Effect of Agricultural Exploitation on the Activity of Alkaline Phosphatase and Its Kinetic Properties in Some Soils

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Abstract: In order to study the role of agricultural exploitation in the activity of alkaline phosphatase in the province of Diwaniyah, six sites that differ in some of their chemical, physical and biological properties were selected (Diwaniyah, Sunniya, Shamiya, Daghara, Afak and Al Budair). Three types of soils were chosen in each location, namely, orchard soil, field soil and jungle soil. The kinetic parameters of the enzymes maximum velocity (Vmax) and Michaelis constant (Km) were estimated in all study soils using increasing concentrations of the controlled substance. The results present that the highest efficacy of this enzyme is in the orchard soil of all sites except the location of the center Diwaniyah and Shamia. The average efficacy values ranged between (208.11 - 234.95) μg P-Nitro phenol. g⁻¹ soil. 1 hour⁻¹. The highest value of maximum velocity (Vmax) recorded at the field soil of Al-Shamia site (108.57) μg P-Nitro phenol. g⁻¹ soil. 1 hour⁻¹. The lowest value recorded at the soil of a jungle field at the Sunniya location (49.62) μg P-nitro phenol. g⁻¹ soil. 1 hour⁻¹. While the orchard soil of Al-Budair had the highest value of the Michaelis constant (Km) of (85.90) mM, the orchard soil of the Sunniya location had the lowest value (24.34) mM.

Keywords: Agricultural Exploitation, L-Asparaginase , Vmax, Michaelis constant.

I. INTRODUCTION

Agricultural exploitation is an important and influential factor in many physical, chemical and biological properties. The variation and the diversity of soil uses are important factors in the distribution of organic matter, organic carbon and nutrient content among soil separators, [1]. One of the bio-traits that are affected by agricultural exploitation is the soil enzymes, since their sources either soil microorganisms or plant roots.

They stimulate enzymatic activity by stimulating microorganisms in the rhizosphere region. Roots are an important source of external soil enzymes, Dodor and Tabatabai, 2003. Soil enzymes are the key to biochemical reactions and the biological interactions of soil and plant roots. Enzymes stimulate all these biological and biochemical reactions. Enzymes are also assistant agents made up of proteins with catalytic properties that increase the velocity of reaction without altering enzyme properties after the end of the reaction, [2].

The alkaline phosphatase is a hydrolysis enzyme that dissolves phosphorus esters. Plants, microorganisms and soil animals produce Basal phosphatase, [3]. The alkaline phosphatase participates in the phosphorus cycle in the soil and reveals the solubility of phosphorus in the soil, [4]. Renala [5] presented that the alkaline phosphatase is excreted abundantly in basal or neutral soils. The enzyme is stabilized by organic colloids and is trapped by humus particles, [6]. The importance of the enzyme in plant nutrition comes from its high efficiency in the area of the rhizosphere compared to the bulk soil area, [7]. Pantelista et al. [8], note that the activity of basal phosphatase increases significantly as the biomass increases, especially during the flowering period. This increase is due to the production of enzymes by microorganisms and root excretions. Ting et al.[9], reported that the efficacy of alkaline phosphatase with different soils that are collected from different crop growth stages was between 186.37-108.34 μg PNP⁻¹. g⁻¹ soil. 1 hour⁻¹.

This is due to a significant difference in the decomposition of organic matter as well as the increase in microbiological activity and the type of the cultivated agricultural crops. Microorganisms in the soil have the ability to dissolve phosphorus and convert it into an available form. Many types of fungi, including Spp. Mucor, Spp Penicillium and Spp. Aspergillus have a role in increasing plant growth by (5-20) % after inoculation, [10],[11],[12],[13],[14]. explained that there are species of Trichoderma spp, fungi that dissolve phosphorus, play an important role in promoting plant growth.

Soil animals, including earthworms, affect the phosphatase enzyme. The presence of these animals increases the activity of the enzyme [15]. The values of the kinetic parameters of the Vmax and KM are some of the most important parameters in the chemistry of enzymes. Vmax is the maximum efficacy of the enzyme, and Km is the concentration of the controlled substance, which gives half of the maximum activity and is an evidence of the ridge between the enzyme and the subject matter, [15]. Frankenberger and Tabatabai [16] reported that the values of the
Michaelis constant and the maximum velocity of the reaction vary depending on the enzyme surrounding conditions. The values of the ionization constant do not rely on the concentration of the enzyme but on its efficacy. Therefore, it is an important measure the enzyme dynamics. The Michaelis constant uses to measure the link to the substance of the interaction. The lower the Michaelis constant value, the greater the homogeneity between the substance of the reaction and the enzyme[17], clarified that high value of Km, which is also called the high affinity constant, means that there is poor indexation between the enzyme and the subject matter. This is due to the strong impedance of the enzyme by the soil components [18]. found that increasing Vmax values of the basal phosphatase in two different soils, the first is loamy clay and the second loamy sand, the maximum velocity values were (522.7 and 398.5) µg PNP-1 soil. 1 hour-1, respectively. The difference in these values is due to the high efficacy of the enzyme in the soil. Km values were low for both soils (1.3 and 1.2), respectively. The reason is either the way the enzyme is evaluated or the difference in the enzyme environment and the soil environment or both. Since there is a lack of studies on the role of agricultural exploitation in the activity of enzymes and kinetics, the study aims to the fellows:

1. Determination of the activity of Alkaline Phosphatase at different agricultural exploitation sites.
2. Determination of kinetic parameters of the (Vmax) maximum velocity and the Michaelis constant (Km) of the Alkaline Phosphatase.

II. Materials and Methods

Soil samples were collected from six locations that differ in some of their chemical, physical and biological characteristics in the Diwaniyah province (Diwaniyah, Sunniya, Shamiya, Daghara, Afak and Al Budair). Three types of soils within the same location that have been different exploited for agricultural purposes were selected, which are orchard, field, and bushes (table 1). Soil samples were randomly taken from the surface layer (0-30) cm from several different spots to each location and stored in polyethylene bags then transferred to the laboratory. They have undergone air-dried, grind, and sifted with a 2 mm diameter sieve then mixed well for homogeneity. Some chemical, physical, and biochemical analysis have been carried out (Table 2, 3, 4, 5, 6 and 7).

Table 1: Agricultural exploitation of the studied location.

| Location       | Sample | Agricultural exploitation                      |
|----------------|--------|-----------------------------------------------|
| Center of Diwaniyah | orchard| Planted with palm trees, fruits, and citrus fruits |
|                 | Field  | Planted with eggplant plants                  |
|                 | jungle | Contains lots of flora                        |
| Sunniya         | orchard| Planted with palm trees, fruits, and citrus fruits |
|                 | Field  | Planted with wheat plants                     |
|                 | jungle | Contains flora plants                         |
| Shamiya         | orchard| Planted with palm trees, fruits, and citrus fruits |
|                 | Field  | Planted with cowpea plants                    |
|                 | jungle | Contains lots of flora                        |
| Dagara          | orchard| Planted with palm trees                       |
|                 | Field  | Planted with the rice crop                    |
|                 | jungle | There are many plants of the alliance          |
| Afak            | orchard| Planted with palm trees                       |
|                 | Field  | Planted with yellow maize plants              |
|                 | jungle | Contains lots of flora                        |
| Al Budair       | orchard| Planted with palm trees and almond trees       |
|                 | field  | Planted with yellow maize plants              |
|                 | jungle | Contains flora plants                         |
III. Results and Discussion

Physical properties volumetric distribution of soil separators Estimated according to the international pipette method according to the method in Black, 1965a. Bulk density Estimated according to the Core Sample method that mentioned in Black 1965a.[19]. Chemical properties Soil pH Measure in 1:1 (soil: water) extract using a pH-meter using the Black 1965a Method. Electrical conductivity (EC) It was estimated at 1:1 (soil: water) extract using an EC-meter according to the method in Black, 1965a. Cation exchange capacity CEC Estimated by Papanicolaou method,1976. Through soil saturation with calcium chloride (0.1) standard solution at pH = 7 and displacement with sodium nitrate (0.1) standard. CalciumCarbonate CaCO₃ Calcium carbonate was measured by calculating the loss of carbon dioxide by treating the soil with hydrochloric acid (3 standards), according to the method in Black, 1965a. Gypsum CaSO₄ Estimated through sedimentation by acetone and by the method given in Black, 1965b. Positive and negative dissolved ions Estimated in 1:1 (soil: water) extract according to the methods stated in Black, 1965b [20].

Sodium Na+ and potassium K+ Estimated by using a flame photometer device. Calcium Ca²⁺ and magnesium Mg²⁺ Estimated by the titration with Na₂EDTA. Chloride (Cl⁻) estimated by the titration with silver nitrate 0.005 standards. Sulfates (SO₄²⁻) Estimated according to turbidity method by using barium chloride and through the Spectrophotometer device. Total nitrogen Estimated by digesting soil samples with concentrated sulfuric acid then using micro-Kjeldahl steam distillation device according to a method of Bremner, 1965. That mentioned in Black, 1965b. Available Phosphorus available soil phosphorus was extracted using 0.5 molar of NaHCO₃ according to Olsen method.

The color was developed with ammonium polysaccharides and ascorbic acid and was evaluated using the Spectrophotometer according to Page et al, 1982. Available potassium Soil potassium was extracted by using (1) molar of ammonium acetate and then extracted potassium estimated by Flame-photometer device according to the method in Page et al.,1982. Organic matter Organic matter was estimated according to the method of Walkely-Black, Black,1965b. by oxidation with potassium dichromate solution with a concentrated sulfuric acid, and reverse titration with ferrous sulfate using D-phenylamine. Determination of the number of bacteria and total fungi A total number of fungi in the soil were estimated by dilution method and counting. 10 grams of soil and were transferred to a dilution bottle containing 90 ml distilled and sterilized water. After that, 1 ml was removed and transferred to another bottle containing 90 ml distilled and sterile water. The dilution process continued to obtain a dilute chain from (10⁻¹ to 10⁻⁷). Dilutions of 10⁻⁵, 10⁻⁶ and 10⁻⁷ was used to estimate the numbers of bacteria, which were grown on Nutrient Agar medium in accordance with the [20] method.

To estimate the number of fungi, dilutions of 10⁻⁵, 10⁻⁶, and 10⁻⁷ were taken was grown in Martin medium, according to Rashidi, 1987. Determination of the activity of alkaline phosphatase in soil. The efficacy of the alkaline phosphatase was estimated according to Eivazi and Tabatabai ,1977 method. It was done by placing 1 g of soil in a 50 ml flask with 0.2 mL of toluene and 4 mL of Modified universal buffer (MUB) (boric acid, citric acid, salicylic acid, And THAM with pH= 11 and add (1) mL of P-nitro phenyl phosphate as an enzyme-dependent substance. After that, it incubates at a temperature of (370) for an hour. After incubation, (1) mL of potassium chloride solution 0.5 molar and 4 mL of 0.5 mM sodium hydroxide were added and the soil suspension filtered. The efficiency of the enzyme is estimated by the amount of P-nitro phenol released, which is measured by the spectrophotometer at a wavelength of (420) nanometres. The efficacy of this enzyme is calculated according to the following equation:

\[ \frac{C \times V}{dwt \times SW \times t} = p\text{-}nitro\text{ phenol (µg. g⁻¹ dwt. h⁻¹)} \]

Where:
- \(C\) = concentration of P-nitrophenol (µg.mL⁻¹)
- \(V\) = soil suspended volume (ml)
- \(dwt\) = dry weight for (1) gram wet soil weight
- \(SW\) = weight of wet soil (g)
- \(t\) = incubation time (1 hour).

Study of the kinetic parameters of alkaline phosphatase Vmax and KM of the basal phosphatase enzyme were estimated by studying the effect of the difference in the concentration of the controlled substance in the activity of the enzyme. Seven concentrations of the controlled substance (12.5, 25, 50, 100, 125 and 150) mM were used, according to [21] method. The enzyme activity was estimated according to Eivazi and Tabatabai, 1977 method.

The values of Vmax and Km were calculated according to the Hanes-Woolf equation of the Michaelis-Menten equation as follows:

\[ V = \frac{Km}{Vmax} + 1/Vmax \cdot [S] \]

Where:
- \(V\) = the speed of the reaction
- \(Vmax\) = the maximum velocity of the enzyme
- \(Km\) = Michaelis constant (M -L⁻¹) 
- \(S\) = concentration of the controlled substance (M -L⁻¹)

Maximal velocity measurements and the Michaelis constant are estimated from the straight-line equation between \(V / [S]\) and \([S]\) and extract the slop value that represents Vmax / 1 and the cutter that represents Km / Vmax.
### Table 2: Some chemical, physical and biological properties of the center of Diwaniyah site.

| Soils Traits       | Orchard | Field | Jungle fields | Unit       |
|--------------------|---------|-------|---------------|------------|
| pH                 | 7.11    | 7.30  | 7.61          |            |
| EC                 | 2.92    | 2.33  | 3.21          | ds .m⁻¹    |
| Ca²⁺               | 0.59    | 0.76  | 0.54          |            |
| Mg²⁺               | 0.92    | 0.67  | 0.66          |            |
| Na⁺                | 1.37    | 0.33  | 0.71          |            |
| K⁺                 | 0.06    | 0.05  | 0.07          |            |
| Cl⁻                | 1.81    | 1.52  | 1.31          |            |
| CO₃²⁻              | Nill    | Nill  | Nill          |            |
| HCO₃⁻              | 0.23    | 0.15  | 0.13          |            |
| CEC                | 34.40   | 45.27 | 33.61         |            |

**Dissolved ions**

| Unit              |            |
|-------------------|------------|
| Organic matter    | 12.06      |
| Organic Carbon    | 7.00       |
| Gypsum            | 3.10       |
| Calcium carbonate | 221.1      |
| Total nitrogen    | 0.60       |
| Available Phosphorus | 14.52    |
| Available Potassium | 128.71   |

**Soil separators**

| Unit |            |
|------|------------|
| pH   | 7.11       |
| EC   | 2.71       |
| Ca²⁺ | 2.50       |
| Mg²⁺ | 3.20       |
| Na⁺  | 0.37       |
| K⁺   | 0.07       |
| Cl⁻  | 2.03       |
| CO₃²⁻| Nill       |
| HCO₃⁻| 0.17       |
| CEC  | 42.22      |

**Soil texture**

| Unit |            |
|------|------------|
| pH   | 7.21       |
| EC   | 2.80       |
| Ca²⁺ | 1.52       |
| Mg²⁺ | 0.91       |
| Na⁺  | 0.33       |
| K⁺   | 0.06       |
| Cl⁻  | 2.10       |
| CO₃²⁻| Nill       |
| HCO₃⁻| 0.15       |
| CEC  | 47.33      |

**Bulk density**

| Unit |            |
|------|------------|
| pH   | 7.60       |
| EC   | 3.22       |
| Ca²⁺ | 1.27       |
| Mg²⁺ | 0.51       |
| Na⁺  | 0.57       |
| K⁺   | 0.13       |
| Cl⁻  | 2.41       |
| CO₃²⁻| Nill       |
| HCO₃⁻| 0.122      |

**Table 3: Some chemical, physical and biological properties of Sunniya site.**

| Soils Traits       | Orchard | Field | Jungle fields | Unit       |
|--------------------|---------|-------|---------------|------------|
| pH                 | 7.11    | 7.21  | 7.60          |            |
| EC                 | 2.71    | 2.80  | 3.22          | ds .m⁻¹    |
| Ca²⁺               | 2.50    | 1.52  | 1.27          |            |
| Mg²⁺               | 3.20    | 0.91  | 0.51          |            |
| Na⁺                | 0.37    | 0.33  | 0.57          |            |
| K⁺                 | 0.07    | 0.06  | 0.13          |            |
| Cl⁻                | 2.03    | 2.10  | 2.41          |            |
| CO₃²⁻              | Nill    | Nill  | Nill          |            |
| HCO₃⁻              | 0.17    | 0.15  | 0.122         |            |
| CEC                | 42.22   | 47.33 | 35.21         |            |

**Dissolved ions**

| Unit |            |
|------|------------|
| pH   | 7.21       |
| EC   | 2.80       |
| Ca²⁺ | 1.52       |
| Mg²⁺ | 0.91       |
| Na⁺  | 0.33       |
| K⁺   | 0.06       |
| Cl⁻  | 2.10       |
| CO₃²⁻| Nill       |
| HCO₃⁻| 0.15       |
| CEC  | 47.33      |

**Soil separators**

| Unit |            |
|------|------------|
| pH   | 7.60       |
| EC   | 3.22       |
| Ca²⁺ | 1.27       |
| Mg²⁺ | 0.51       |
| Na⁺  | 0.57       |
| K⁺   | 0.13       |
| Cl⁻  | 2.41       |
| CO₃²⁻| Nill       |
| HCO₃⁻| 0.122      |

**Soil texture**

| Unit |            |
|------|------------|
| pH   | 7.21       |
| EC   | 2.80       |
| Ca²⁺ | 1.52       |
| Mg²⁺ | 0.91       |
| Na⁺  | 0.33       |
| K⁺   | 0.06       |
| Cl⁻  | 2.10       |
| CO₃²⁻| Nill       |
| HCO₃⁻| 0.15       |
| CEC  | 47.33      |

**Bulk density**

| Unit |            |
|------|------------|
| pH   | 7.60       |
| EC   | 3.22       |
| Ca²⁺ | 1.27       |
| Mg²⁺ | 0.51       |
| Na⁺  | 0.57       |
| K⁺   | 0.13       |
| Cl⁻  | 2.41       |
| CO₃²⁻| Nill       |
| HCO₃⁻| 0.122      |

**Table 2:** Some chemical, physical and biological properties of the center of Diwaniyah site.

**Table 3:** Some chemical, physical and biological properties of Sunniya site.
### Table 4: Some chemical, physical and biological properties of Shamiya site.

| Soils Traits | Orchard | Field | Jungle fields | Unit |
|--------------|---------|-------|--------------|------|
| pH           | 7.71    | 7.53  | 7.91         |      |
| EC           | 2.34    | 2.27  | 3.52         | ds.m⁻¹ |
| Ca²⁺         | 1.05    | 1.57  | 2.03         | Cmol. Kg⁻¹ Soil |
| Mg²⁺         | 0.91    | 0.44  | 1.02         |      |
| Na⁺          | 0.03    | 0.07  | 0.06         |      |
| K⁺           | 1.80    | 1.21  | 0.07         |      |
| Cl⁻          | 1.71    | 2.22  | 3.20         |      |
| CO₃²⁻        | Nil     | Nil   | Nil          |      |
| HCO₃⁻        | 0.14    | 0.27  | 0.15         |      |
| CEC          | 25.22   | 24.01 | 27.22        |      |
| Organic matter | 12.1    | 15.68 | 12.06        | g.Kg⁻¹ Soil |
| Organic Carbon | 2.4      | 9.10  | 7.10         |      |
| Gypsum       | 4.70    | 3.10  | 3.30         |      |
| Calcium carbonate | 224.4  | 241.1 | 276.6        |      |
| Total nitrogen | 0.90   | 0.70  | 0.60         |      |
| Available Phosphorus | 18.71 | 15.27 | 9.33         |      |
| Available Potassium | 123.32 | 101.21 | 93.12       |      |
| Soil separators | silt: 15.51 | clay: 15.58 | sand: 12.24 | g.Kg⁻¹ Soil |
| Soil texture  | Silty loam | Loam  | loam         |      |
| Bulk density  | 1.20    | 1.46  | 1.38         | Mg.m⁻³ |
| Total bacteria | 12.6×10⁶ | 17.0×10⁶ | 4.0×10⁶     | CFU. g⁻¹ Soil |
| Total fungi   | 3.0×10⁶ | 2.21×10⁷ | 1.25×10⁸   |      |

### Table 5: Some chemical, physical and biological properties of Shamiya site.

| Soils Traits | Orchard | Field | Jungle fields | Unit |
|--------------|---------|-------|--------------|------|
| pH           | 7.62    | 7.36  | 7.51         |      |
| EC           | 2.35    | 3.13  | 6.21         | ds.m⁻¹ |
| Ca²⁺         | 2.03    | 1.61  | 0.74         | Cmol. Kg⁻¹ Soil |
| Mg²⁺         | 1.56    | 0.76  | 0.60         |      |
| Na⁺          | 0.62    | 0.52  | 0.67         |      |
| K⁺           | 1.61    | 0.09  | 0.80         |      |
| Cl⁻          | 1.73    | 2.093 | 4.49         |      |
| CO₃²⁻        | Nil     | Nil   | Nil          |      |
| HCO₃⁻        | 0.28    | 0.79  | 0.67         |      |
| CEC          | 40.30   | 42.20 | 34.50        |      |
| Organic matter | 15.51  | 15.58 | 12.24        | g.Kg⁻¹ Soil |
| Organic Carbon | 9.00     | 9.10  | 7.0         |      |
| Gypsum       | 0.32    | 0.54  | 0.45         |      |
| Calcium carbonate | 257.7  | 281.3 | 211.2        |      |
| Total nitrogen | 0.87   | 0.77  | 0.71         |      |
| Available Phosphorus | 13.44 | 15.03 | 11.07        |      |
| Available Potassium | 169.71 | 163.01 | 86.31       |      |
| Soil separators | silt: 661 | clay: 223 | sand: 224 | g.Kg⁻¹ Soil |
| Soil texture  | Silty loam | Loam  |          |      |
| Bulk density  | 1.20    | 1.40  | 1.30         | Mg.m⁻³ |
| Total bacteria | 12.5×10⁶ | 17.0×10⁶ | 12.2×10⁶    | CFU. g⁻¹ Soil |
| Total fungi   | 7.0×10⁶ | 6.0×10⁶ | 4.2×10³   |      |
Table 6: Some chemical, physical and biological properties of Afak site.

| Soils Traits          | Orchard | Field | Jungle fields | Unit       |
|-----------------------|---------|-------|---------------|------------|
| pH                    | 7.11    | 7.31  | 7.61          |            |
| EC                    | 2.24    | 2.61  | 4.55          | ds.m⁻¹     |
| Ca²⁺                  | 0.64    | 0.65  | 1.30          |            |
| Mg²⁺                  | 0.30    | 0.35  | 0.95          |            |
| Na⁺                   | 0.33    | 0.21  | 0.31          |            |
| K⁺                    | 0.07    | 0.06  | 0.04          |            |
| Cl⁻                   | 1.31    | 1.60  | 3.13          |            |
| CO₃²⁻                 | Nill    | Nill  | Nill          |            |
| HCO₃⁻                 | 0.30    | 0.47  | 0.48          |            |
| CEC                   | 32.55   | 30.13 | 21.17         |            |
| Organic matter        | 25.86   | 14.48 | 10.34         | g.Kg⁻¹ Soil|
| Organic Carbon        | 15.00   | 8.60  | 6.00          |            |
| Gypsum                | 0.31    | 0.36  | 0.32          |            |
| Calcium carbonate     | 25⁺.1   | 27⁺.3 | 25⁺.3         |            |
| Total nitrogen        | 1.73    | 0.60  | 0.63          |            |
| Available Phosphorus  | 12.08   | 14.82 | 11.13         |            |
| Available Potassium   | 68.23   | 138.27| 121.71        | mg.Kg⁻¹ Soil|
| Soil separators       |         |       |               |            |
| silt                  |         |       |               | g Kg⁻¹ Soil|
| clay                  |         |       |               |            |
| sand                  |         |       |               |            |
| Bulk density          | 1.12    | 1.30  | 1.20          | Mg.m⁻³     |
| Total bacteria        | 17.0x10⁶| 13.0x10⁶| 12.1x10⁶     | CFU.g⁻¹ Soil|
| Total fungi           | 5.0x10⁵ | 5.2x10⁵| 4.9x10⁵       |            |

Table 7: Some chemical, physical and biological properties of Al-Budair site.

| Soils Traits          | Orchard | Field | Jungle fields | Unit       |
|-----------------------|---------|-------|---------------|------------|
| pH                    | 7.23    | 7.31  | 7.61          |            |
| EC                    | 1.23    | 2.41  | 5.12          | ds.m⁻¹     |
| Ca²⁺                  | 0.31    | 1.23  | 1.56          |            |
| Mg²⁺                  | 0.32    | 0.91  | 0.71          |            |
| Na⁺                   | 0.21    | 0.31  | 0.79          |            |
| K⁺                    | 0.01    | 0.07  | 0.02          |            |
| Cl⁻                   | 0.87    | 4.78  | 3.20          |            |
| CO₃²⁻                 | Nill    | Nill  | Nill          |            |
| HCO₃⁻                 | 0.08    | 0.63  | 0.62          |            |
| CEC                   | 40.25   | 44.20 | 20.41         |            |
| Organic matter        | 13.27   | 13.79 | 9.44          |            |
| Organic Carbon        | 7.70    | 8.00  | 6.00          |            |
| Gypsum                | 1.70    | 11.60 | 13.50         |            |
| Calcium carbonate     | 262.2   | 333.4 | 352.1         |            |
| Total nitrogen        | 0.70    | 0.71  | 0.53          |            |
| Available Phosphorus  | 12.19   | 17.03 | 16.77         |            |
| Available Potassium   | 105.61  | 159.81| 97.22         | mg.Kg⁻¹ Soil|
| Soil separators       |         |       |               |            |
| silt                  |         |       |               | g Kg⁻¹ Soil|
| clay                  |         |       |               |            |
| sand                  |         |       |               |            |
| Bulk density          | 1.22    | 1.30  | 1.41          | Mg.m⁻³     |
| Total bacteria        | 12.3x10⁶| 12.5x10⁶| 17.1x10⁶     | CFU.g⁻¹ Soil|
| Total fungi           | 5.0x10⁵ | 6.0x10⁵| 2.3x10⁴       |            |
The highest efficacy values were recorded in the soil of the Shamiya location (234.95) mg P-Nitro phenol. g⁻¹ soil. 1 hour⁻¹. Orchard soils had the highest values of all the studied locations except for the location of Diwaniyah and Shamiya. Field soils that were cultivated with eggplant and cowpea plants had the height values in these two locations.

### Table 8: Activity of Alkaline Phosphatase of the study sites.

| Location          | Orchard | Field | Jungle fields | Average |
|-------------------|---------|-------|---------------|---------|
| Center of Diwaniyah | 227.73  | 237.80| 206.93        | 223.48  |
| Sunniya           | 227.73  | 220.23| 214.20        | 220.72  |
| Shamiya           | 239.83  | 243.43| 221.60        | 234.95  |
| Al-Daghara        | 244.00  | 231.33| 172.00        | 215.77  |
| Afak              | 234.90  | 185.60| 203.83        | 208.11  |
| Al Budair         | 272.20  | 238.90| 190.63        | 233.91  |
| **Average**       | ṭ�� ṭ�� | ṭ�� opolitan | 201.8  | ṭ�� opolitan |

| L.S.D 0.05 | Location | Exploitation type | L*Ε interaction |
|------------|----------|-------------------|-----------------|
| 1.30       |          |                   |                 |
| 0.80       |          |                   |                 |
| 2.22       |          |                   |                 |

This is consistent with results [22] who found that leguminous plants secrete more basal phosphatase enzyme than cereal crops. This because the high need for phosphorus in legumes, due to vagueness stabilizations of nitrogen compared to cereal crops [23]. The results also indicate that the nature of agricultural exploitation has had a direct effect on the increase or decrease of the efficiency of the enzyme, [24] and [25]. Table (8) shows the average enzymatic activity. The highest values were recorded in the soils of the Shamiya location (234.95) mg P-Nitro phenol. g⁻¹ soil. 1 hour⁻¹, and the lowest in the soils of the location of Afak (208.11) mg P-Nitro phenol. g⁻¹ soil. 1 hour⁻¹. Comparing the values of this enzymatic activity in these soils, the highest efficacy values were found in soils with low electrical conductivity (EC) and the lowest in soils with high electrical conductivity (Tables 2-7). The effect of number total bacteria in the activity of alkaline phosphatase enzyme is varied among and within the location. Increasing in the number of bacteria in the soil will increase the activity of basal phosphatase enzyme and vice versa, as in the soil of Shamiya, which had the highest enzyme efficacy value (Table 4). This indicates that the production of alkaline phosphatase is from two sources are either microbiology or plant secretions implanted. This is consistent with [26] the results that enzymatic activity is influenced by several factors, including soil, biomass, climate factors, soil ecosystem functions and crop cultivars.

It also in line with what Oujda Bayerjee and Sanyal [3] found, which is that this enzyme is secreted by plants, microorganisms, and soil animals. Sinsabaugh and Moorhead ,[27] stated that microbial microorganisms, which act as a double function by breaking down organic matter into simpler forms and then acquiring sources of produced enzymes, secrete enzymes. The effect of the fungus in the activity of alkaline phosphatase varies within and among locations. Enzyme activity increased by decreasing the number of fungi in some locations, but the reverse in some other locations. This variation in the efficacy values with the preparation of fungi may because the source that secretes the enzyme is innate rather than bacterial. The increase in the number of fungi increases the activity of the enzyme, and the fungi would increase the secretion and thus increase its activity. This supports what Fitriatin et al.[28] find, which present that the efficacy of phosphatase produced by certain species of fungi is higher than that produced by bacteria.

The difference in the values of enzyme activity is significant at the level of 5% within and among soils of different locations. The values of the alkaline phosphatase enzyme are generally higher in all studied sites and this is consistent with the findings of [5], who found that basal phosphatase is abundantly produced in neutral or alkaline soils. This is consistent with the results of the pH values in tables (2-7). Plant type, root secretions, and a number of bacteria influence the efficacy of the enzyme. Electrical conductivity (EC) and the pH had a significant effect on its efficacy and secretion at all studied locations.

### IV. Kinetics measures of alkaline phosphatase

Figures 1-6 present the linear relationship between the concentrations of the P-nitro phenol (S) and the activity of this enzyme V / (S) enzyme in accordance with the Hanes-Wolf formula for the studied locations. Where V represents the velocity of decomposition of the P-Nitro phenol that is measured according to the unit of mg P-Nitro phenol. g⁻¹ soil. 1 hour⁻¹. The efficiency of the enzyme increases with the addition of applied concentrations of the controlled substance in all soils of the studied location. Vmax and Km values were estimated from the slope Vmax / 1 and the cut Vmax / Km , which their values are shown in the table (9). Results in this table indicate differences in the values of the kinetic parameters of the enzyme according to the studied locations and to the agricultural exploitation of these soils at the same site. The highest value of Vmax is found in the field soil in the location of Al-Shamiya (108.57) mg P-Nitro phenol. g⁻¹ soil. 1 hour⁻¹. The lowest value recorded at the bush dominated location of the Sunniya and (49.62) mg P-Nitro phenol. g⁻¹ soil. 1 hour⁻¹. The orchard soil of the Al Budair location had the highest values (85.90) mM and the lowest in orchard soils.
There is a difference in Vmax values within and among locations. This variation may due to the difference in the nature of agricultural exploitation and/or soil properties, table (2-7). This is consistent with the Al-Taweel, 2001 study of Amidase in different soils of northern, central and southern Iraq, as well as her study in 2007. about the study of Vmax values of basal phosphatase in the two different soil textures, which attributed the increase in Vmax values to the increasing in the activity of soil enzyme. These results were less valuable than those found by [29] in their study of some soils in the Basrah province (not cultivated), which Vmax values ranged from (400) to (560) μg P-nitro phenol. g\(^{-1}\) soil. 1 hour\(^{-1}\). Km values also differed within and among locations.

This is due to the high or low affinity between the enzyme and the controlled substance, which is affected by the molecular structure of the enzyme, which varies according to the biological source, [30]. It is natural that the source of the enzyme and the molecular structure according to the dominant group will change the microorganisms, the soil animals and plants, which are affected by the conditions and properties of the soil, as well as the difference of the root plant secretions. In general, Km is an indication of affinity between the substances matter and the enzyme as mentioned. The low value of Km means that the affinity is high between the enzyme and the controlled substance. The enzyme needs a lower amount of the controlled substance to reach its maximum velocity, and the high-value Km means the weak affinity between the controlled substance and the enzyme, [31].

Table (9) shows that the highest Vmax value in the orchard soils in all locations except for the locations of the Diwaniyah and the Shamiya, where the values of the field were superior. These results agree with the activity values of this enzyme in those soils. The factors that influenced the enzyme's effects are the same that affected the maximum velocity values of the enzyme. These results correlate with [32], found that soil fertility and crop diversity in soils significantly affect biological processes occurring in the soil, which affect soil enzyme efficacy.

### Table 9: Maximum speed (Vmax) and Michaelis constant (KM) of the alkaline phosphatase in the study soils.

| Location          | Velocity values | Vmax μg P-nitro phenol. g\(^{-1}\) soil. 1 hour\(^{-1}\) | Km Molly Muller |
|-------------------|-----------------|----------------------------------------------------------|-----------------|
| Center of Diwaniyah | Orchard         | 76.21                                                    | 26.63           |
|                   | Field           | 90.00                                                    | 44.55           |
|                   | Jungle          | 82.16                                                    | 24.94           |
| Suniya            | Orchard         | 82.57                                                    | 24.34           |
|                   | Field           | 58.37                                                    | 37.85           |
|                   | Jungle          | 49.62                                                    | 60.65           |
| Shamiya           | Orchard         | 86.80                                                    | 47.34           |
|                   | Field           | 108.57                                                   | 33.24           |
|                   | Jungle          | 65.57                                                    | 54.13           |
| Dagara            | Orchard         | 81.49                                                    | 49.44           |
|                   | Field           | 66.22                                                    | 45.60           |
|                   | Jungle          | 49.67                                                    | 60.59           |
| Afak              | Orchard         | 104.71                                                   | 38.29           |
|                   | Field           | 73.20                                                    | 64.33           |
|                   | Jungle          | 89.92                                                    | 33.91           |
| Al Budair         | Orchard         | 95.41                                                    | 41.62           |
|                   | Field           | 88.33                                                    | 36.45           |
|                   | jungle          | 67.98                                                    |                |

The values of Km do not take a specific direction in all soils. This may because different sources of processing of the enzyme and that these values do not depend on the concentration of the enzyme. Km is the highest in the soils that predominate in the bush except for the location of the Diwaniyah and Afak where the soils of the field are superior and the location of Al-Budair the orchard soil overtook it. The lowest values of Km are recorded in the field soils and orchard soils among the different sites and does not take a specific direction. This indicates the obvious differences in the sources of enzyme processing, and the basis of this is the dominant organisms and root excretions, [33],[34].
Figure 1: The relationship between concentrations of the controlled substance (P-nitro phenol) (S) and V / (S) for the center of the Diwaniya location. Where (V) represents the velocity of P-nitro phenol decomposition (μg P-nitro phenol. g⁻¹ soil. 1 hour⁻¹).
Figure 2: The relationship between concentrations of the controlled substance \((P\text{-nitro phenol})\) \((S)\) and \(V / (S)\) for the center of the Sunniya location. Where \((V)\) represents the velocity of \(P\)-nitro phenol decomposition \((\mu g \text{-} P\text{-nitro phenol. } g^{-1}\text{ soil. } 1\text{ hour}^{-1})\).
Figure 3: The relationship between concentrations of the controlled substance ($P$-nitro phenol) $(S)$ and $V / (S)$ for the center of the Shamya location. Where $(V)$ represents the velocity of $P$-nitro phenol decomposition ($\mu g$ $P$-nitro phenol. g$^{-1}$ soil. 1 hour$^{-1}$).
Figure 4: The relationship between concentrations of the controlled substance (P-nitro phenol) (S) and V / (S) for the center of the Daghara location. Where (V) represents the velocity of P-nitro phenol decomposition (μg P-nitro phenol. g⁻¹ soil. 1 hour⁻¹).
Figure 5: The relationship between concentrations of the controlled substance (P-nitro phenol) (S) and \( V/(S) \) for the center of the Afak location. Where (V) represents the velocity of P-nitro phenol decomposition (\( \mu g \) P-nitro phenol. g\(^{-1}\) soil. 1 hour\(^{-1}\)).
Figure 6: The relationship between concentrations of the controlled substance (P-nitro phenol) (S) and \( \frac{V}{S} \) for the center of the Al-Budair location. Where (V) represents the velocity of P-nitro phenol decomposition (µg P-nitro phenol. g\(^{-1}\) soil. 1 hour\(^{-1}\)).

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