



## Alkaline phosphatase activity and kinetics in organic residues – impacted soils

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### Abstract

Soil phosphomonoesterases play an important role in controlling phosphorus cycling for crops, especially in P- deficient soils. Phosphomonoesterases markedly affected by addition of organic residues depending on source, rate, and maturity and stability of these residues. An incubation experiment was conducted to evaluate alkaline phosphatase activity and kinetics of two soils (silty clay and loamy sand) after addition of different organic sources (cow residue, alfalfa leaves, wheat straw and poultry residue) at rate of 2 %. The experiment was set out in randomized complete design with three replicates. Amended and control soils were incubated at 30°C for 30 days. Results showed that alkaline phosphatase activity of amended soils was significantly higher than that of control soil, except soil amended with wheat straw. Enzyme activity was differed according to the type of organic residue with superiority of poultry residue. Alkaline phosphatase activity of silty clay soil was significantly higher than that of loamy sand soil. Data also revealed that V<sub>max</sub> and K<sub>m</sub> values of amended soils were higher than these of unamended soil. Higher V<sub>max</sub> and K<sub>m</sub> value were associated with using of poultry residue. Estimated V<sub>max</sub> and K<sub>m</sub> values varied with the type of transformation used to linearize Michaelis–Menten equation which followed the order: Hanes – Wolf > Eadie – Hofstee > Line Weaver – Burk.

**Keywords:** alkaline phosphatase, organic residues, kinetics, soil, poultry residue

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### INTRODUCTION

In arid and semi-arid regions, where there is low input of organic materials, soil quality can be strongly affected by wide range of land management practices, especially using of inorganic fertilizers that may enhance yield at the short time, but do not enhance soil quality at the long term (Gumus and Seker, 2015; Muslim, et al, 2016). Extraction and purification of L-asparaginase produced by acinetobacter baumannii and their antibiofilm activity against some pathogenic bacteria. The International Journal of Biotechnology, 5(1), 7-14.). Addition of organic residues is commonly used to improve the quality and productivity of degraded soils. Organic materials are important to improve soil physical, chemical and biological characteristics, then control essential nutrient cycles such as phosphorus. Phosphorus is a limiting nutrient element to sustain crop growth and yield in most soils since it is bound with Ca<sup>+2</sup>, Fe<sup>+2</sup>, and Al<sup>+3</sup> as well as adsorb by calcium carbonate and clay particles, remaining insufficient amount to plants. Generally, organic materials decrease phosphate precipitation in calcareous soil, for example the low molecular weight organic acids competitor orthophosphates, thus delaying phosphorus adsorption on active surfaces (Geelhoed et al. 1999). On the other

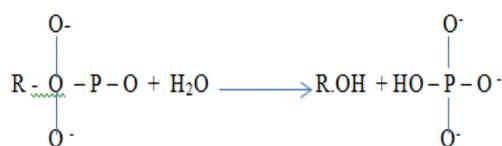
hand, there is evidence that organic matter complexes Ca<sup>+2</sup> and therefore makes more phosphorus available to plant (Prasad & Power, 1997).

A large part of phosphorus in soil has been found in organic forms which hydrolyzed into available forms by soil microorganisms and plant root exudates (Liang et al. 2015). Phosphomonoesterases (alkaline phosphatase and acid phosphatase) hydrolytic enzymes that catalyzed the hydrolysis of phosphate bond in organic compounds are widely disturbed in soils (Al-Ansari et al. 1999 b). These enzymes play an important role in the process of mineralizing organic phosphorus and plant nutrition. The general equation of the reaction catalyzed by alkaline and acid phosphatase is:

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Once enzymes released into soil undergo synthesized, accumulated inactivated and / or decomposed in the soil system (Jayan, 2012). Among the different factors affecting enzyme levels in soil systems, the organic matter has potential to impact enzyme activities. Organic residues influence microbial population as they provide soil with C for microbial growth as well as provide other essential nutrients (Perucci and Scarponi, 1984; Kizilkaya and Dengizy, 2010). Kizilkaya and Bayrakli (2005) and Chang et al. (2007) found that addition of organic residues maintained high levels of alkaline phosphatase activity in soil. Gray and Bahl (2008) reported a significant stimulation in alkaline phosphatase by addition of poultry manure as compared with other residues. On the other hand it is well known that extracellular enzymes such as phosphatases adsorbed on clay and organic colloids. Soil enzymes are largely immobilized by soil colloid particles, hence are different from homogenous system (Jayan, 2012). Soil organic carbon traps soil enzymes and protect them from degradation by microorganisms or proteinases (Plante et al. 2006). However, Zhang et al. (2010) reported that preventing enzymes by soil organic matter sometimes promotes catalytic reaction and sometimes inhibits catalytic reaction.

Theories and Mathematical analysis of enzyme reactions are based on the concept that enzyme acts by forming a complex with substrate, and perchance the complex is unstable and proceeds through one or more steps or rearrangement to form the product plus the original enzyme (Jayan, 2012). Studies on kinetic parameters help to understand the change in enzymes affinity to substrate at different concentration, catalytic activity and sensitivity to temperature (Juan et al. 2010; Zhang et al. 2010). The two fundamental kinetic parameters of an enzyme catalyzed reaction are  $V_{max}$  indicated the maximum velocity obtained when the substrate concentration is high enough to saturate the enzyme and  $K_m$ , the Michaelis – Menten constant which reflects the binding affinity between enzyme and substrate (Liang et al. 2015).  $K_m$  gives the substrate concentration at which the reaction rate reaches half of its maximum value ( $V_{max} / 2$ ). Organic matter of soil can alter kinetic parameters ( $V_{max}$  and  $K_m$ ) and Catalytic efficiency of enzyme reactions (Cartes et al. 2009). Tabatabai (1994) stated that both  $V_{max}$  and  $K_m$  are constant for the enzyme, but may vary independently of each other under different condition. Tietgen and watzel (2003) reported that soil enzyme  $K_m$  and  $V_{max}$  were affected by soil texture and organic matter content.

**Table 1.** Some properties of soil and organic materials used

property	Soil		Organic material			
	Silty clay	loamy sand	Cow residue	alfalfa leaves	wheat straw	poultry residue
Sand %	4.63	86.60	-	-	-	-
Silt %	53.89	5.19	-	-	-	-
Clay %	41.48	8.20	-	-	-	-
E.C*, dSm <sup>-1</sup>	5.99	2.93	11.10	10.10	11.70	11.02
pH*	7.79	8.00	7.20	5.80	6.00	6.50
organic, C, g kg <sup>-1</sup>	3.94	0.33	258.60	416.00	392.60	265.40
Total N, g kg <sup>-1</sup>	0.36	0.03	18.40	27.70	4.20	27.30
Phosphorus-p*, g kg	0.018	0.0091	13.80	13.13	2.93	17.32
C/N	10.94	11.00	14.00	15.00	92.70	9.72
Alkaline phosphatase, $\mu\text{g p-nitrophenole g}^{-1}\text{soil hr}^{-1}$	150.16	120.30	-	-	-	-
Total carbonate, g kg <sup>-1</sup>	316.88	82.31	-	-	-	-

\*Electrical conductivity (E.C.) and pH were estimated in suspension of 1:1 ratio

Zhang et al. (2010) also stated that type and content of organic matter and clay in soils affect the diffusion of enzyme or substrate, and hence the enzyme  $V_{max}$  and  $K_m$ .

The objective of this study was to determine the effect of different organic sources (cow residue, alfalfa leaves, wheat straw and poultry residue) on alkaline phosphatase activity and kinetic parameters ( $V_{max}$  and  $K_m$ ) in two agricultural soils (silty clay and loamy sand).

## MATERIALS AND METHODS

### Soil and Organic Materials

Surface soils (0 – 20 cm) were taken from private farms at Basrah province based on wide range in their properties. The first soil was loamy sand and another was silty clay. Each soil was air – dried, crushed, then sieved to 2 mm and stored at 4°C until used. Some chemical, physical and biological properties of soils were obtained according to methods described by Black (1965) and page et al. (1982) and presented in **Table 1**. Four different organic residues (cow residue, alfalfa leaves, wheat straw and poultry residue) were obtained from the Experimental Station Farms of Agriculture College of Basrah University, south of Iraq. The organic residues were composted for few months, dried at 50°C and sieved to 1 mm, then stored in polyethylene bags at 4°C until used. Some chemical properties of residues were obtained following standard methods described by page et al. (1982) and presented in **Table 1**.

Soil treatments: The soil samples (50g air-dried) were placed in 100 cm<sup>3</sup> plastic pots, and the organic residues were thoroughly mixed with the soil at the rate of 2 % based on dried weight. The amended soils were moistened to field capacity and incubated at 30 ± 2°C for 30 days to undergo biochemical equilibrium. The moisture content was maintained throughout the incubation period. Soil without organic residues was used as a control. Sub samples were removed to determine alkaline phosphatase activity.

For soils and 1:5 ratio for organic materials; phosphorus –p was estimated as available fraction for soils and as total for organic material.

Alkaline phosphatase (EC 3.1.3.1) activity was determined according to procedures of Tabatabai and Bremner (1969) and Eivazi and Tabatabai (1977). One g of soil sample was incubated with 0.25 ml toluene, 4 ml modified phosphate buffer (pH 11) and 1 ml of 12.5, 25, 50, 100, 125 or 150 mM of P-nitrophenylphosphate solution (substrate) at  $37 \pm 2^\circ\text{C}$  for 1 hr. After incubation alkaline phosphatase was inhibited by 0.5 M  $\text{CaCl}_2$  solution and 0.5 M NaOH solution. Samples were swirled and soil suspension was filtered through Whatman No.42 paper. The formation of P-nitrophenol was assayed spectrophotometrically at 420 nm.

Kinetic parameters ( $K_m$  and  $V_{max}$ ) reported in the study were calculated from the results obtained from the effect of various substrate concentrations on enzyme activity.  $K_m$  and  $V_{max}$  values were calculated using Michaelis-Menten equation for enzyme reactions

$$V = V_{max} \cdot [S] / (K_m + [S])$$

Where  $V$  is initial velocity,  $[S]$  is substrate concentration,  $K_m$  is Michaelis constant, and  $V_{max}$  is the maximum velocity. The three linear transformations of Michaelis-Menten equation given below were used to calculate  $K_m$  and  $V_{max}$ .

#### 1-LineWeaver – Burk transformation

$$1/V = 1/V_{max} + K_m / (V_{max} \cdot [S])$$

$V_{max}$  was calculated from  $1/(\text{intercept})$  and  $K_m$  calculated from  $(\text{slope}) / (\text{intercept})$  of the plot  $1/V$  vs  $1/S$ .

#### 2- Hanes – Wolf transformation

$$([S])/V = K_m/V_{max} + 1/V_{max} \cdot [S]$$

$V_{max}$  was calculated from  $1/(\text{slope})$  and  $K_m$  calculated from  $(\text{intercept}) / (\text{slope})$  of the plot  $([S])/V$  vs  $[S]$ .

#### 3- Eadie – Hofstee transformation

$$V = V_{max} - K_m \cdot V/[S]$$

$V_{max}$  was calculated from the intercept and  $K_m$  calculated from the slope of the plot  $V$  vs  $V/[S]$ .

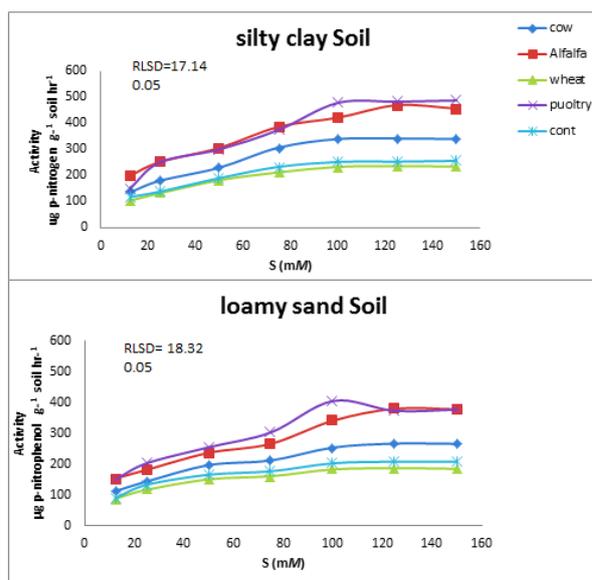
### Statistical Analysis

The enzyme activity,  $K_m$  and  $V_{max}$  data were subjected to analysis of variance (ANOVA) using a randomized complete plot design with three replications by using SPSS ver.11.0 program. Means were compared using Revised Least Significant Difference (R.L.S.D) test at a significance level of 0.05.

## RESULTS

### Alkaline Phosphatase Activity

Alkaline phosphatase activity affected by various substrate concentrations are shown in Fig. 1. Enzyme activity increased by increasing substrate concentration till reached maximum values at 100 mM for all organic residues except that of alfalfa leaves which reached maximum activity at 125 mM. This trend was similar at the two soils under study. However, further increase in substrate concentration beyond these concentrations did not significantly affect the activity of enzyme. It has



**Fig. 1.** Effect of substrate concentration and type of organic residue on alkaline phosphatase activity in soils

been suggest that rate of reaction catalyzed by alkaline phosphatase for all treatments at the two soils approached first order reaction at low substrate concentration ( $< 100$  or  $125$  mM) followed by zero order reaction at high substrate concentrations. That means, the kinetics behavior of alkaline phosphatase in these soils followed the hyperbolic kinetics which can be described through Michaelis-Menten equation (Tabatabai, 1994). Malcolm (1983) stated that the concentration of substrate in the soil reaction mixture should remain sufficiently high to maintain zero kinetics. Jayan (2012) also reported that substrate concentration determines the velocity of enzyme reaction, and it used for enzyme assay should wherever possible be sufficient for saturation of the enzyme, so that kinetics in the standard assay approaches zero order. Similar results were reported by Al-Ansari et al. (1999 a); Jayan (2012) and Alhajjaj (2014) for soil alkaline phosphatase.

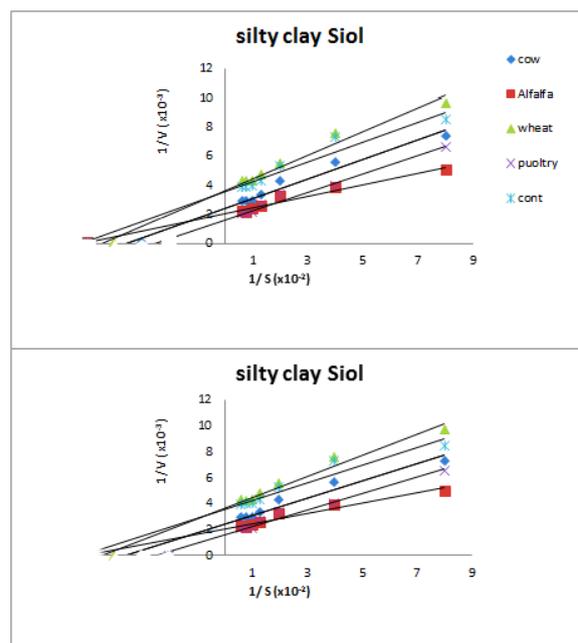
Alkaline phosphatase activity in soil amended with cow residue, alfalfa leaves, or poultry residue were significantly ( $P \leq 0.05$ ) higher than that of control at all substrate concentrations and at both soils. While, enzyme activity in soil amended with wheat straw was lower than that of control at all substrate concentration and at both soils, but without significant differences (Fig. 1). In both soils, the effect of organic residue types was in the following order: poultry manure  $\geq$  alfalfa leaves  $>$  cow residue  $>$  cont.  $>$  wheat straw. The higher alkaline phosphatase activity in soils treated with organic residues can be attributed to high amount of substrate in organic matter for increased microbial population and activity. Chang et al. (2007) reported that the increase in soil organic matter as a result of compost application, in addition to the incorporation of stable enzyme by compost favors the formation of complexes with free

enzymes, and consequently, the soil enzyme activities increase. In addition, soil enzymes seemed to be immobilized on soil organic matter, and significant correlation between organic carbon and enzyme activities is reported (Frankenberger and Tabatabai, 1991). This significant correlation is because organic carbon is the basis of microbial proliferation and activity. Kizilkaya and Dengiz (2010) found a positive correlation of alkaline phosphatase activity with organic matter content. This result was in agreement with the results of Kizilkaya and Bayrakli (2005) and Chang et al. (2007) who found that the addition of organic residues maintained high levels of alkaline phosphatase activity in soil. Variation in enzyme activity in soils treated with different organic residues could be due to chemical composition of organic residues. The higher decomposition of organic residues with low C/N ratio (Morena et al. 1999). Chandra (2011) concluded that presence of legume recorded higher microbial biomass carbon and enzyme activity which was attributed to favorable effect on microbial activity due to rapid utilization of its residues by microbes being of narrow C/N ratio. Kizilkaya and Bayrakli (2005) have demonstrated that low C/N ratio is an indicator of a high decomposition rate. Furthermore, the differences in enzyme activity with different organic residues can be explained on the basis of their content of total N (**Table 1**). The higher the N content, the higher the enzyme activity. Nitrogen changes the composition of microbial community leading to stimulate the growth and multiplication of microorganisms which then building up of enzyme level.

Djordjevic et al. (2002) and Akmal et al. (2012) showed a positive correlation of soil phosphatase with total N. Busato et al. (2015) found a higher phosphatase activity in filter cake residue as compared with cattle manure because of higher content of water soluble phosphorus in filter cake along the experiment time. A similar stimulation in alkaline phosphatase activity with addition of poultry manure as compared with other residues was reported by Gary and Bahl (2008) who attributed that to low lignin content and high C, N and cellulose content in poultry manure.

Alkaline phosphatase activity in silty clay soil at different organic residues were significantly higher than those of their counterparts of loamy sand soil, with an overall averages of 276.13 and 219.31  $\mu\text{g P-nitrophenol g}^{-1} \text{ soil hr}^{-1}$  for silty clay and loamy sand soils, respectively (**Fig. 1**). These difference's may be attributed to initial characteristics of soils Jayan (2012) reported a significant correlation.

Between alkaline phosphatase activity and organic C and total N. That means, the enzyme activity can be increased through addition of energy sources. Furthermore, Gary and Bahl (2008) stated that soil available P is the main factor controlling variation in

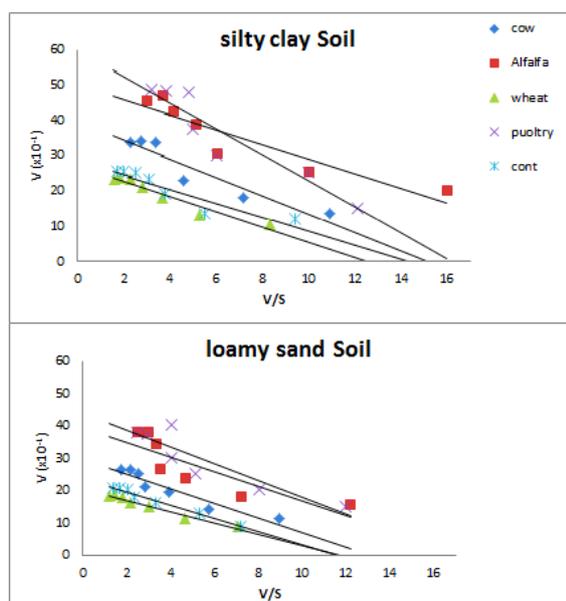


**Fig. 2.** Line Weaver–Burk plot of alkaline phosphatase in soils treated with different type of organic residues. velocity (V) expressed as  $\mu\text{g p-nitrophenol g}^{-1} \text{ soil hr}^{-1}$  and substrate concentration (s) in mM

alkaline phosphatase in respect to soil type with increasing available P in soil, the release of P-nitrophenol phosphate increased. Beside this, soil organic C and clay particles can adsorb enzyme molecules and protect them from microbial degradation (Zhang et al. 2010). In our study, silty clay soil have higher amount of organic C, total N, available P as well as higher clay content as compared with loamy sand soil (**Table 1**). The results agree with Al-Ansari et al. (1999a) and Alhajjaj (2014).

#### Kinetic parameters

Plots of the three linear transformations of Michaelis-Menten equation for alkaline phosphatase were presented in **Figs. 2** and **3**. It is observed that with all organic residues, the linearity of these plots followed the Michaelis – Menten equation at both soils. In this context, coefficient of determination ( $R^2$ ) of different transformations indicated significant correlations between substrate concentration and enzyme activity ranged from 0.890 and 0.985 for Line weaver-Burk transformation, from 0.954 to 0.996 for Hanes–Wolf transformation and from 0.729 to 0.978 for Eadie–Hofstee transformation (**Table 2**). The  $V_{\text{max}}$  and  $K_m$  values were determined by using the three linear transformations and presented in **Table 3**. The maximum enzyme reaction velocity ( $V_{\text{max}}$ ) was markedly different for different organic residues. The  $V_{\text{max}}$  for the organic residues ranged from 263.15 to 625.00  $\mu\text{g P-nitrophenol g}^{-1} \text{ soil hr}^{-1}$  at silty clay soil and ranged from 192.30 to 476.19  $\mu\text{g P-nitrophenol g}^{-1} \text{ hr}^{-1}$  at loamy sand soil and follow the order poultry residue >



**Fig. 3.** Eadie – Hofstee plot of alkaline phosphatase in soils treated with different type of organic residues. velocity (V) expressed as  $\mu\text{g p-nitrophenole g}^{-1} \text{ soil hr}^{-1}$  and substrate concentration (s) in mM.

alfalfa leaves > cow residues > control > wheat straw . Study of Liang et al. (2015) compared activity of phosphatase in soil amended with swine manure with unamended soil has shown an increase in  $V_{max}$  in amended soil. The organic matter of soil significantly alters kinetic parameters ( $V_{max}$  and  $K_m$ ) and catalytic

efficiency of enzyme reactions (Cartes et al. 2009). Zhang et al. (2010) also reported that the  $V_{max}$  increased after addition of organic residues because the calorific movement of enzyme molecules harried and the collisions between enzyme and substrate sped up, resulting in more substrate transformed. They also reported that the content and kinds of organic matter and clay may affect the substrate or enzyme diffusion, and hence the enzyme  $V_{max}$  and  $K_m$ .

$V_{max}$  values for alkaline phosphatase significantly varied for soils under study at all treatments (Table 3).  $V_{max}$  values in silty clay soil were greater than their respective values of loamy sand soil with an average of 412.91 and 319.97  $\mu\text{g p- nitrophenol g}^{-1} \text{ soil hr}^{-1}$ , respectively. Al-Ansari et al. (1999b); Jayan (2012) and Alhajjaj (2014) found that  $V_{max}$  of soil alkaline phosphatase differed with different soil types. AL-Ansari et al. (2019) suggested that soil properties play a major role in determining the effect of organic residues on soil enzymes activity. Zaman et al.(1999) also stated that organic matter composition and clay content of different soils can control the substrate concentration and enzyme distribution in soil, resulting in an alteration of  $V_{max}$  and  $K_m$ . Furthermore, Jayan (2012) reported that soils with different origin and under different environmental conditions show wide variation in the amount of enzyme , hence the enzyme concentration (E) is to be the most relevant factor to explain  $V_{max}$  .

Michaelis constant ( $K_m$ ) of soil alkaline phosphatase calculated using the three transformation plots differed

**Table 2.** Coefficient of determination ( $R^2$ ) and Linear equation for the relationship between substrate concentration and alkaline activity at the three transformations

Soil	Organic residue	Line Weaver- Burk	Hanes - Wolf	Eadie – Hofstee
Silty Clay	Cow residue	$Y=0.0623X+0.0026, R^2=0.962$	$Y=0.0024X+0.0742, R^2=0.986$	$Y=-26.33X+399.15, R^2=0.886$
	Alfalfa leaves	$Y=0.039X+0.0021, R^2=0.946$	$Y=0.0018X+0.0544, R^2=0.983$	$Y=-20.934X+500.97, R^2=0.851$
	Wheat straw	$Y=0.0765X+0.0038, R^2=0.971$	$Y=0.0036X+0.0848, R^2=0.995$	$Y=-21.654X+270.33, R^2=0.937$
	Poultry residue	$Y=0.0607X+0.0017, R^2=0.982$	$Y=0.0016X+0.069, R^2=0.974$	$Y=-34.579X+569.65, R^2=0.900$
	control	$Y=0.0666X+0.0036, R^2=0.929$	$Y=0.0033X+0.0811, R^2=0.992$	$Y=-19.91X+285.66, R^2=0.867$
Loamy Sand	Cow residue	$Y=0.0717X+0.0035, R^2=0.962$	$Y=0.0031X+0.0904, R^2=0.990$	$Y=-22.516X+296.06, R^2=0.905$
	Alfalfa leaves	$Y=0.0533X+0.0027, R^2=0.890$	$Y=0.0021X+0.0856, R^2=0.954$	$Y=-22.700X+394.77, R^2=0.729$
	Wheat straw	$Y=0.0656X+0.0052, R^2=0.938$	$Y=0.0047X+0.0913, R^2=0.996$	$Y=-17.604X+205.93, R^2=0.966$
	Poultry residue	$Y=0.0582X+0.0023, R^2=0.947$	$Y=0.0021X+0.0680, R^2=0.969$	$Y=-25.761X+436.67, R^2=0.818$
	control	$Y=0.0712X+0.0045, R^2=0.985$	$Y=0.0042X+0.0894, R^2=0.996$	$Y=-20.141X+235.10, R^2=0.978$

**Table 3.**  $V_{max}$  and  $K_m$  values of alkaline phosphatase in soils treated with different organic residues. Different letters indicates significant differences among means of main factors (capital letters) and among means of interaction (small letters)

soil	Organic residues	$V_{max}$ ( $\mu\text{g p-nitrophenol g}^{-1} \text{ soil hr}^{-1}$ )				$K_m$ ( mM )			
		Line Weaver-Burk	Hanes- Wolf	Eadi-Hofstee	mean	Line weaver-Burk	Hanes- Wolf	Eadi-Hofstee	mean
Silty clay	Cow residue	384.61c	416.66d	399.15cd	400.14B	23.96d	30.91f	26.33e	27.06B
	Alfalfa leaves	476.19e	555.55f	500.97e	510.90C	18.59a	30.22f	20.93b	23.24B
	Wheat straw	263.15a	277.77ab	270.33a	270.41A	20.13ab	23.55cd	21.65bc	21.77A
	Poultry residue	588.23g	625.00h	569.65fg	594.29D	35.70g	43.12h	34.57g	37.79C
	control	277.77ab	303.03b	285.66ab	288.82A	18.50a	24.57de	19.91ab	20.99A
	mean	397.99A	435.60B	405.15A		23.37A	30.47B	24.67A	
	Loamy sand	Cow residue	285.71e	322.58f	296.06e	301.45B	20.48de	29.16h	22.51ef
Alfalfa leaves	370.37g	476.19i	394.77g	413.77C	19.74cd	40.76j	22.70f	27.73D	
Wheat straw	192.30a	212.76abc	205.93ab	203.66A	12.61a	19.53cd	17.60bc	16.58A	
Poultry residue	434.78h	476.19i	436.67h	449.21D	25.30g	32.80i	25.76g	27.95D	
control	222.22bed	238.09d	235.10cd	231.80A	15.82b	21.28def	20.14d	19.08B	
mean	301.07A	345.16B	313.70A		18.79A	28.70B	21.74C		

with different organic residues followed the order poultry residue > cow residue > alfalfa leaves > control > wheat straw. That means, addition of organic residues to soil significantly affected the formation of enzyme – substrate complex since  $K_m$  value is used as a measure of the affinity between the enzyme and its substrate. The  $K_m$  values ranged from 18.50 to 43.12 mM for silty clay soil and from 15.82 to 40.76 mM for loamy sand soil. The  $K_m$  values found in our study were higher than these reported by many authors, this is due to difference in soil physical and chemical characteristics. Tabatabai (1994) reported that both  $V_{max}$  and  $K_m$  are constant for the enzyme, but may vary independently of each other under different conditions. However, similar values of  $K_m$  (10.8 – 40.3 M) of alkaline phosphatase were reported by Alhajjaj (2014).

It seems that the change of  $K_m$  had a positive trend with change of corresponding  $V_{max}$  (**Table 3**), indicating that the affinity between alkaline phosphatase and substrate had negative relationship with alkaline phosphatase amount in soils under experiment conditions, which agrees with the results found by Zhang et al. (2010) and Alhajjaj (2014). The higher soil organic C after addition of organic residues holds soil enzyme and prevent it from interacting with the substrate, and increased  $K_m$  values (Handrikova et al, 1996). However, preventing enzyme by soil organic matter sometimes promotes catalytic reaction and sometimes inhibits such catalytic reaction (Zhang et al. 2010). Furthermore, Irving and Cosgrove (1976) attributed the higher  $K_m$  value of acid phosphatase to the adsorption of the substrate (p- nitrophenylphosphate) to soil, which lower the concentration of substrate in soil solution and lead to increase  $K_m$  value.

Data obtained in **Table 3** indicated that  $K_m$  values of alkaline phosphatase in silty clay soil were significantly higher than those of loamy sand soil, with all over

averages of 26.17 and 23.07 mM, respectively. Al-Ansari et al. (1999b) reported that variation in  $K_m$  values among soils might be due to differences in soil properties. Higher soil organic C and clay content can adsorb enzyme molecules and protect enzyme from reacting with the substrate, resulting in an increase in  $K_m$  value.

Data of **Table 3** showed that  $V_{max}$  and  $K_m$  value were varied with the type of Michaelis – Menten transformation plots. The  $V_{max}$  and  $K_m$  value were consistently higher at the following order: Hanes – Wolf > Eadie – Hofstee > LineWeaver – Burk. Jayan (2012) examined the three transformations for alkaline phosphatase in different soils and found that the plot of LineWeaver – Burk is superior. Al-Ansari et al. (1999b) also found that the high values of  $V_{max}$  and  $K_m$  of alkaline phosphatase in soils followed the order Line Weave-Burk > Eadie – Hofstee > Hanes – Wolf. However, Irving and Cosgrove (1976) found that the linear transformation of Eadie– Hofstee gave the higher  $K_m$  values of acid phosphatase. Each transformation gives differing weigh of error in the variables, and this reflected in the variation of estimated  $V_{max}$  and  $K_m$  values for any soil enzyme by using different plots (Dowd and Riggs, 1965).

## CONCLUSION

The results indicated a higher alkaline phosphatase activity as a result of addition of organic residues. poultry manure stands out as a superior organic source compared to cow residue, alfalfa leaves or wheat straw in organic p mineralization and make phosphorus more available for plant. Kinetic parameters ( $V_{max}$  and  $K_m$ ) values were positively consistent at all treatments because of trapping the enzyme molecule and / or the substrate by organic carbon.

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