifuge and 1-mL of nitrophenol phosphate was added as substrate. Then, 4 mL buffer solution with pH = 11 and pH = 6.5 was utilized for alkaline and acidic phosphatase, respectively. In the next step, the suspension was mixed and incubated at 37 °C for one hour. In the next procedure, the samples were centrifuged for three minutes. In addition, the clear supernatant was taken, following by adding 1 mL of $CaCl_2$ solution and 4 mL of NaOH. Finally, the color intensity was measured by spectrophotometry method at 430 nm (Ölinger et al., 1996).

Statistical analysis

The data were analyzed applying SPSS 23.0 (SPSS, Inc., Chicago, IL). To this aim, two-way ANOVA was employed to test the effects of treatment, time, and their interactions on soil P fractions. Thus, the mean P in different fractions was statistically analyzed by the Duncan test at $P < 0.01$ at three intervals (zero, fourth week, and end of incubation). Finally, Microsoft Office Excel 13.0 was used to draw the figures.

RESULTS AND DISCUSSION

Physical and chemical properties of soil

Table 1 indicates some of the chemical and physical properties of the soil. The soil was slightly alkaline with a pH value of 8.2. The values of TC (Total carbon) and TP (Total phosphorous) were 0.14 and 0.04%, respectively. The soil texture was loam.

FTIR, SEM, and EDX results

FTIR analysis was utilized to recognize the main functional groups and characterize covalent bonding information in PM and PM-BC samples. The FTIR spectra of PM showed that the frequency range between 400-800 cm^{-1} reveals the peaks corresponding to C-H aromatic bonds (Fig. 1a). Clear peak at 1024.61 cm^{-1} is attributed to C-O ester aliphatic bonds, as well as hemicellulose and cellulose polarity. The peak at 1407.59 cm^{-1} is attributed to lignin due to rings of type C-O (phenolic group) (Rasoulpoor et al., 2020). C=O stretching vibrations are observed at 1638.76 cm^{-1} that is related to the carbonyl and carboxylic acid groups (Tipson and Cohen, 1968). This vibration was always present until the temperature exceeded 600 °C. The wide absorption band at 3414 cm^{-1} was attributed to the presence of an O-H bond in samples (Fleming and Williams, 1966). The wide adsorption derives from the presence of water in samples.

The FTIR spectra of PM-BC showed that the frequency range between 400-800 cm^{-1} reveals the peaks corresponding to C-H aromatic bonds. Clear peaks from 1039.35-1055.63 cm^{-1} are attributed to C-O ester aliphatic bonds, as well as hemicellulose and cellulose polarity. Peak at 1423.05 cm^{-1} is attributed to lignin due to rings of type C-O (phenolic group) (Rasoulpoor et al., 2020). The peak at

2503.73 is attributed to SO_2, while peak area 3436.62-3488.65 is related to O-H stretching vibration (Fleming and Williams, 1966) (Fig.1b).

The SEM images represented that BC contained bigger pores compared to PM after pyrolysis process (Fig. 2a and Fig. 2b). The temperature plays a critical role in the changes in morphological, physical, and chemical properties of raw materials. The formation of bigger pores in BC treatment is attributed to the elimination of volatile organic compounds.

The results of EDX analysis are presented in Fig 3a. Based on EDX results the dominant elements in PM treatments were C, O, Ca, N, and P. Fig. 3b shows the EDX of PM-BC treatments. The enhancement of C, Ca, and K content, and a decrease in N and O amounts of elements were detected after the pyrolysis process in the PM-BC sample. It was found that the composition of BC samples was influenced by pyrolysis temperature. Most BC samples contain a low content of N and S since N and S compounds are released as NH_3 and SO_2 in pyrolysis.

Characterizing PM and PM-BC

PM and PM-BC, which were ground to < 2mm for homogenizing, were analyzed (Table 2). The mass of biochar decreased due to the pyrolysis process, resulting in enriching the P content in biochar that was 2.5 times in the raw poultry. The pH value was elevated to 11.3, while EC reached 6.1 dS m^{-1} in generated biochar. Finally, the total N content in PM was about 5.6%, which was higher than that of PM-BC (1.1%).

Table 1. Physical and chemical properties of soil

Clay	Silt	Sand	Texture	pH	EC	TN	TP	OM	$CaCO_3$	FC	CEC
	(%)				(dSm^{-1})			(%)			($cmol_c\ kg^{-1}$)
19.2	38.0	42.8	Loam	8.2	0.25	0.04	0.14	2.2	5.8	17.5	14.4

TN: Total Nitrogen; TP: Total Phosphorus; OM: Organic Matter; FC: Field Capacity; CEC: Cation Exchange Capacity

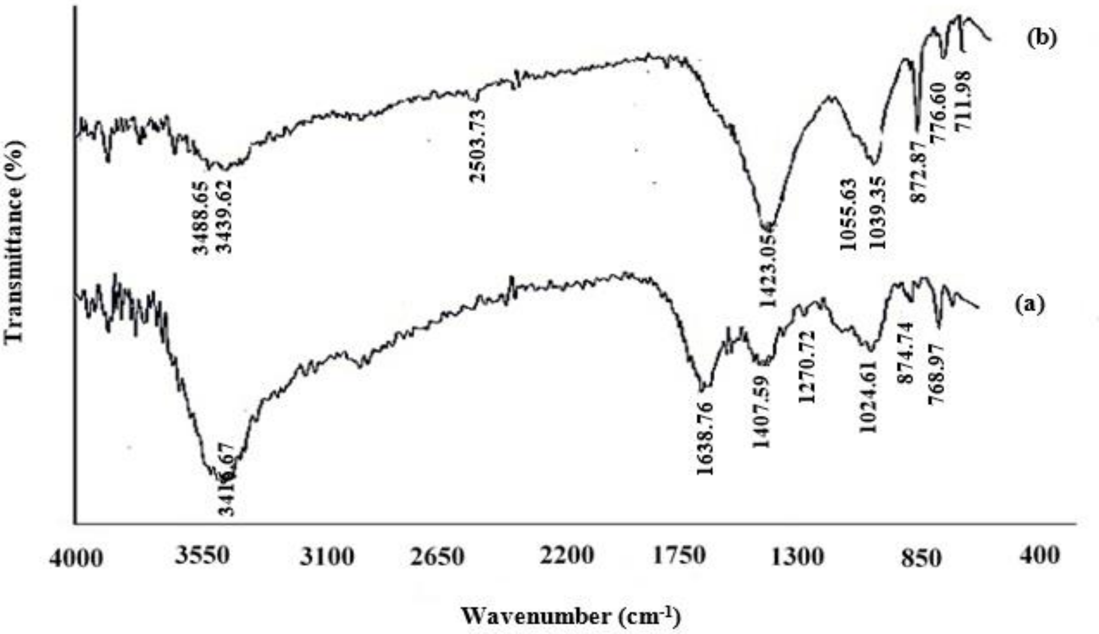

Fig. 1. (a) FTIR spectra of poultry manure and (b) its derived biochar.

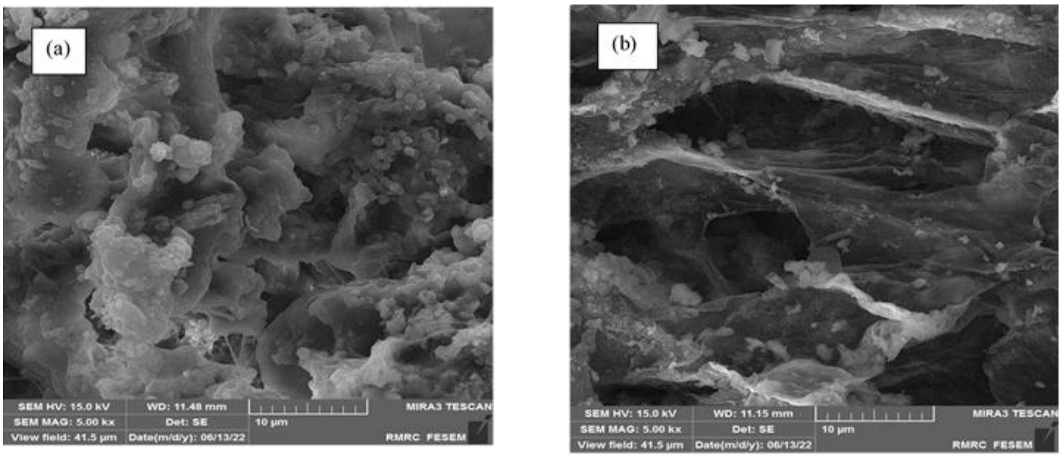

Fig. 2. Scanning electron microscopy of (a) poultry manure and (b) its derived biochar.

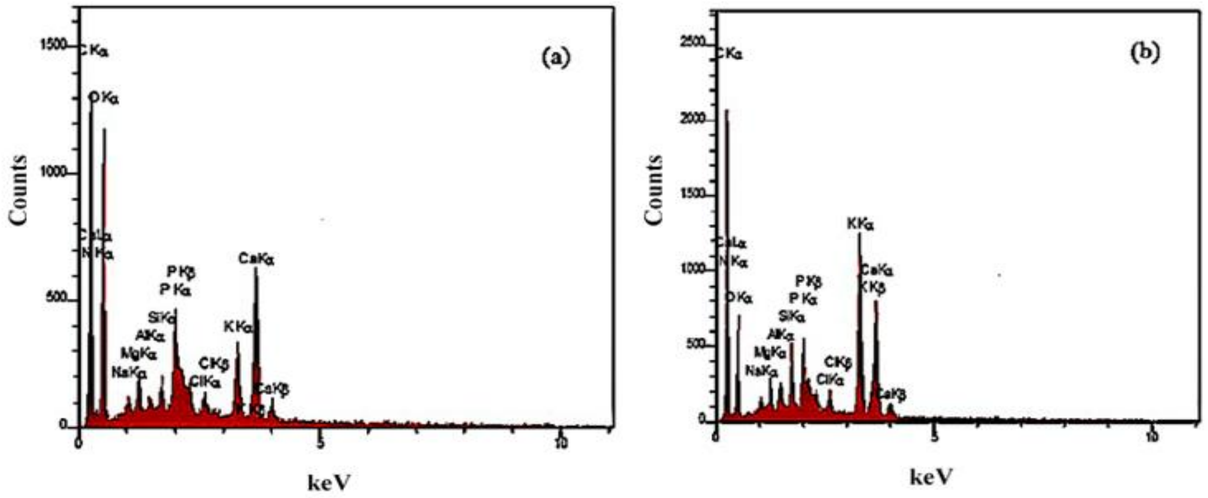

Fig. 3. Energy dispersive X-ray spectra of (a) poultry manure and (b) its derived biochar.

Table 2. Characteristics of poultry manure and its biochar

Organic compound	**pH**	**EC**	**TN**	**TP**
	-	(dSm^{-1})	(%)	
Poultry manure	9.3	5.02	5.9	1.02
Biochar derived from poultry manure	11.1	6.10	1.1	2.60

EC: Electrical conductivity; TN: Total nitrogen; TP: Total phosphorus

Impact of PM and PM-BC on soil pH

Fig. 4 displays the changes in the pH values of PM and PM-BC treatments, as well as control soil at different rates (0.5%, 1%, and 1.5%) during the incubation. Compared to the control soil, an increase of 0.2 unit was reported after adding PM from the beginning of the experiment to 72 hours. Then, a decreasing trend occurred during the incubation. Feizi et al. (2017) reported that the pH value of PM amendment increased after 24 hours of soil incubation and continued to decrease in long-term incubation. The pH value decreased during the first three days of incubation in all of the PM-BC treatments, despite PM ones. This decline continued until the 14th day of incubation for soil with 0.5% BC, while the enhancement of pH occurred between 14-28 days of incubation for soil treated with 1% and 1.5% BC. The pH value was constant (7.8) for all of the treated and control soil after 28 days of incubation up to the 48th day. As indicated, the treatments affected soil pH significantly, while PM and PM-BC decreased the pH of the soils. Generally, the pH value in PM treatments was lower than that in PM-BC ones because of low molecular acid generation due to the PM decomposition (Cassity-Duffey et al., 2015). Some researchers found that soil pH increased early in the incubation after applying organic material amendments due to the nitrification of NH_4^+ ions generated during mineralizing organic N early in the incubation, followed by an apparent decrease during the incubation (Yuan et al., 2011). The initial soil pH increases the impact of biochar on soil pH value. Acidic soils responded to biochar applications better than alkaline soil due to alkaline nature of biochar (Farhangi-Abriz et al., 2021). There was limited information on biochar application to alkaline soils since most of the biochars benefit from alkaline pH with a liming capacity for acidic soils (Yu et al., 2019). In addition, Liu and Zhang (2012) declared that adding alkaline biochar to the alkaline soils generated a decreasing pH trend, especially with higher biochar application rates, instead of increasing the soil pH. They found that generating acidic materials during the oxidation of biochar and organic matter may lead to the pH decrease (Liu and Zhang, 2012). Biochars generated at higher temperatures are more alkaline, show lower nutrient availability, and present higher specific surface area. Biochars generated at low temperatures stimulate microbial activity, resulting in increasing CO_2 emission. Generally, the difference in biochar properties and application rates, soil fertility and texture, and climatic conditions, as well as experimental setup and incubation duration play critical roles in the impact of biochar on the soil properties (Sun et al., 2022).

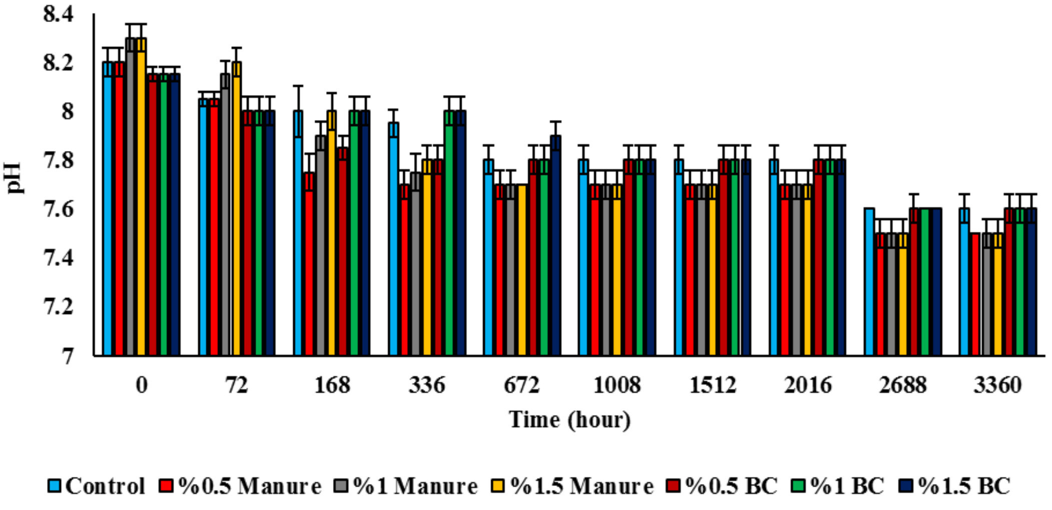

Fig. 4. Effect of poultry manure and its derived biochar (BC) on pH value during incubation.

Changes in P fraction

The P distribution in different fractions was compared in the presence of PM and PM-BC treatments, as well as control soils. As shown in Fig. 5a, P in exchangeable (EXC) fraction increases in treated soil compared to the control one, with the greatest rise observed in 1.5% of BC treatment (35 mg kg^{-1}). Based on the results, P availability increased by 191% in soil treated with 1.5% PM-BC. Adding PM to the soil samples can increase available P since the decomposition of organic matter leads to the soil acidity and phosphate mineral dissolution (Johan et al., 2021). The effect of biochar and compost on available P during incubation showed that mineralizing organic compounds plays a vital role on enhancing available P in soil samples (Ch'ng et al., 2014). Some studies focused on the effects of biochar on P availability. Liu et al. (2017) indicated that employing rice hull biochar amendments to red clay and alkaline soil increased the P availability by 52.63% and 33.37%, respectively. In addition, Atkinson et al. (2010) argued that biochar can increase the availability of macro-nutrients such as N and P. The increase of Olsen-P has been reported after biochar addition (Toor et al., 2006). The reasons for the increase in P availability have not been fully understood. However, different mechanisms have been proposed for high P availability including direct nutrient input from biochar and indirect impact of biochar due to the increasing cation exchange capacity or altering soil pH (Enders et al., 2012). Desorption of P from unstable soil sources in the presence of biochar and changes in soil anion exchange capacity (DeLuca et al., 2024) are regarded as other reasons for the increase in available P (Cui et al., 2011; Schneider and Haderlein, 2016). Further, Ch'ng et al. (2014) incubated soil samples with organic amendments and observed the increase in available P in soil treatments due to the P mineralization. Furthermore, Chan et al. (2008) found that soil nutrient retention improved after adding biochar due to the BC properties such as pH, CEC, porosity, and specific surface area. The soil microbial community is affected by biochar through supplying nutrient content and providing an appropriate growth environment due to the porous structure of biochar, resulting in impacting nutrient availability (Yu et al., 2019). Based on some studies, using PM-BC leads to increased available P due to its release from biochar, resulting in promoting its desorption and dissolution from (Fe, Al) (hydro) oxides and stimulating the mineralization of organic P by increasing phosphatase activities (Jin et al., 2016).

Fig. 6 illustrates the periodic releases of P from soils treated by PM and PM-BC amendments during incubation. The available P was the lowest at PM-BC treatments at the beginning of the incubation, while soil with 1.5% PM benefitted from the highest content of available P. As demonstrated, an increased trend is displayed with time in all of the treated and control soils. Both PM-BC and PM treatments increased up to the 4th week of incubation and the greatest changes were reported in PM-BC treatments. The P is released from soil samples during a rapid phase followed by a slow one. The P release curve in EXC and carbonate (CAB) fractions showed rapid release during the first incubation up to the 672 hours, and plateaued as an apparent equilibrium (Fig.5).

The amount of available P during 672 hours was 1.3% and 1.9% for soil treated with 1.5% PM and PM-BC, respectively, compared to 0.8% in control soil. However, this value reached 1.9% and 1.8% for PM and PM-BC treatments at the end of incubation, respectively. P release increased within 672 hours for PM-BC and PM treatments, and tended to plateau after 1700 hours in all of the treatments. The highest difference was observed in soil treated with PM-BC that had approximately 2.5 times more available P than the control one at the end of the incubation. In addition, the highest average P release was recorded under PM with 45% compared to 39% and 33.5% for BC and control soils, respectively. BC treatments reduced the amount of extractable P fraction by the end of the incubation (140 days), while the amount of P in PM amendment increased in available fraction. However, the control soil had the lowest available P at the end of incubation (5 mg kg^{-1}). Generally, the EXC fractions in soil amendments were observed in the order 1.5% > 1% > 0.5%. However, no significant difference was reported in the three levels of treatments. In another study, Jin et al. (2016) claimed that P release increased by 15% after adding biochar treatment. PM and PM-BC addition increased available P significantly, with PM-BC being more effective in this regard. Further, the release of available P was more affected by the incubation period than the utilized treatments. Li et al. (2019) declared a short-term and rapid P release and found that more than half of P in BC was distributed in stable forms. Thus, BC represented a slower P rate compared to the PM. The increase of EXC or available P in the presence of PM-BC is related to the raised surface area and porosity. Based on the results, the rate of P release in PM-BC treatments was higher than that in the presence of PM at the beginning of the experiment. A slower rate of P release was observed at PM-BC treated soils over time. The P release from biochar is characterized by two mechanisms including an instantaneous direct release and a long-term slow one by alteration of soil pH, microbial mineralization, and co-precipitation with cations present in the soil (Cao et al., 2021; Xu et al., 2014).

The P content in Fe-Al oxide (OX) fraction increased up to the 28th day of incubation followed by a decrease up to its end (Fig. 5b). Soil with 1.5% manure benefitted from the highest content of OX fraction (161.7 mg kg^{-1}), while the lowest content was reported in the control soil. Compared to the PM-BC treatments, PM ones increased OX fraction in soil more effectively. Liu and Zhang (2012) reported the conversion of the OX fraction to the available P during the pyrolysis process. The increase in the OX fraction can be attributed to the transformation of crystalline Fe oxides (goethite and hematite) to the amorphous ones (Eduah et al. 2019). Amorphous Fe oxides exhibit a greater tendency to P sorption compared to the crystalline ones (Ruttenberg and Sulak 2011; Zhang et al. 2003). However, Fe-P decreased after 25th day of incubation, which can be attributed to the lower pH value after the 28th day. The dissolution of OX minerals is accelerated at low pH, resulting in stimulating P desorption (Cao et al., 2021). P fractions were affected by the incubation period. The data indicated that OX fraction reached its highest value at the 28th day of incubation and gradually continued to decrease. The increase in OX fraction in soil treated with PM was higher than that of PM-BC (1.5 times).

CAB is considered as the dominant P fraction in calcareous soil (33% of total P), which increased in the presence of PM (about 12-15%). An increase of 12-14% for PM-BC treatments was measured. Compared to the control, all of the treatments represented CAB fraction increase during the incubation. CAB fraction increased sharply within 28 days of incubation. Then, a gradual upward trend occurred until the 8th week, leading to the stabilization in most of the treatments. The enrichment of CAB fraction followed the order 1.5% > 1% > 0.5% in both treatments (Fig. 5c), indicating that PM increased the soil CAB fraction more than PM-BC. According to Hong and Lu (2018), the CAB fraction of P increased after utilizing biochar. In another study, Xu et al. (2014) found that adding biochar increases the CAB fraction significantly. At the end of incubation, the amount of CAB fraction in soil with 1.5% PM and PM-BC was 73.6 and 53.5%, respectively. The CAB fraction in soils was influenced by the P content of biochar. Accordingly, biochar with a higher P content in CAB fraction affected the CAB content of treated soils significantly. Change in soil pH due to the biochar addition is among the most common mechanisms for altering P fractions in soils. In addition, a dramatic competition is observed between P anions with large amounts of different anions for the surface of biochar, which affects the activity of cations simultaneously. Biochar increases the concentration of free divalent cations in soil, as well as raising the P precipitation as Ca or Mg phosphates (Manolikaki et al., 2016). The increase in CAB fraction may stem from the raised ionic strength and Ca concentration in the soil solution after biochar addition. Based on some studies, the CAB fraction in biochar was dominant, leading to its rise in treated soils (Li et al., 2019).

Unlike other fractions, organically bound one decreased continuously over time in all of the treatments. This fraction, which was dominant among other ones, included approximately 54-65% of the total P. The aforementioned fraction declined within 600 hours and gradually continued to decline. The low content of OM fraction was found in PM treatment over incubation time although PM treatments contained a higher OM fraction than PM-BC ones (Fig. 5d). The OM fraction decreased at 1.5% PM treatment by 10% compared to the control soil, which was regarded as the highest reduction among all of the treatments. Sarfaraz et al. (2020) reported the increase in P mineralization due to the rise in microbial biomass. Some studies focused on the gradual transformation of organic P to the inorganic one due to the microbial activity. P released from organic treatments rapidly at the beginning of the experiment. Then, slow release was reported. The lowest content of organically bound P fraction was found in soil treated with 1.5% PM at the end of the experiment. The decrease in OM fraction was higher in PM in comparison with PM-BC. Further, BC treatments showed more OM fraction than control soil during the incubation. OM fraction was significantly lower in PM treatment than in control and PM-BC ones. No significant difference was observed among the PM application rates. However, a significant difference was reported in all of the treatments among interval times of incubation.

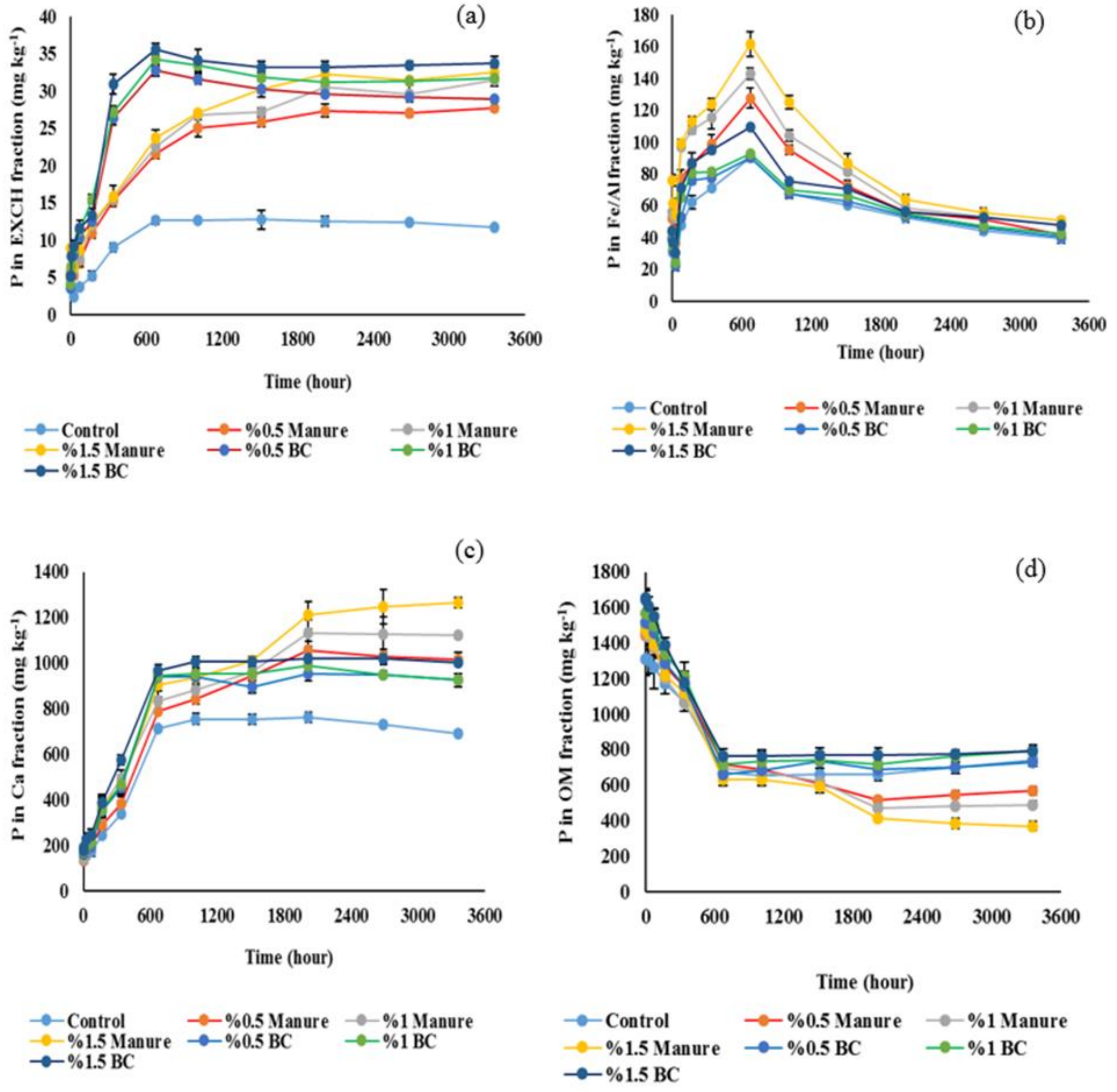

Fig. 5. Effect of poultry manure and its derived biochar (BC) on (a) exchangeable, (b) oxides, (c) carbonate, and (d) organically-bond fractions.

P release kinetics

Few studies have been conducted on the P release kinetics in all of the fractions. The gradual changes in P in different fractions were described based on kinetic models. The fast and slower rates of P release are related to the rapid desorption of surface unstable P and slow dissolution of the crystalline P compounds (Yang et al., 2018). However, the soil amended with PM released more P at a higher rate than biochar amendment even though PM added less P than PM-BC. Slower P release in PM-BC amended soils derived from their porosity and structure (Jin et al., 2016). PM-amended soils contain more organic matter, which increases humic and fulvic acids, resulting in raising nutrient release (Arancon et al., 2006). The sequential P fractionation is employed to compare the fluxes of various P forms in soil. P fractionation was used to determine the presence of P forms relevant to the kinetics of inorganic P release (McDowell and Sharpley, 2003).

The rate of P release can be described utilizing kinetic equations. Here, all of the kinetic models were applied to simulate the relationship between released P and time. In fact, two equations including the first and power models fitted properly to the experimental data. Fig. 3 shows the best-fit model parameters. Comparing coefficients of determination (R^2) and standard errors of estimate (SEE) for kinetic models represented that release kinetics were better fitted with the first order (R^2 = 0.97 and 0.98; S.E. = 0.14 and 0.11 for PM and PM-BC, respectively), power equation (R^2 = 0.96 and 0.98; S.E. = 0.15 and 0.21 for PM and PM-BC, respectively), and parabolic model, despite obtaining a high R^2 (R^2 = 0.95 and 0.97). A significant difference between experimental and calculated data with large square error (SE = 561 and 459) indicated the lack of fit in kinetic model. Based on the lower SE value, the first-order equation was considered as the most appropriate one. Compared to the power equation, the determination coefficient of the first-order model for PM and PM-BC treated soil was higher with the lower SE value and fitted better than the power equation. Table 3 shows the kinetic parameters. The kinetic parameters calculated from power and first-order models (a and b) were higher when soil was treated with PM, representing that inorganic and organic P release in the presence of PM treatments occur more rapidly than that under PM-BC treatments. An increase in (b) or a decrease in (a) parameters can exhibit an increase in the reaction rate, while the slope of the equation shows the impact of the condition reaction. Based on the kinetic curves, the slope of the equation simulated each other. The size of BC particles plays a critical role in the rate of P release. Some studies revealed that BC with a smaller particle size exhibited a larger P release due to the greater specific area (Ma and Matsunaka, 2013; Manolikaki et al., 2016; Morshedizad et al., 2018). Fig. 5a, Fig. 5c, and Fig. 5d illustrate two regions. A rapid release step occurred within 28 days of incubation and continued slowly over the following weeks. Soluble organic and mineral compounds dissolve after adding PM and PM-BC to the soil due to the water entering their pores. Dissolution of salts, ion exchange, submicrometer particle detachment, and preferential dissolution at crystal imperfections account for rapid initial dissolution. The slope of kinetic models indicated by constant b could be employed as the rate of P species transformation (Wang et al., 2020). The treatments with steeper slope showed the highest power of transformation. In the sum of EXC and CAB fractions, b in PM and PM-BC treatments ranged from -0.005 to -0.007 and -0.007 for first order equation, respectively. The above-mentioned values were from 0.83-0.93 and 0.85-0.88 for power equation in PM and PM-BC treatments, respectively (Table 3).

Statistical analysis of fractions

The results represented that EXC fraction was significantly affected by treatments and time incubation. No significant difference was observed among different levels of treatments. As demonstrated in Fig. 6a, treatments affect ECX fraction. In addition, a stronger effect is observed under PM-BC in the fourth week. Comparing P in OX fraction at three time points among treatments revealed a significant difference and the greatest significance was found in the fourth week. However, their different levels did not affect P in OX fraction significantly (Fig. 6b). Furthermore, a significant difference was reported in CAB fraction between treatments at different points of time. Soil treated with PM benefitted from a higher content of CAB fraction compared to PM-BC (Fig. 6c). However, no significant difference was observed between P content at 1% and 1.5% PM and PM-BC application. The mean P in inorganic fraction (ECX + OX + CAB) was 36.3%, 48.2%, and 42.2% for control, PM, and PM-BC treatments, respectively, indicating a significant difference. However, no significant difference was reported within various levels of PM and PM-BC treatments. In addition, a significant difference was revealed in organic fraction with increasing time (Fig. 6d).

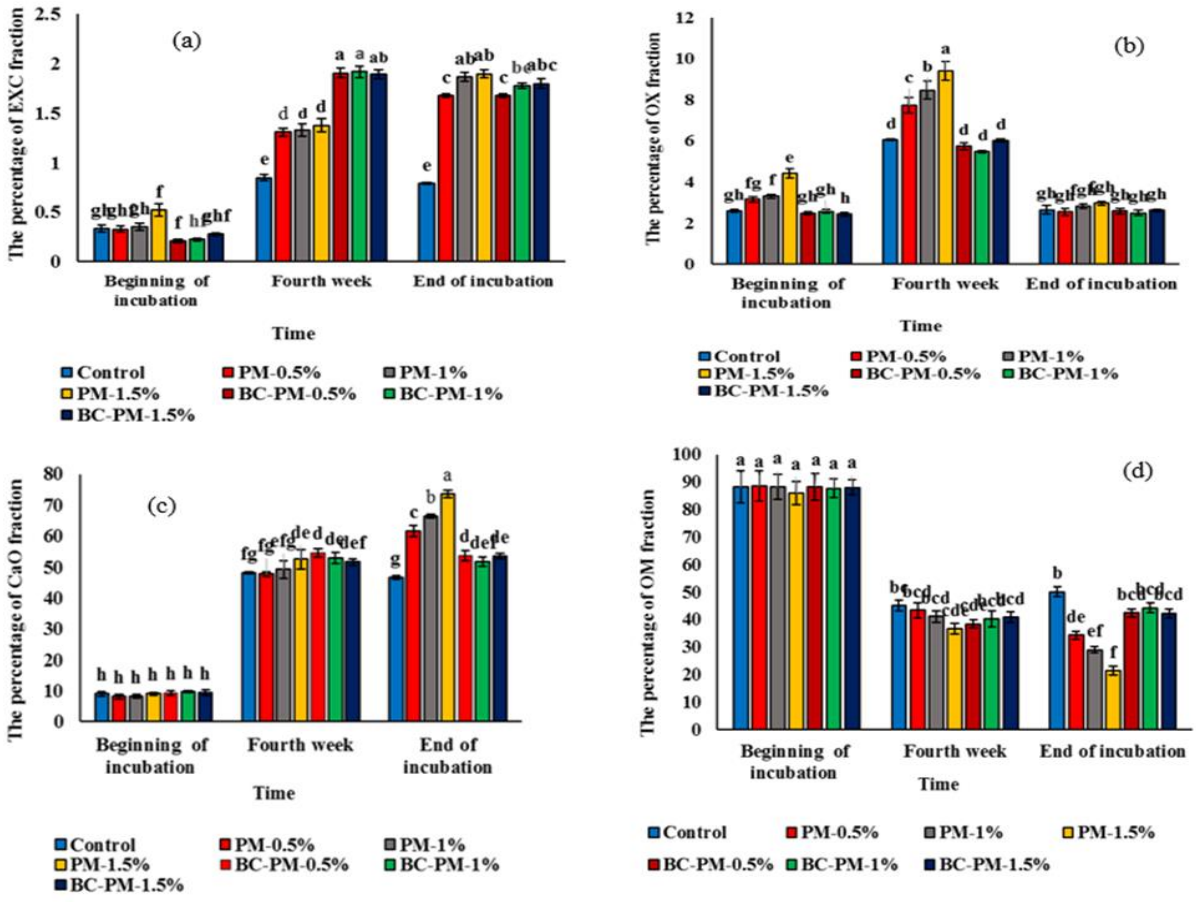

Fig. 6. The comparison mean values of different fractions in poultry manure (PM) and poultry manure-derived biochar (BC-PM) treatments (values accompanied by different letters are significantly different within columns at the level of $P < 0.01$) ((a) exchangeable, (b) oxides, (c) carbonate, and (d) organically-bond fractions)

Table 3. Parameters of kinetic equations used to describe the release of phosphorous

Sample	**Model**	**EXC**		**OX**		**CaO**		**OM**	
		a	**b**	**a**	**b**	**a**	**b**	**a**	**b**
Control	First order	4.578	-0.0007	6.278	-0.0010	8.618	-0.0007	8.881	-0.0009
	Power	3.558	0.391	36.866	0.347	109.29	0.459	2096.4	0.208
	Parabolic	1.088	1.740	59.674	10.492	-177.00	94.701	3245.1	155.93
Manure 0.5%	First order	5.287	-0.0007	6.539	-0.0009	8.857	-0.0005	8.838	-0.0009
	Power	4.397	0.447	55.169	0.358	110.86	0.478	2393.5	0.199
	Parabolic	-4.396	3.387	98.682	14.141	-328.11	116.64	3715.2	158.28
Manure 1%	First order	5.366	-0.0007	6.658	-0.0009	8.934	-0.0005	8.791	-0.0009
	Power	4.881	0.442	65.983	0.357	116.19	0.486	2465.4	0.193
	Parabolic	-4.772	3.640	125.400	16.281	-334.80	126.61	3774.2	152.520
Manure 1.5%	First order	5.420	-0.0007	6.751	-0.0009	9.002	-0.0005	8.731	-0.0009
	Power	6.430	0.414	81.589	0.351	125.05	0.482	2499.6	0.190
	Parabolic	-2.263	3.833	147.990	17.827	-374.15	134.27	3845.4	147.91
Control	First order	4.578	-0.0007	6.275	-0.0010	8.618	-0.0007	2096.4	-0.0009
	Power	3.558	0.391	36.866	0.347	109.29	0.459	3245.1	0.208
	Parabolic	1.088	1.740	59.674	10.492	-177.00	94.701	8.941	155.93
BC 0.5%	First order	5.486	-0.0007	6.320	-0.0009	8.857	-0.0007	248.2	-0.0009
	Power	4.397	0.416	3.640	0.351	126.68	0.471	3812.7	0.192
	Parabolic	-6.208	3.308	65.545	11.097	-244.56	114.49	8.993	148.82
BC 1%	First order	5.550	-0.0007	6.370	-0.0009	8.881	-0.0007	2558.0	-0.0009
	Power	4.881	0.418	3.770	0.344	139.03	0.463	3924.7	0.192
	Parabolic	-4.848	3.600	77.231	11.748	-226.79	123.52	9.023	146.85
BC 1.5%	First order	5.598	-0.0007	5.598	-0.0009	8.945	-0.0007	2688.9	-0.0009
	Power	6.430	0.416	3.815	0.352	148.25	0.465	4096.4	0.199
	Parabolic	-3.978	3.811	82.635	13.024	-220.09	132.31	2096.4	142.08
Sample	**Model**	**EXC**		**OX**		**CaO**		**OX**	
		R^2	**SE**	**R^2**	**SE**	**R^2**	**SE**	**R^2**	**SE**
Control	First order	0.988	0.087	0.995	0.093	0.983	0.092	0.947	0.207
	Power	0.938	0.234	0.976	0.126	0.949	0.246	0.997	0.028
	Parabolic	0.993	3.129	0.987	360.57	0.983	260.45	0.917	990.11
Manure 0.5%	First order	0.984	0.091	0.993	0.096	0.975	0.118	0.944	0.235
	Power	0.957	0.220	0.982	0.106	0.947	0.264	0.994	0.035
	Parabolic	0.986	8.367	0.975	508.56	0.972	414.86	0.888	1185.93
Manure 1%	First order	0.983	0.102	0.991	0.119	0.976	0.111	0.947	0.232
	Power	0.956	0.222	0.988	0.087	0.957	0.239	0.994	0.035
	Parabolic	0.984	9.698	0.966	599.14	0.977	413.75	0.881	1187.66
Manure 1.5%	First order	0.982	0.103	0.992	0.104	0.975	0.101	0.948	0.251
	Power	0.948	0.226	0.984	0.095	0.952	0.251	0.990	0.044
	Parabolic	0.984	10.356	0.968	666.02	0.972	478.51	0.862	1252.45
Control	First order	0.988	0.087	0.995	0.093	0.983	0.092	0.947	0.207
	Power	0.938	0.234	0.976	0.126	0.949	0.246	0.997	0.028
	Parabolic	0.993	3.129	0.987	360.57	0.983	260.45	0.917	990.11
BC 0.5%	First order	0.992	0.086	0.992	0.086	0.985	0.085	0.944	0.211
	Power	0.983	0.154	0.980	0.154	0.952	0.248	0.995	0.032
	Parabolic	0.995	6.258	0.995	115.80	0.984	319.29	0.903	1169.06
BC 1%	First order	0.992	0.077	0.992	0.077	0.985	0.086	0.944	0.211
	Power	0.983	0.150	0.980	0.150	0.952	0.242	0.996	0.031
	Parabolic	0.996	5.848	0.996	125.17	0.985	322.08	0.907	1199.53
BC 1.5%	First order	0.992	0.075	0.992	0.075	0.986	0.082	0.946	0.207
	Power	0.976	0.917	0.980	0.175	0.961	0.220	0.996	0.029
	Parabolic	0.996	6.676	0.996	131.77	0.988	314.06	0.907	1791.48

EXC: Exchangeable; OX: Oxide; CaO; Carbonate; OM: Organic. BC: Biochar; R^2: Determination coefficient; SE: standard errors

Effect of PM-BC and PM on phosphatase enzyme

Based on two-way ANOVA, a significant difference was observed on time-depended data of alkaline phosphatase activity at the 1% probability level between the treated soils with PM-BC and PM. An increase in the phosphatase activity raised the PM application rates at all of the time intervals after using PM (Fig. 7). The highest and lowest phosphatase activity was found at zero time for 1.5% manure treatment and 1008 hours of incubation time for control soil (754 µg NP $Cg^{-1}h^{-1}$, 209.9 µg NP $Cg^{-1}h^{-1}$). Generally, the phosphatase activity represented a downward trend from 0-1008 hours of incubation time. Then, the trend was upward in different levels of treatments. The intensified phosphatase activity was reported in soil with a higher percentage of PM. Alkaline and acid phosphatase can catalyze the hydrolysis of phospholipids to release free P (Sun et al., 2022). Based on the literature review, alkaline phosphatase is exclusively derived from microorganisms, especially bacteria, while acid phosphatase is mainly generated and secreted by living plants (Krämer and Green, 2000). Phosphatase plays a vital role in P mineralization (Jin et al., 2016). A positive correlation was observed between the decomposition of organic compounds and phosphatase activity. Jin et al. (2016) reported an increase in soil-soluble P after adding manure, which was related to the high activities of alkaline phosphatase. The increase of P in CAB and OX fraction after utilizing PM stemmed from stimulating phosphates generation and rising phosphates solubility related to Al and Fe. In addition, Waldrip et al. (2011) reported an increase in phosphatase activity in soil treated with PM. Further, Marinari et al. (2000) argued that applying manure increased the phosphatase activity almost threefold due to the mineralization of native soil OM and subsequent release of soluble nutrients for microbial growth. Enzyme activities are commonly influenced by temperature, humidity, nutrient availability, pH, and organic matter content. Soil P availability is controlled by the phosphatase activity. Low content of available P leads to stimulated phosphatase generation, while its activity stops at high available P content (Nannipieri et al., 2012). A greater pH value increased the phosphatase activity which is consistent with the results presented by others (Garg and Bahl, 2008; Jin et al., 2016). Soil with higher organic matter and larger pH value leads to the generation of increasing phosphatase enzymes (Garg and Bahl, 2008). PM-BC affected phosphatase activity similar to that in PM treatments, yet with lesser severity due to the higher available P in PM-BC treatments. Furthermore, Jin et al. (2016) claimed that 0.5% and 1.5% application rates of pig manure biochar increased the soil alkaline phosphatase activity by 28.5% and 95.1%, respectively. In another study, Lehmann et al. (2011) asserted that the synthesis phosphatase activity increased by 615% employing corn stalk biochar. According to Du et al. (2014), the phosphatase activity increased after adding biochar to soil, leading to enhanced soil pH. Jin et al. (2016) declared that phosphatase activity increased by 28.5% with a rise in soil pH from 6.9 to 7.5 after using biochar, meaning that the biochar pH buffer and nutrient inputs can affect the soil microbial community and phosphatase activity, resulting in influencing the hydrolysis of organic P in the soil (Gul and Whalen, 2016).

The treatments, time, and their interactions affected the acidic phosphatase activity at 1% probability. The highest activity was investigated in control soil. In addition, an increase in time decreased the acid phosphatase activity in all of the soil samples due to the raised available P. The maximum and minimum phosphatase activity was studied in control soil at zero time containing low inorganic P (192.3 mg kg^{-1}) and 1.5% PM treatments containing the greatest inorganic P (1348.7 mg kg^{-1}) at the end of incubation, respectively. Jin et al. (2016) argued that 0.5% and 1.5% application rates of pig manure biochar decreased the acid phosphatase activity by 18.6% and 34.0%, respectively, due to the biochar effect on soil pH and inorganic P concentration.

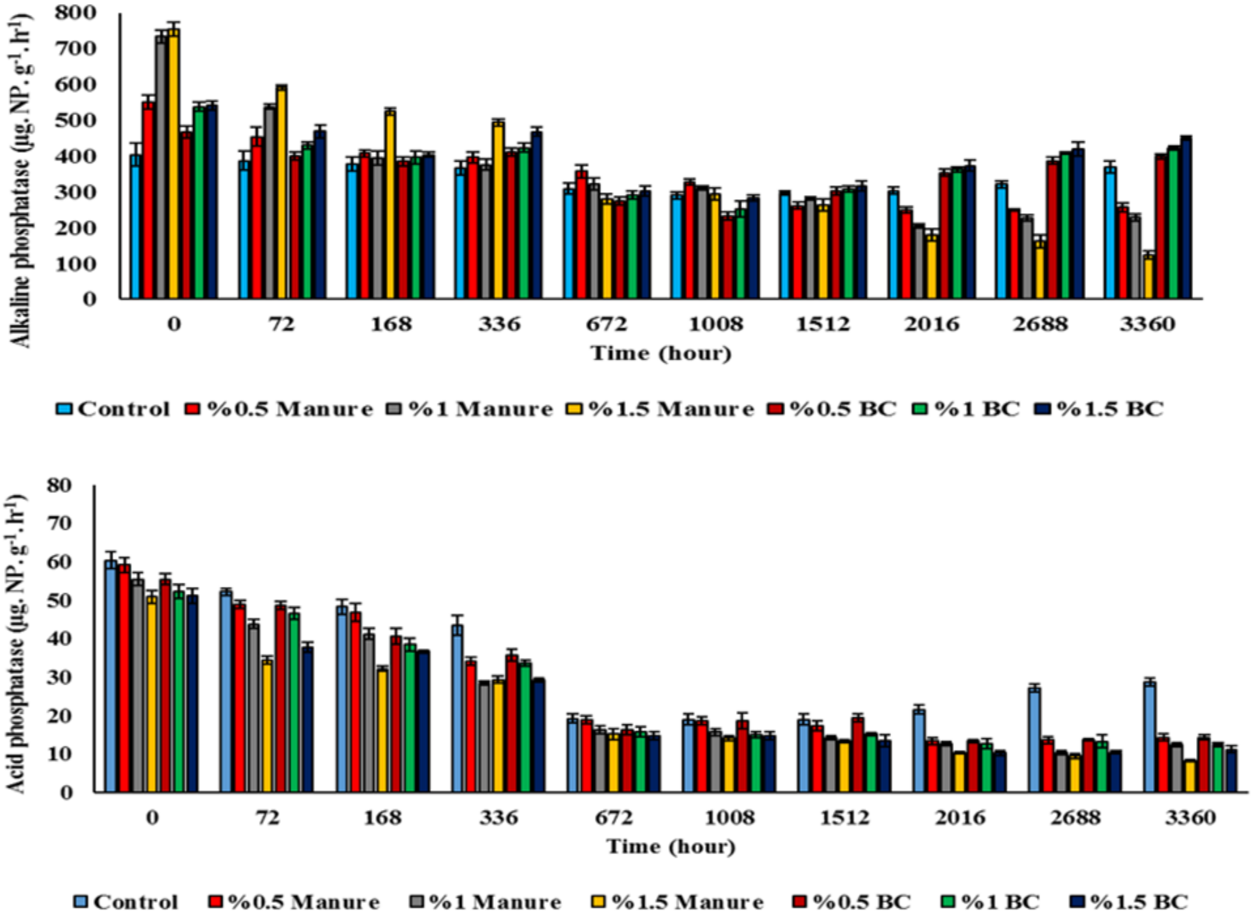

Fig. 7. Effect of poultry manure and its derived biochar (BC) on alkaline and acid phosphatase activity.

CONCLUSION

The results indicated that pyrolyzing PM into biochar at 400 °C is regarded as an effective method to increase slow-release and long-term P concentration in soil. A rapid release was shown at the beginning of the experiment for PM-BC treatments although the stable P in BC-PM displayed a slow one. Soil P fractions were influenced by PM and PM-BC treatments and the amount of EXC fraction in soil treated with PM-BC was more than that in PM treatments. However, OX and CAB fractions were higher in PM treatments. Unlike acid phosphatase activity, alkaline phosphatase activity increased after adding PM and PM-BC to the soil. Using 0.5% PM-BC is recommended to maximize economic benefit due to the insignificant difference in the three levels of treatments. Based on the literature review, utilizing BC affected soil properties both positively and negatively. Therefore, further studies should review the effect of feedstock type in biochar and pyrolysis conditions on improving soil P availability and generating slow-release phosphorous biochar.

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Conceptualization: Akbar Asadyan, Zahra Varasteh Khanlari, Shahriar Mahdavi; Methodology: Akbar Asadyan, Zahra Varasteh Khanlari, Shahriar Mahdavi; Data collection, and analysis: Akbar Asadyan; Writing—original draft preparation: Zahra Varasteh Khanlari; Writing—review and editing: Zahra Varasteh Khanlari; Supervision: Zahra Varasteh Khanlari; Project administration: Zahra Varasteh Khanlari; All authors read and approved the final manuscript.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY

Not applicable

ETHICAL STATEMENT

All authors have approved the manuscript and agree with the submission to "**Iran Agricultural Research**" Journal. Manuscript was prepared in compliance with Ethics in Publishing Policy and we used Guide for Authors in preparing submitted manuscript.

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