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MARSH BULLETIN

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Study of Charasteristics of Inorganic Pyrophosphatase Activity in Marshland, South of Iraq

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Abstract

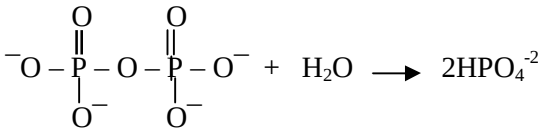
Marsh land (south of Iraq) is of great interest at present time, therefore studying some of microbial properties, including enzyme activities, can be useful as indicator of soil quality of this area. Hence, this study was carried out to compare inorganic pyrophosphatase (EC 3. 6. 1. 1) activity in this soil with their counterparts of other soils of south Iraq (Al–Zubair and Abul–Khsib soils). Results indicated that inorganic pyrophosphatase activity at all substrate concentrations in marsh land soil was higher than those of Al–Zubair soil, but lower than those of Abul–Khasib soil. Expressed as PO_4^{-3} – P released per gram soil per 5 hrs, the average Vmax values were 21.93, 37.23, and 33.33 while average Km values were 19.82, 18.26 and 29.77 for Al–Zubair, Abul–Khasib and marsh land soils, respectively. The results also revealed that the threshold of salinity negative effect on inorganic pyrophosphatase activity varies according to initial E.C. of soils (6 and 12 $dS.m^{-1}$ for Abul–Khasib and marsh land soils, respectively).

1-Introduction

Soil can be thought as a biological entity (i.e, a living tissue with complex biochemical reaction). Complex structure of organic compounds in soils must be first hydrolyzed through the activity of enzymes into low molecular weight compounds can be directly transfered to cells, oxidized and use as an energy source (Chrost, 1991). Enzymes are known to be involved in the cycling of nutrients and can be used as potential indicators of nutrient cycling processes (McLatchey and Reddy, 1998). Soils contain free enzymes, immobilized extracellular enzymes stabilized by a three-dimensional net work of macromolecules, and enzymes within microbial cells.

Several phosphtase enzymes occur in soil, among them is pyrophosphatase (pyrophosphate phosphohydrolase) E. C. (3. 6. 1. 1), which catalyze the hydrolysis of pyrophosphate

synthesized by microorganisms (Pepper *et al*., 1976) or added to soil as fertilizers (Dick and Tabatabai, 1978) to orthophosphate. The over all reaction is:



Pyrophosphatase is widely distributed in nature and its activity in bacteria, insect, mammalian and plants has been reported by several workers as reported by Tabatabai (1994). Pyrophosphatase activity in soils has been reported by Gilliam and Sample (1968) and Hossner and Phillips (1971).

In general the total enzymatic activity of soil are influences by many factors among them organic and mineral fractions in both bulk soil and the rhizosphere, cropping history and soil amendments. Moreovere, Tabatabai (1994) reported that several studies on extracellular enzyme activities in ecosystem have shown that vegetation, agricultural chemicals and industry pollutants have marked effects on soil enzymes. The effect observed differs markedly and depend on many factors including soil type, ionic strength, temperature and pH.

Marsh land (south of Iraq) is of great interest at present time therefore, studying some of microbiological properties, including enzyme activities, can be useful as indicator of soil quality of this area. A search of literatures revealed that there is no previous studies on enzymes activates in this area. Therefore, series of experiments were conducted to study enzymes activities in this area as compared with their counterparts of other soils,. Soil samples from these areas were collected during late 1999 and early 2000 (at which time, the marsh land was dried and brought into cultivation) to study enzyme activities in this area.

Activities and characteristics of urease, acid and alkaline phosphatase were reported in previous papers (Al – Ansari, 2000 and Al – Ansari *et al.*, 1999). However, the purpose of this paper is to present inorganic pyrophosphatase activity in soil samples collected during 2005 (when the marshes has been reestablished) from marshes and other soils under study.

2- Materials and Methods

Soil samples were collected from marsh land (south of Iraq) to study inorganic pyrophosphatase activity in such a soil. Samples were also collected from two other areas (namely, Abul–Khasib and Al–Zubair, both areas are located in south of Iraq) to compare enzyme activity in marsh land with their counterparts of other selected soils. Soils were collected to obtain a wide range in organic matter, total nitrogen, $CaCO_3$, electrical conductivity,and texture. Soil E.C. range from 13 at marsh land to 5.8 $dS.m^{-1}$ at Abul–Khasib area. Organic matter and total nitrogen ($gm.kg^{-1}$) range from 35.0 and 5.0 to 22.0 and 1.5 at marshland at Abul–Khasib area, respectively. While textures were clay at marsh land and silty clay at Abul–Khasib area. Soil properties of Al–Zubair area were: electrical conductivity 9.0, organic matter 2.0, total nitrogen 0.30 with texture of loamy sand. pH of all soils under study was about 7.8. Above

reported soil properties were determined as described by page *et al*. (1982).

Assay of inorganic pyrophosphatase activity in soil was made on <2mm soil samples following procedure of Dick and Tabatabai, (1978) described by Tabatabai (1994).

The Km and V_{max} value reported in this paper were calculated from the results obtained in studies of the effect of varying substrate concentrations in assay of inorganic pyrophosphatase activity. Km and V_{max} were obtaind by using three linear transformations to enzyme activity in soils under study. The Michaelis–Menten equation:

$$V = V_{max} .[S]/(Km + [S])$$

(Where: V is initial velocity, [S] is substrate concentration, Km is the Michaelis constant, and V_{max} is the maximum velocity) was applied and expressed in the linear forms representing the three transformations :-

$$\frac{1}{V} = \frac{1}{V_{max}} + \frac{Km}{V_{max}} \cdot \frac{1}{[S]}$$

(Lineweaver–Burk)

$$\frac{[S]}{V} = \frac{Km}{V_{max}} + \frac{1}{V_{max}} . [S]$$

(Hanes – Woolf)

$$V = V_{max} - Km . \frac{V}{[S]}$$

(Eadie – Hofstee)

In testing the effect of salinity on inorganic pyrophosphatase activity, samples of Abul–Khasib and marsh land soils were leached with distilled water until E.C. of saturated extract reached 3 or 6 $dS.m^{-1}$. Raising E. C. of soils to 12 and 24 $dS.m^{-1}$ were achieved by leaching soils with saline solution until equilibrium was reached between the soil extracts and saline solution. Then 1 gm of each salinity level was incubated with substrate solution for 5 hours at 37˚C, then inorganic pyrophosphatase activity was assayed following procedure of Dick and Tabatabai (1978).

3-Results and Discussion

Fig. (1) shows that pyrophosphatase activity (µg PO_4^{-3} – P / gm soil / 5 hr) in all soils under study increased by increasing substrate concentration till reach maximum at 40, 60, 80 mM at Al–Zubair, Abul–Khasib and march land soils, respectively. However, increasing substrate concentration beyond these concentrations did not significantly affect the amount of PO_4^{-3} – P produced. This suggested that the rate of reaction catalyzed by inorganic pyrophosphatase approached a limiting velocity at higher concentration of substrate following zero–order kinetics at higher substrate concentration. However, it has been suggested that soil phosphatase enzymes do not follow the hyperbolical kinetics described by the mathematical analysis of Michaelis–Menten because the enzymatic reaction in soil takes place at solid–liquid interfaces (Irving and Cosgrove 1979). Our previous studies on alkaline and acid phosphatase in soils, showed similar effect of substrate concentrations on their activity in soil (Al – Ansari *et al*., 1999). These results suggested that kinetics behaviour of inorganic phosphatase in soils followed the

hyperbolical kinetics which can be described through Michaelis – Menten equation.

Inorganic pyrophosphatase activity at all substrate concentrations in marsh land soil (average 20.0 µg PO_4^{-3} – P / gm soil / 5 hr) was higher than those of Al–Zubair soil (average 15.4 µg PO_4^{-3} – P / gm soil / 5 hr), but lower than those of Abul–Khasib soil (average 27.2 µg PO_4^{-3} – P / gm soil / 5 hr). Differences in values of pyrophosphatase activity in soils under study may be due to differences of soil properties. Tabatabai and Dick (1979) reported that pyrophosphatase, similar to other enzymes activities in soils is concentrated in surface soils and decreases with depth, it is significantly correlated with organic carbon in surface soils and soil profile. They also reported that pyrophosphatase activity in soil positively correlated with percentage clay and mole fraction of Mg / (Mg + Ca) in water extracts of surface soils but negatively correlated with percentage of $CaCO_3$equivalent in surface soils. Bolton *et al*. (1985) reported that phosphatase activity in soil fluctuated over sampling time. Results of Herrisson and Pearce (1979) supported the trend of phosphatase activity fluctuation over time and they suggested that increase in activities were mainly due to phosphatase production by roots and soil microorganisms.

Kinetic parameters (Km and V_{max}):-

Quantitative measurements of the activity of soil enzymes are necessary for understanding of their biological function and for determining the amount of activity present in soil samples. Kinetics properties of the reaction catalyzed by enzymes in soil is desirable because these properties are used to characterized enzyme reactions and to predict the reaction rate and substrate concentration needed under certain conditions. Once the Km and V_{max} are known for particular enzymatic reaction under a given set of condition, the reaction velocity (V) can be calculated for any substrate concentration by using linear transformation of Michaelis–Menten equation

$$V = \frac{V_{max}\ [S]}{Km + [S]}$$

Michaelis–Menten equation is such that approximately 10 and 90 % of V_{max} is achieved at substrate concentration corresponding to Km X 10^{-1} and Km X 10, respectively.

Fig. (2) shows plot of the three possible linear transformations of Michaelis – Menten equation applied to inorganic pyrophosphatase activity in marsh land soil as compared to other soils under study. Both V_{max} and Km values were obtained graphically and presented in table (1).

Data in table (1) shows that V_{max} of inorganic pyrophosphatase activity in marsh land was higher than those of Al–Zubair area soil but lower than those of Abul–Khasib area soil. Expressed as PO_4^{-3} – P released per gram soil per 5hr, the average V_{max} values were 21.93, 37.23 and 33.33 while average Km values were 19.82, 18.26 and 29.77 for Al–Zubair, Abul–Khasib and marsh land soils, respectively.

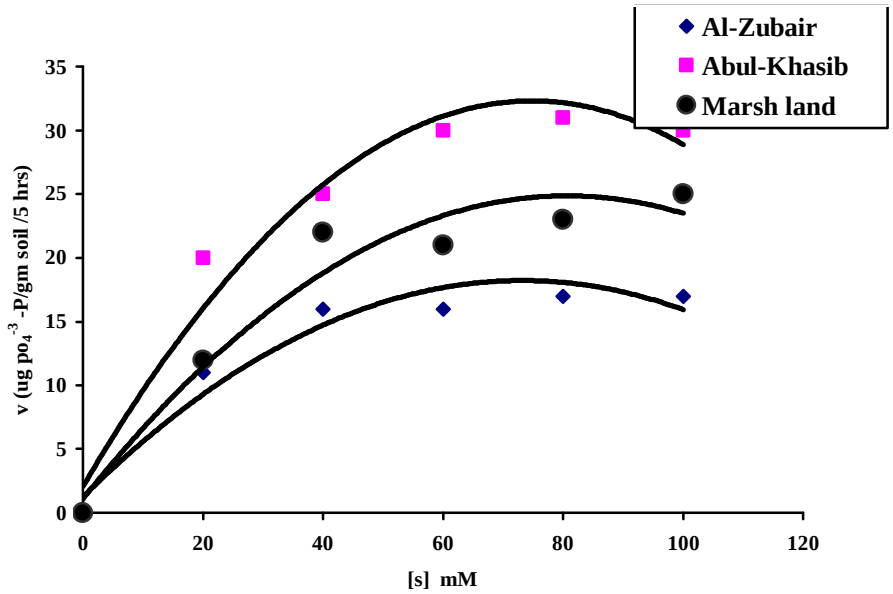
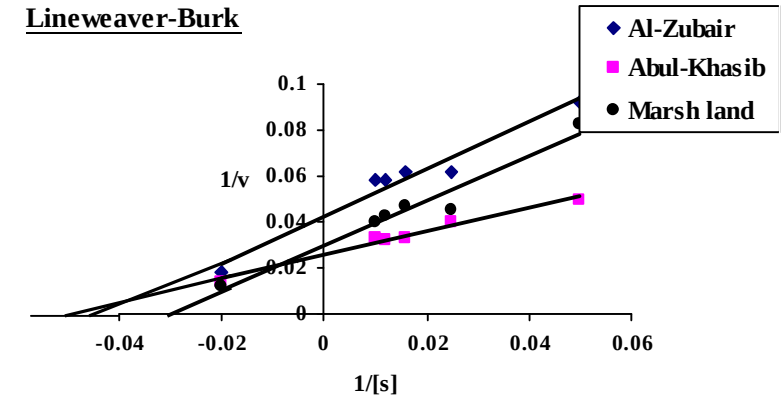


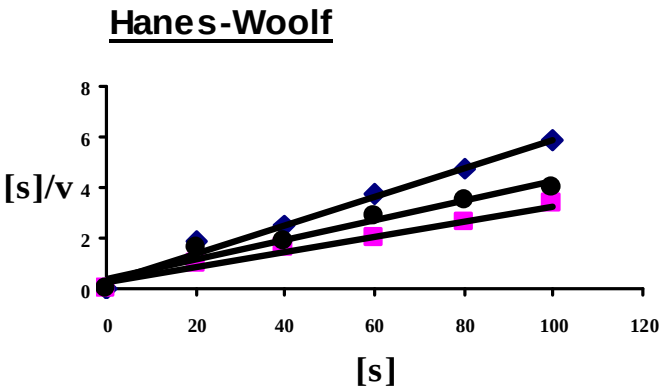
Fig.(1):Effect of substrate concentrations on inorganic pyrophosphatase activity of soils under study

Table 1: V_{max} and Km values of inorganic pyrophosphatase calculated from the three linear ransformations of Michaelis – Menten equation.

Soil	Lineweaver–Burk		Hanes Woolf		Eadie- Hofstee		Average	
	Km *	V_{max}**	Km	V_{max}	Km	V_{max}	Km	V_{max}
Al–Zubair	29.20	26.31	16.00	20.00	14.28	19.50	19.82	21.93
Abul–Khasib	30.00	45.45	12.90	32.25	11.90	34.00	18.26	37.23
Marsh land	44.40	40.00	17.14	28.57	27.77	31.42	29.77	33.33

*** Km: mM ; **V_{max}: µg PO_4^{-3} – P / gm soil / 5 hr**





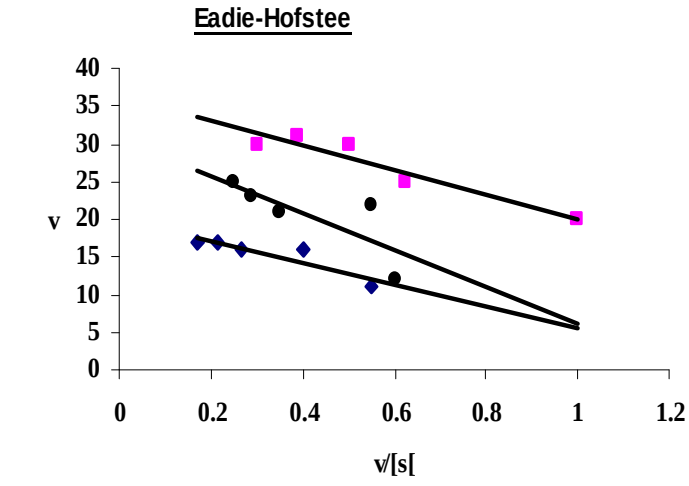


Fig.(2); Linear plots of Michalis-Menten equesion for inorganic pyrophosphatase activity in different soils .Velocity (V) expressed as µg PO_4^{-3}-p/gm soil /5 hrs .and substrate concentration [S]in mM

Data also shows that magnitude of V_{max} and Km values were somewhat varied with type of transformation. The magnitude of V_{max} and Km values were consistently higher (more abvious in marsh land) in the following order: - Lineweaver > Eadie–Hofstee > Hanes–Woolf. Irving and cosgrove (1979) examined the graphical technique used in calculation of the Km values of acid phosphatase enzyme and concluded that the linear transformation of Eadie–Hofstee should be used. Tabatabai (1994) reported that three linear transformations are equally applicable for estimation of apparent Km values of enzymes in soils. Dowd and Rigges (1965) reported that each transformation gives different weight of errors in the variable and this reflected in the variation of estimated V_{max} and Km values derived from any soil by using different plots. Tabatabai, (1994) reported that kinetics studies with surface soils have shown that the apparent Km values of pyrophosphatase in soils range from 20 to 51 mM. Results of this study showed lower Km value than reported by Tabatabai (1994). This differences in Km values obtained for the soils under study and that reported by other researchers seems due to environmental conditions under which enzymes present. Paulson and kurtiz (1970) and Tabatabai (1994) reported that Km and V_{max} for particular enzyme influenced by environmental conditions and may vary independently of each other under different condition. Durand (1964) suggested that Km values for adsorbed enzymes is greater than those of unadsorbed enzymes.

Effect of Salinity:-

The effect of salinity on pyrophosphatase activity in samples of Abul–Khasib soil and marsh land soil were used in this study due to their different initial E.C. values (5.8 vs 13 $dS.m^{-1}$). Data in Fig. (3) clearly shows that pyrophosphatase activity in Abul – Khasib soil under all studied salinity levels were higher than those of marsh land. Average values were 19.5 and 15.2 µg PO_4^{-3} – P / gm soil / 5 hr at Abul–Khasib and marsh land soils, respectively. At both soils increasing salinity level beyond 3 $dS.m^{-1}$ significantly increase pyrophosphatase activity. However, the maximum activity in soil were reached at E.C. 6 $dS.m^{-1}$ for Abul – Khasib soil and 12 $ds.m^{-1}$ for marsh land soil. These values were similar to initial soil E.C. Nevertheless, increasing salinity levels beyond these values significantly decreased pyrophosphatase activity. This results revealed that the threshold of salinity negative effect on pyrophosphatase activity varies according to initial E. C. of medium. Similar trends of salinity effect were observed on studying the effect of salinity on urease activity (Al – Ansari, 2000) and phosphatase activity (Al – Ansari *et al.*,1999).

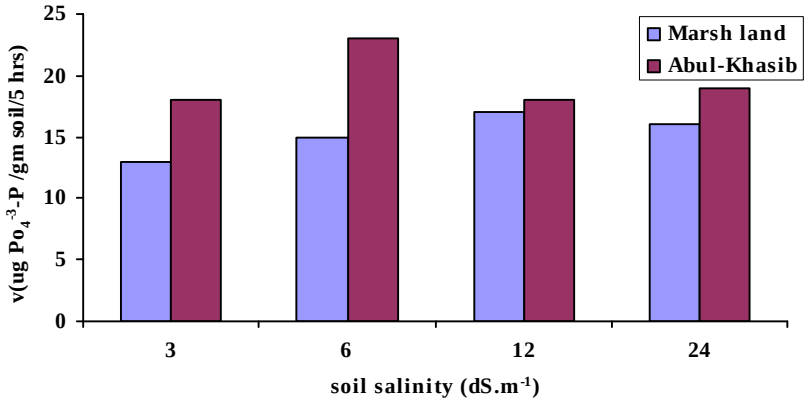


Fig.(3):Effect of soil salinity on inorganic pyrophosphatase activity in soils under study.

Excess salinity can affect soil enzyme activity (Dick, 1998). Gracia *et al*. (1994) found a negative correlation between soil electrical conductivity and different enzymes activities. This reduction in enzyme activity can be due a lower microbial biomass and to direct effects of osmotic potential and specific ions on enzyme activity. Frankenberger and Bingham (1982) reported that the level of soil enzymes activity decreased with increasing salinity level from 3 to 24 $dS.m^{-1}$ and the degree of inhibition varied among the enzymes assayed and the nature and amount of salts added. Salts containing Cl^- showed more inhibition to enzyme activities than salts containing SO_4^{-2}. From results of their work, they suggest that both ionic and osmotic effect are important in affecting the proliferation of microorganisms, the synthesis of enzymes, and in changing the ionic conformation of enzymes.

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دراسة خواص أنزيم البايروفوسفاتيز المعدني في تربة الاهوار جنوبي العراق

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الملخص

نظراً للاهتمام الكبير الذي استحوذت علية مناطق اهوار جنوبي العراق في الفترة الأخيرة فمن المهم دراسة بعض الصفات المايكروبايولوجية ومنها فعالية الأنزيمات كمؤشر للدلالة على خواص ترب هذه المناطق ونظراً لمحدودية الدراسات في مجال أنزيمات التربة لمثل هذه الترب فقد نفذت هذه التجربة لدراسة فعالية أنزيم البايروفوسفاتيز المعدني (EC 3. 6. 1. 1) في تربة الاهوار ومقارنتها بترب أخرى من جنوب العراق (الزبير وأبو الخصيب). أوضحت النتائج أن نشاط أنزيم البايروفوسفايتز المعدني لتربة الاهوار أعلى من نشاط الأنزيم لتربة الزبير ولكنه اقل مما هو لتربة ابي الخصيب, عند جميع تراكيز المادة الخاضعة المستخدمة في الدراسة. بلغت قيمة V_{max} 21.93, 37.23, 33.33 وقيم Km 19.82, 18.26, 29.77 لترب الزبير وأبي الخصيب والاهوار على التوالي. وأشارت نتائج الدراسة ايضاً إلى أن بداية التأثير السلبي لملوحة الترب على نشاط أنزيم البايروفوسفاتيز المعدني قد اعتمد على الملوحة الأصلية للترب المدروسة (6 ديسمنز / م لتربة أبي الخصيب و 12 ديسمنز / م لتربة الاهوار).