

Relationship Between Phosphatase Activity and Phosphorus Fractions in Agricultural Soils

¹Rıdvan Kızılkaya, ²Fethi Bayraklı and ³Abdulkadir Sürücü

¹Department of Soil Science, Faculty of Agriculture,
Ondokuz Mayıs University, 55139 Samsun, Turkey

²Department of Environmental Engineering, Faculty of Engineering,
Ondokuz Mayıs University, 55139 Samsun, Turkey

³Directorate of Rural Services Research Institute, 63040, Sanlıurfa, Turkey

Abstract: Soil phosphatases play a major role in the mineralization processes of organic phosphorus. The activity of soil phosphatases can be influenced by numerous factors and soil physico-chemical properties play a key role among them. Our research adds to the growing knowledge on soil acid (ACPA), neutral (NEPA) and alkaline phosphatase with some soil properties and total P (P_{total}), organic P (P_{org}), bioavailable P and inorganic phosphorus (P_{inorg}) fractions such as soluble and loosely bound phosphorus ($P_{soluble}$), aluminum phosphates (Al-P), iron phosphates (Fe-P), reductant soluble phosphorus ($P_{reductant}$) calcium phosphates (Ca-P) and strongly adsorbed Fe and Al phosphates (Fixed-P) fractions in agricultural soils of Çarşamba Plain, Turkey. ALPA, 24.6-138.6 $\mu\text{g phenol g}^{-1} \text{ 3 h}^{-1}$, was significantly higher than NEPA, 11.5-84.1 $\mu\text{g phenol g}^{-1} \text{ 3 h}^{-1}$ and ACPA, 8.6 - 64.1 $\mu\text{g phenol g}^{-1} \text{ 3 h}^{-1}$, in the studied soils. P_{total} contents in the soils ranged from 400.8 - 745.0 $\mu\text{g P g}^{-1}$ with an average 570 $\mu\text{g P g}^{-1}$. Phosphorus was mostly concentrated in the P_{inorg} (57%) although it was also present in P_{org} (12%) and $P_{residual}$ (31%). On the average, percent of P_{inorg} associated with different fractions in these soils was in the decreasing order of: Ca-P > Fixed-P > $P_{reductant}$ > Al-P > Fe-P > $P_{soluble}$. On the basis of results obtained in this study, phosphatase activities showed significant correlations with the P_{total} , P_{org} , bioavailable P and some P_{inorg} fractions (Ca-P and $P_{soluble}$). These results suggested that Ca-P is major P_{inorg} compound on the effects of soil phosphatase activity in agricultural soils of Çarşamba Plain, Turkey.

Key words: Phosphatase activities, total phosphorus, organic and inorganic phosphorus fractions

INTRODUCTION

The element of phosphorus is essential for plant growth and metabolism. It is generally accepted that plant roots take up phosphorus as soluble inorganic phosphate (Bielecki and Ferguson, 1983). Since a large proportion of the P in soil is organically bound (a very important amount of P in soils especially of arid climates is bound inorganically), the mineralization of this organic fraction is major agricultural importance (Speir and Ross, 1978). Several enzymes are involved in the decomposition of organic phosphorus compounds. Those enzymes that catalyze the hydrolysis of both P and anhydrides of H_3PO_4 esters are commonly called phosphatases (Alexander, 1977). Phosphatases (orthophosphoric monoester phosphohydrolase, E.C. 3.1.3) are important in soils because phosphatase catalyze the hydrolysis of organic esters and anhydrides of H_3PO_4 to orthophosphate; thus they form an important link between plant-unavailable and soluble P fractions in soil

Corresponding Author: Rıdvan Kızılkaya, Department of Soil Science,
Faculty of Agriculture, Ondokuz Mayıs University, 55139 Samsun, Turkey
Tel: (+90) 362 312 19 19/1358 Fax: (+90) 362 457 60 34

(Amador *et al.*, 1997). Phosphatase activities in soil can be associated with active cells (animal, plant, microbial), entire dead cells and cell debris as well as being complexed with clay minerals and humic colloids (Pascual *et al.*, 2002). In addition, the sorption of phosphatases on clay, oxides or humic substances can change enzyme conformation and reduce activity (Dick and Tabatabai, 1987; Nannipieri *et al.*, 1988). Phosphatases are distinguishable not only by the chemical nature of the substrates hydrolyzed but also by pH ranges for their optimal activity. Among them are: acid phosphatase, optimal pH 4-6; neutral phosphatase, optimal pH 7; and alkaline phosphatase, optimum pH 8-10 (Speir and Ross, 1978).

Phosphatase activity is affected by soil physico-chemical (clay content, soil moisture, soil depth, temperature, organic matter, pH and nutrients) and biological (microbial population and their activities) properties (Speir and Ross, 1978) and these properties play a key role among them. As far as physico-chemical soil properties are concerned, numerous studies have focused on the carbon content and its positive impact phosphatase activity (e.g., Herbien and Neal, 1990; Pagliai and De-Nobili, 1993; Marinari *et al.*, 2000), relationships between organic matter and the other elements in the organic bounds (e.g., N and P) and pH. Relationships between phosphatase activities and total P, organic P available P have been described by Gavrilova *et al.* (1973), Speir and Ross (1978), Haynes and Swift (1988) and Nahas *et al.* (1994). On the contrary, little information on the relationships between phosphatase activities and inorganic P fractions in soils is available. In moderately well drained soils with a relatively low level of inorganic P, Amador *et al.* (1997) has observed a positive correlation between inorganic P and phosphatase activity. The high concentration of inorganic P in soils has been shown to reduce phosphatase activity. For example, orthophosphate inhibited phosphatase activity in soils (Juma and Tabatabai, 1978), as well as the synthesis and catalytic action of phosphatases of microorganisms in soil (Woolhouse, 1969). Chen (2003) suggested that the positive correlations between phosphatase activities (acide and neutral) and inorganic P fractions (iron and aluminium phosphates) in acidic Chinese forest soils.

There is currently great interest in the use of extracellular enzymes as biological indicators of soil quality, because they are relatively simple to determine, have microbial ecological significance, are sensitive to environmental stress and respond rapidly to changes in land management (Dick, 1997; Yakovchenko *et al.*, 1996). Phosphatase activity may be a particularly useful enzyme for soil quality monitoring because of its central role in soil organic matter cycling, which is generally regarded as an important component of soil quality. Research has shown that phosphatase is the most abundant and easily detected of the enzymes involved in organic P compounds decomposition in soil and is rarely substrate limited, thus making it ideal to examine the importance of soil P status. Indeed, it provides an early indication of changes in organic P and organic matter status and turnover (Gavrilova *et al.*, 1973; Speir and Ross, 1978).

The adoption of soil phosphatase activities in soil quality monitoring requires information on activities from a wide range of soil types and land uses under steady-state conditions, in addition to mechanistic understanding of how soil properties control these activities (Trasar-Cepeda *et al.*, 2000). The aim of this study were, (I) to determine phosphatases (acide, neutral and alkaline) in wide range of agricultural soils with contrasting total P, organic P and inorganic P fractions such as aluminium phosphates, iron phosphates, calcium phosphates, strongly adsorbed Fe and Al phosphates, phosphorus involved in reductant and slightly bounded phosphorus, but under similar land use and (ii) to investigate the relations between activity of the phosphatases and total P, organic P, inorganic phosphors fractions in those soils.

MATERIALS AND METHODS

Study Sites

The study sites, Çarşamba plain (Latitude, 41°11'N; longitude, 36°45'W), are located in the Black Sea Region, Northern Turkey (Fig. 1). Soil parent materials are alluvial in sampling sites; deposited by Yeşilırmak River and also on recent stream outwash generally. As choosing sites, we considered

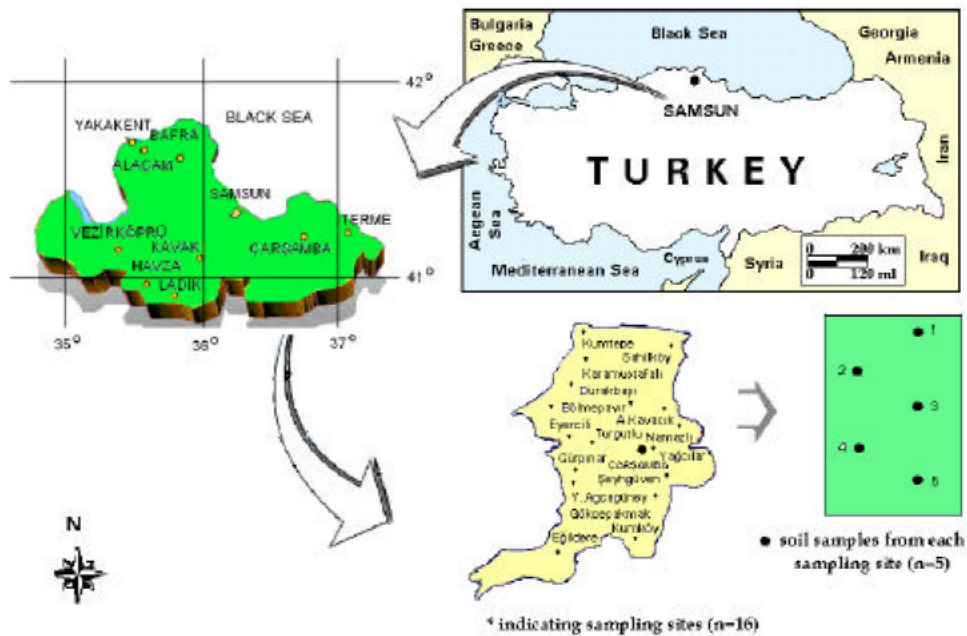


Fig. 1: Location of the sampling sites in Çarşamba Plain, Turkey

consistency in soil texture (sandy loam to clay loam) and slope (0-3%). Historically, all sampling sites were managed as agricultural land for several hundred years. This management regime included the use of intensively cultivated vegetable fields and cereals such as wheat and corn have been allocated in these sites with more intensive cultivation practices and were irrigated. All sites face with chemical fertilizer (e.g., diammonium phosphate, triple super phosphate, compose fertilizer and calcium ammonium nitrate) and herbicides applications for 30-35 years. The climate is semi humid with monthly mean temperatures ranging from 9.6°C in January to 19.3°C in July. The annual mean temperature is 14.3°C and annual mean precipitation 1045.3 mm (Anonymous, 2000).

Soil Sampling

In May 2000, 16 sampling sites were collected randomly from the agricultural soils of Çarşamba Plain. Samples of 0-20 cm depth below the litter (or where soil surface where no litter existed) were taken form five points within each plot using a 20×5 cm soil corer. The five replicate samples were homogenized by hand mixing. Major live plant materials (roots and shoots) and pebbles in each sample were separated by hand and discarded. About 1 kg mixed samples were returned to the laboratory in polyethylene bags at 4°C. The samples were brought to the laboratory on the same day and kept in the refrigerator at 4°C for no longer than 72 h prior to phosphatase analysis. These soil samples were used for analyzing physico-chemical properties and total, organic and inorganic phosphorus fractions and phosphatase activities.

Soil Physico-chemical Analyses

Prior to physico-chemical analysis, all soil samples were air dried at approximately 25°C, lightly ground and sieved through a 2 mm stainless steel sieve. All analyses were performed on the size fraction smaller than 2 mm and stored at room temperature. Selected soil physico-chemical properties

were determined by means of appropriate methods: soil particle size distribution by the hydrometer method (Bouyoucos, 1951), pH in 1: 2.5 (w/v) in soil: water suspension by pH-meter (Peech, 1965) and Cation Exchange Capacity (CEC) by Bower method (Rowell, 1996), CaCO₃ content by Scheibler calcimeter (Soil Survey Staff, 1993). Bioavailable phosphorus (Olsen-P) was extracted by shaking 2.5 g of soil for 30 min with 50 mL of 0.5 M NaHCO₃ (Olsen *et al.*, 1954). An aliquot of the extract was analyzed for P with molybdenum blue method (ascorbic acid method) proposed by Murphey and Riley (1962). Whole soil samples were sieved through a 150 µm mesh to determine total organic carbon by the wet oxidation method (Walkley-Black) with K₂Cr₂O₇ (Rowell, 1996).

Phosphorus

The soil samples were air-dried, then passed through 2 mm sieves for determination of total, organic and inorganic P fractions as soil physico-chemical properties.

Total and Organic Phosphorus

Total phosphorus (P_{total}) was determined perchloric acid (HClO₄) digestion method according to Olsen and Sommers (1982). Organic phosphorus (P_{org}) content was measured by the ignition method according to Saunders and Williams (1955).

Inorganic Phosphorus Fractions

The procedure of Chang and Jackson (1957) and Peterson and Corey (1966) selected for this study, is designed to separate inorganic phosphorus (P_{inorg}) fractions into six operationally defined fractions: soluble and loosely bound phosphorus, aluminum phosphates, iron phosphates, reductant soluble phosphorus, calcium phosphates and strongly adsorbed Fe and Al phosphates fractions. A summary of the procedure is as follows:

One gram of each soil is weighed into 100 mL centrifuge tube and the following fractions obtained.

Soluble and Loosely Bound Phosphorus (P_{soluble})

Soil samples extracted with 50 mL of 1 M NH₄Cl for 30 min. Centrifuging and decanting the supernatant into a 50 mL volumetric flask and bringing to volume with deionized water (extract A).

Aluminum Phosphates (Al-P)

The residue from P_{soluble} is extracted with 50 mL of 0.5 M NH₄F (pH 8.2) for 1 h. Centrifuging and decanting the supernatant into a 100 mL volumetric flask (extract B).

Iron Phosphates (Fe-P)

After washing the soil sample twice with 25 mL portion of saturated NaCl and centrifugation combining the washing with extract B and bringing to volume. The residue from Al-P is extracted with 50 mL 0.1 M NaOH for 17 h. Centrifuging and decanting the supernatant solution into a 100 mL volumetric flask (extract C). Washing the soil twice with 25 mL portions of saturated NaCl and centrifuging. Combining the washing with extract C and bringing to volume.

Reductant Soluble Phosphorus (P_{reductant})

The residue from Fe-P is heating in water bath at 85°C with 40 mL of 0.3 M Na₂ C₆H₅O₇ and 5 mL of 1 M NaHCO₃ and adding 1.0 g of Na₂ S₂O₄ and stirring rapidly to extract P_{reductant}. Continuing to heat for 15 min and then centrifuging. Decanting the supernatant solution into a 100 mL volumetric flask (extract D). Washing the soil twice with 25 mL portion of saturated NaCl and centrifuging. Combining the washings with extract D and diluting D to volume.

Calcium Phosphates (Ca-P)

The residue from $P_{\text{reductant}}$ is extracted with 50 mL of 0.25 M H_2SO_4 for 1 h. Centrifuging the suspension for 10 min and decanting the supernatant into a 100 mL volumetric flask (extract E). Washing the soil twice with 25 mL portions of saturated NaCl and centrifuging. Combining the washings with the extract E and diluting to volume.

Strongly Adsorbed Fe and Al Phosphates (Fixed-P)

The residue from Ca-P is extracted with 50 mL of 0.1 M NaOH for 1 h. Centrifuging and decanting the supernatant into a 50 mL volumetric flask and bringing to volume with deionized water (extract F).

Phosphorus concentrations in all the extracts were carried out using the molybdenum blue method (ascorbic acid method) proposed by Murphey and Riley (1962). The amount of inorganic P (P_{inorg}) was calculated based on the sum of P_{soluble} , Al-P, Fe-P, $P_{\text{reductant}}$, Ca-P and Fixed-P. Residual phosphorus (P_{residual}) was calculated as follows; $P_{\text{residual}} = P_{\text{total}} - (P_{\text{org}} + P_{\text{inorg}})$. Results were expressed as $\mu\text{g P g}^{-1}$.

Phosphatases

The phosphatase activities were determined for the each of the field-moist soil. All results are expressed on a moisture-free basis. Moisture was determined after drying at 105°C for 48 h.

Acid, neutral and alkaline phosphatase activities (ACPA, NEPA and ALPA, respectively) were assayed by Hoffmann's method (Hoffmann, 1968; Alef and Nannipieri, 1995). Two milliliter toluene, 20 mL buffer (acetate buffer, pH 5.0 to acid phosphatase; citrate buffer, pH 7.0 to neutral phosphatase; borate buffer, pH 9.6 to alkaline phosphatase) and 10 mL of 0.675% disodiumphenyl phosphate substrate solution were added in to the 10 g soil and the samples were incubated for 3 h at 37°C . The volume was made up to 100 mL with distilled water at 37°C . Following filtration through Whatman No. 42 filter papers, 1 mL of filtrate was diluted to 10 mL with distilled water and 5 mL of borate buffer and 1 mL of 2.6 dibromchinon chlorimid solution were added. The volume was made up to 50 mL with distilled water. Finally the formation phenol was determined spectrophotometrically at 578 nm. Acid, neutral and alkaline phosphatase activity was expressed as μg hydrolyzed phenol g^{-1} dry soil for 3 h at 37°C .

Statistical Analysis

All data were analyzed using SPSS 11.0 statistical software (SPSS Inc.). The significance of differences between the different soils or sampling sites was tested by one-way ANOVA. Pearson correlation coefficients and P-values were calculated for all possible variable pairs. The asterisks *and ** indicate significance at $p < 0.05$ and 0.01 , respectively.

RESULTS

Soil Physico-chemical Properties

The sixteen sampling sites representing mineral soils were collected from agricultural land. For all sites, the five replicate sampling points showed different values in soil physico-chemical properties. The mean value of soil pH was 7.35 (slightly alkaline), which has slightly above the well-established values (6.7-7.3) in cultivated soils of this area of Turkey Table 1. All soils were clay loam with $>27\%$ clay content, except for few sites that were sandy clay loam. Soils were moderately low in total organic carbon. The content of CaCO_3 ranged from low to moderate (8-15%) in these soils. CEC was ranged from 32.5 to 42.2 $\text{cmol}(+) \text{kg}^{-1}$.

Table 1: Maxima, minima, means and standard deviations (S.D.) of the soil physico-chemical properties studied

Parameters	Minimum	Maximum	Mean±SD
Sand (%)	21.85	56.53	40.35±9.37
Silt (%)	17.28	42.20	24.57±6.85
Clay (%)	28.06	45.05	35.06±7.13
CaCO ₃ (%)	1.00	13.80	4.28±3.67
Organic matter (%)	1.05	2.70	1.78±0.55
pH	6.00	8.40	7.35±0.69
CEC (cmol(+)kg ⁻¹)	32.50	42.20	37.14±3.45
Bioavailable P (µg g ⁻¹)	3.75	16.26	7.86±3.38

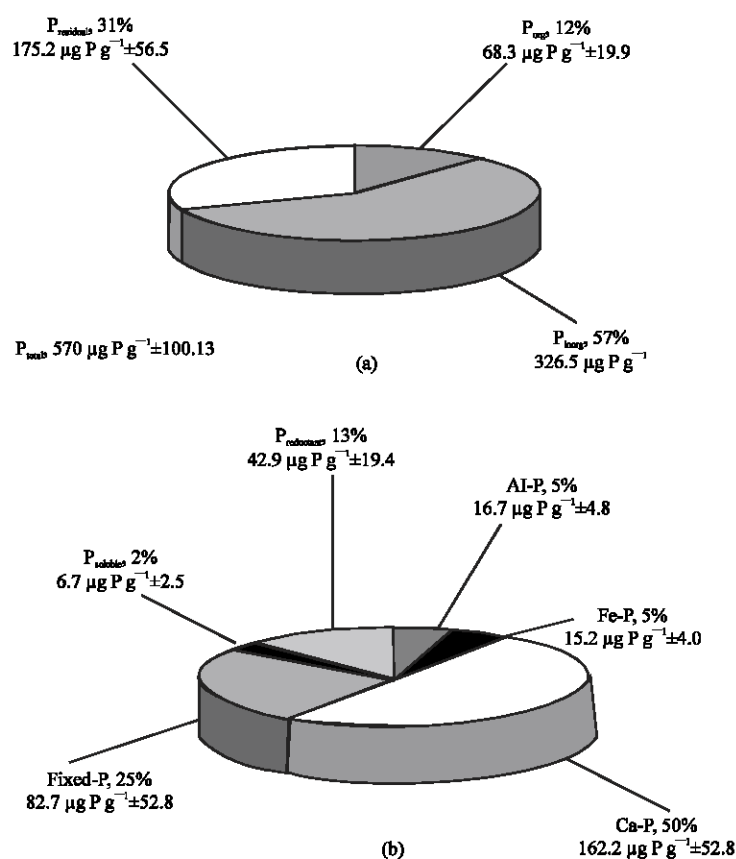


Fig. 2: Percentage of phosphorus fractions in soils studied, (a) P_{org}, P_{residual} and P_{inorg} by P_{total} (b) Ca-P, Fe-P, Al-P, Fixed-P, P_{reductant} and P_{soluble} by P_{inorg}

The Contents of Soil Phosphorus Fractions

Like soil physico-chemical properties, P distribution showed different values in P_{total}, P_{org} and inorganic P fractions relative to sampling sites. Bioavailable phosphorus contents ranged from low (<8 µg P g⁻¹) to marginal (8-15 µg P g⁻¹). P_{total} contents ranged from 400.78 to 745.03 µg P g⁻¹; P_{org} contents ranged from 32.96 to 100.08 µg P g⁻¹; P_{inorg} contents ranged from 131.63 to 504.84 µg P g⁻¹; P_{residual} contents ranged from 46.09 to 227.43 µg P g⁻¹. The amounts of phosphorus in P_{org}, P_{inorg} and P_{residual} fractions were 12.0, 57.3 and 30.7% of P_{total}, respectively (Fig. 2a). Al-P contents ranged from 9.56 to 26.77 µg P g⁻¹; Fe-P contents ranged from 9.69 to 21.77 µg P g⁻¹; Ca-P contents ranged from

67.42 to 235.09 $\mu\text{g P g}^{-1}$; Fixed-P contents ranged from 31.45 to 131.83 $\mu\text{g P g}^{-1}$; $\text{P}_{\text{soluble}}$ contents ranged from 2.40 to 12.00 $\mu\text{g P g}^{-1}$; $\text{P}_{\text{reductant}}$ contents ranged from 11.08 to 77.38 $\mu\text{g P g}^{-1}$. The amounts of phosphorus in Al-P, Fe-P, Ca-P, Fixed-P, $\text{P}_{\text{soluble}}$ and $\text{P}_{\text{reductant}}$ fractions were 5.1, 4.7, 49.7, 25.3, 2.3 and 13.1% of the total P_{inorg} . On the average, percent of P_{inorg} associated with different fractions in the sixteen sampling sites was in the following order: Ca-P>Fixed-P> $\text{P}_{\text{reductant}}$ >Al-P>Fe-P> $\text{P}_{\text{soluble}}$ (Fig. 2b). Analysis of variance (ANOVA) indicated significant differences in P_{total} , P_{org} and inorganic P fractions among different soil samples ($p<0.01$).

Phosphatase Activities

ALPA, 64.8 $\mu\text{g phenol g}^{-1} 3 \text{ h}^{-1}$ (range 24.6 to 138.6, S.D. 16.8), was significantly higher than NEPA, 47.5 $\mu\text{g phenol g}^{-1} 3 \text{ h}^{-1}$ (range 11.5 to 84.1, S.D. 22.3) and ACPA, 37.4 $\mu\text{g phenol g}^{-1} 3 \text{ h}^{-1}$ (range 8.6 to 64.1, SD 16.8), in the studied soils (Fig. 3). ANOVA indicated that there were significant differences in all phosphatase (ACPA, NEPA and ALPA) in the different soils ($p<0.01$).

The results indicated that there was a statistically significant Pearson correlations between physico-chemical properties and soil phosphatase activities. ACPA, NEPA and ALPA were strongly positively correlated with bioavailable P and soil organic matter (Table 2). Pearson correlation analysis indicates highly significant positive relationships among phosphatase activities (ACPA, ALPA and NEPA), P_{total} , P_{org} , Ca-P and $\text{P}_{\text{soluble}}$ but not significantly correlated with the other inorganic P fractions (Al-P, Fe-P, Fixed-P, $\text{P}_{\text{reductant}}$) (Table 3).

Table 2: Correlation coefficients between phosphatase activity and soil physico-chemical properties

Parameters	ACPA	NEPA	ALPA
Sand	0.530*	0.492*	0.414
Silt	-0.425	-0.527*	-0.405
Clay	-0.289	-0.139	-0.154
pH	-0.145	-0.271	-0.246
CaCO_3	-0.149	-0.253	-0.257
Organic matter	0.550*	0.653**	0.672**
CEC	0.364	0.471	0.493*
Bioavailable P	0.867**	0.813**	0.978**

*, ** indicate significant at $p<0.05$ and 0.01 level

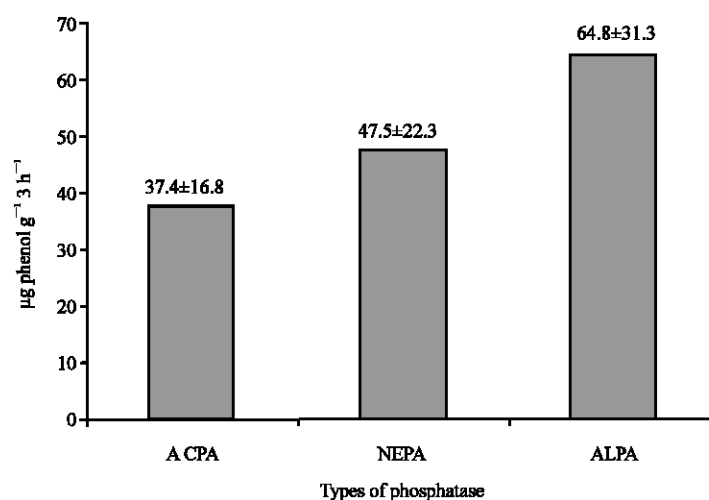


Fig. 3: Distribution of phosphatase activity (ACPA, ALPA and NEPA) in soils

Table 3: Correlation coefficients among P_{total} , P_{org} and inorganic phosphorus fractions with phosphatase activity in soils studied

Parameters	ACPA	NEPA	ALPA
P_{total}	0.656**	0.831**	0.573**
P_{org}	0.930**	0.932**	0.903**
Al-P	0.014	-0.172	-0.207
Fe-P	0.178	-0.009	0.291
Ca-P	0.549**	0.651**	0.522**
Fixed-P	0.017	0.186	0.201
$P_{soluble}$	0.885**	0.828**	0.939**
$P_{reductant}$	0.302	0.270	0.105
$P_{residual}$	0.158	0.324	0.025

** : Significant at 0.01 level

DISCUSSION

The Contents of Soil Phosphorus

Mean P_{total} in soil studied was $570.01 \mu\text{g P g}^{-1}$ (Fig. 2a). The phosphorus content of common soils varies from 100 to $2000 \mu\text{g P g}^{-1}$ (Kabata-Pendias and Pendias, 1992). Soils can be classified according to Tripathi *et al.* (1970) as low ($<600 \mu\text{g P g}^{-1}$) to moderate ($600-1000 \mu\text{g P g}^{-1}$). Bayraklı (1975) determined that P_{total} contents were $641 \mu\text{g P g}^{-1}$ in Bayburt Plain soils, were $521 \mu\text{g P g}^{-1}$ in Erzincan plain soils and were $676 \mu\text{g P g}^{-1}$ in Rize region soils, Turkey.

Mean P_{org} in soil studied was $68.3 \mu\text{g P g}^{-1}$ (Fig. 2a). Most soils contain between 50 to $500 \mu\text{g P g}^{-1}$. The average content of P_{org} in cultivated soils ranges from 5-50 % of P_{total} (Adepetu and Corey, 1976; Harrison, 1987). In this research the contents of P_{org} was 12% of the P_{total} .

Mean P_{inorg} in soil studied was $326.5 \mu\text{g P g}^{-1}$ (Fig. 2a). P_{inorg} was mostly concentrated in the Ca-P fraction, although it was also present in the other fractions. A small percentage of P_{inorg} was associated with $P_{soluble}$, Al-P and Fe-P fractions (Fig. 2b). Similarly, in the study of alluvial soils by Sing *et al.* (1968), also found that the most P_{inorg} was associated with calcium phosphates (Ca-P) and only very low amounts of P was in soluble and slightly bounded phosphorus ($P_{soluble}$) forms. Uriyo and Kasseba, (1973), Bayraklı (1975) and Udo and Ogunwale, (1977) also found a majority of the P_{inorg} in soils to be associated with the Ca-P. Nearly all P_{inorg} exists in the form of orthophosphates, derivatives of phosphoric acid (Black, 1968). The compounds are believed to be chiefly phosphates of calcium, aluminum and iron with minor proportions of others. Some of the P_{inorg} is present in the lattices of silicate minerals and as inclusions in minerals, e.g., in quartz crystals (Black, 1968). The initial form of P_{inorg} is predominantly some form of the mineral apatite, perhaps most commonly calcium fluorapatite (Larsen, 1967). Approximately 90% of the soil phosphorus occurs in soluble or fixed forms (primary phosphate minerals, humus P, phosphates of Ca, Fe, Al, phosphates fixed by colloidal oxides and silicate minerals). On the contrary, only a small fraction of phosphorus occurs in labile forms (Fox and Kamprath, 1970).

Phosphatase Activities and Their Relationship to Physico-chemical Properties and Different P Fractions in Soil

The pH values of soil samples varied from 6.00 to 8.40 with a mean of 7.35. ALPA was higher than the ACPA and NEPA in the investigated soil sites (Fig. 3), because the soil reaction was slightly alkaline in nature. ACPA, NEPA and ALPA are common in nature; pH optima are generally within the ranges pH 4-6, 7 and 8-10, respectively (Speir and Ross, 1978). Soil organic matter and bioavailable P contents gave the significant positive correlations with ACPA, NEPA and ALPA. On the contrary, phosphatase activities were not significantly correlated with the pH, CaCO_3 and clay content. In agricultural soils phosphatase activity is affected by physico-chemical soil properties. As far as chemical characteristics are concerned, numerous studies have focused on carbon content and its

positive impact on phosphatase activity (Jordan and Kremer 1994; Pascual *et al.*, 2002), relationships between organic matter content and bioavailable phosphorus content. Similarly, Aon and Colaneri (2001) described positive correlations of phosphatase activity with organic matter and a negative relationship with soil pH. In this research no correlation was found between phosphatase activity and clay content. Various studies have shown either positive or negative correlations between the above soil properties and phosphatase activity. Generally, soil phosphatase activity is related to content of organic matter and bioavailable P in soil.

Phosphatase activities (ACPA, NEPA and APA) gave significant positive correlations with P_{total} and P_{org} but phosphatase activity not significantly correlated with $P_{residual}$ (Table 3). The relationship of phosphatase activity to soil P_{total} , P_{org} has been the subject of several studies. Marinari *et al.* (2000) described positive correlations of phosphatase activity with total organic matter and organic phosphorus. On the contrary, Nahas *et al.* (1994) suggested that the activity of phosphatase correlated with organic matter and total phosphorus content, but not with organic phosphorus content. In most studies, however, significant positive correlations have been found between phosphatase activity and P_{org} . (Gavrilova *et al.*, 1973).

ACPA, NEPA and ALPA were strongly positively correlated with Ca-P and $P_{soluble}$ ($P < 0.01$) but were also not significantly correlated with Al-P, Fe-P, $P_{reductant}$ and Fixed-P (Table 3). Correlation coefficients between phosphatase activity and P_{inorg} fractions had not been measured in earlier studies, probably because of the difficulty of measuring soil phosphate. However, correlations between phosphatase activity and the so-called plant available fraction of inorganic phosphate, variously called available-P, mobile-P and soluble-P, had been measured contradictory results. Hofmann and Kasseba (1962) found a highly significant correlation between phosphatase activity and soluble-P in German soils but an equally significant positive correlation between these factors in Egyptian soils. Haynes and Swift (1988) described negative correlations of phosphatase activity with available phosphorus. Nahas *et al.* (1994) suggested that the activity of phosphatase not correlated with available phosphorus. Inverse relationships have found by other workers (Speir and Ross, 1978) found positively correlations between inorganic PO_4^{2-} anions and phosphatase activity.

In literatures it was shown that phosphatase activity is directly related to organic phosphorus and bioavailable P content in soil. The strong correlation among Ca-P, P_{total} with activity of phosphatases (ACPA, NEPA and APA) may be sourced by relationship among Ca-P, P_{total} with P_{org} and bioavailable-P. The relationships among the P_{total} , P_{org} , Ca-P, $P_{soluble}$ with bioavailable P in soil studied are given Table 4.

The P_{org} content of a soil, could be affected by parent material, climatic zone and human activities such as cultivation, land use and fertilizer, is related to some soil physico-chemical and biological properties. The contents of organic matter and P_{org} in soil studied were correlated positively ($r = 0.589^{**}$). In addition, organic matter and P_{org} were positively correlated with the bioavailable P. A significant relationship between the P_{org} and the available form of soils has been found by a number of researchers (Adepetu and Corey, 1976; Harrison, 1987). A large reservoir of P_{org} exists which is unavailable to alive. Here, the microbial oxidation of organic substrates is an important supplementary source of bioavailable P.

Table 4: Pearson correlation coefficients and regression equations among P_{total} , P_{org} , Ca-P, $P_{soluble}$ with bioavailable P in soil studied

Parameters	Correlation coefficients	Regression equation
P_{total}	0.552**	$y = 16.372x + 441.33$
P_{org}	0.869**	$y = 5.1074x + 28.152$
Ca-P	0.544**	$y = 8.5027x + 95.405$
$P_{soluble}$	0.933**	$y = 0.6867x + 1.3174$

** : Significant at 0.01 level

As might be expected, there is no direct relationship Ca-P and P_{total} with the bioavailable P. However, positive correlation was also found Ca-P and P_{total} was significantly correlated with bioavailable P (Table 4). As soils develop, the total P undergoes changes that are related to the weathering environment. We expect the amount and forms of P_{total} to change with soil-forming processes. It was demonstrated that the driving force for conversion of primary to secondary and occluded forms is weathering and available of the soil. In addition, bioavailable P concentrations might be largely controlled by solubility of P minerals that are dominated by Ca-P in neutral to high pH soils studied. $P_{soluble}$ (ammonium chloride extractable phosphorus content) can be easily desorbed from soil and thus may be also considered bioavailable (Maida 1978). For this reason, the correlations between bioavailable P and $P_{soluble}$ are expected results in soils.

In summary, in this study has shown that ACPA, NEPA and ALPA were correlated to P_{total} , P_{org} , Ca-P, $P_{solubles}$ bioavailable P contents in soils studied but not significantly correlated with $P_{residual}$, Al-P, Fe-P and Fixed-P. These results obtained that Ca-P is major P_{inorg} compound on the effects of soil phosphatase activity in agricultural soils of Çarşamba Plain, Turkey. In addition, further studies are needed to explore the relevancy in using phosphatase activity to reflect different type soils and climatic zone.

ACKNOWLEDGMENT

This work was supported by the OMU Center of Scientific Research (Z-286).

REFERENCES

- Adepetu, J.A. and R.B. Corey, 1976. Organic phosphorus as a predictor of plant available phosphorus in soils of southern Nigeria. *Soil Sci.*, 122: 159-164.
- Alef, K. and P. Nannipieri, 1995. *Methods in Applied Soil Microbiology and Biochemistry*. Academic Press, New York, pp: 335-337.
- Alexander, M., 1977. *Introduction to Soil Microbiology*. Second Edition, John Wiley and Sons. New York, pp: 467.
- Amador, J.A., A.M. Glucksman, J.B. Lyons and J.H. Gores, 1997. Spatial distribution of soil phosphatase activity within a riparian forest. *Soil Sci.*, 162: 808-825.
- Anonymous, 2000. Samsun climatic data (1974-2000), State Meteorological Services (DMI), Unpublist, Ankara, Turkey.
- Aon, M.A. and A.C. Colaneri, 2001. Temporal and spatial evolution of enzymatic activities and physico-chemical properties in an agricultural soil. *Applied Soil Ecol.*, 18: 255-270.
- Bayraklı, F., 1975. Studies on soil phosphorus of Bayburt, Erzincan and Rize soils. Atatürk University Publication Number 398, Erzurum, Turkey, pp: 95.
- Bieleski, R.L. and I.B. Ferguson, 1983. Physiology and Metabolism of Phosphate and its Compounds. In: *Inorganic Plant Nutrition*. Läuchli, A. and R.L. Bieleski (Eds.), Springer-Verlag Berlin, pp: 415-449.
- Black, C.A., 1968. *Soil-Plant Relationships*. New York, Wiley, pp: 558-653.
- Bouyoucos, G.J., 1951. A recalibration of hydrometer method for making mechanical analysis of soils. *Agron. J.*, 43: 434-438.
- Chang, S.C. and M.L. Jackson, 1957. Fractionation of soil phosphorus. *Soil Sci.*, 84: 133-144.
- Chen, H., 2003. Phosphatase activity and p fractions in soils of an 18-year-old Chinese fir (*Cunninghamia lanceolata*) plantation. *Forest Ecol. Manag.*, 178: 301-310.
- Dick, R.P. and M.A. Tabatabai, 1987. Factors affecting hydrolysis of polyphosphates in soils. *Soil Sci.*, 143: 97-104.

- Dick, R.P., 1997. Soil Enzyme Activities as Integrative Indicators of Soil Health. In: Biological Indicators of Soil Health. Pankhurst, C.E., B.M. Doube and V.V.S.R. Gupta (Eds.), CAB International Wallingford, UK, pp: 121-156.
- Fox, R.L. and E.J. Kamprath, 1970. Phosphate sorption isotherms for evaluating the phosphorous requirements of soils. *Soil Sci. Soc. Am. Proc.*, 34: 902-907.
- Gavrilova, A.N., N.A. Shimko and V.F. Savchenko, 1973. Dynamics of organic phosphorus compounds and phosphatase activity in pale yellow sod-podzolic soil. *Soviet Soil Sci.*, 3: 320-328.
- Harrison, A.F., 1987. Soils organic phosphorus: A Review of world literature. C.A.B. International Wallingford, UK, pp: 257.
- Haynes, R.J. and R.S. Swift, 1988. Effect of lime and phosphate additions on changes in enzyme activities, microbial biomass and levels of extractable nitrogen, sulphur and phosphorus in acid soil. *Biol. Fertil. Soils.*, 6:153-158.
- Herbien, S.A. and J.L. Neal, 1990. Soil pH and phosphatase activity. *Commun. Soil Sci. Plant Anal.*, 21: 439-456.
- Hofmann, E. and A. Kasseba, 1962. Enzymes in Egyptian soils. *Z. Pflanzenernähr. Bodenkd.* 99: 9-20.
- Hoffmann, G., 1968. Eine photometrische Methode zur Bestimmung der Phosphataseaktivität in Böden. *Z. Pflanzenernähr. Bodenkd.*, 118: 161-172.
- Jordan, D. and R.J. Kremer, 1994. Potential Use of Soil Microbial Activity as an Indicator of Soil Quality. In: *Soil Biota: Pankhurst, C.E., B.M. Doube, V.V.S.R. Gupta and P.R. Grace (Eds.), Management in Sustainable Farming Systems, CSIRO, Australia*, pp: 245-249.
- Juma, N.G. and M.A. Tabatabai, 1978. Distribution of phosphomonoesterases in soils. *Soil Sci.*, 126: 1001-108.
- Kabata-Pendias, A. and H. Pendias, 1992. Trace Elements in Soils and Plants. 2nd Edn, Lewis Pub. Inc. Boca Raton, FL.
- Larsen, S., 1967. Soil phosphorus. *Adv. Agron.*, 19: 151-210.
- Maida, J.H.A., 1978. Phosphate availability indices related to phosphate fractions in selected Malawi Soils. *J. Sci. Food Agric.*, 29: 423-428.
- Marinari, S., G. Masciandaro, B. Ceccanti and S. Grego, 2000. Influence of organic and mineral fertilizers on soil biological and physical properties. *Bioresour. Technol.*, 72: 9-17.
- Murphy, J. and J.P. Riley, 1962. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chem. Acta*, 27: 31-36.
- Nahas, E., J.F. Centurion and L.C. Assis, 1994. Efeito das características químicas dos solos sobre os microorganismos solubilizadores de fosfato e produtores de fosfatases. *Revista Brasileira de Ciencia de Solo.*, 18: 49-53.
- Nannipieri, P., B. Ceccanti and D. Bianchi, 1988. Characterization of humus-phosphatase complexes extracted from soil. *Soil Biol. Biochem.*, 20: 683-691.
- Olsen, S.R., C.V. Cole, F.S. Watanabe and L.A. Dean, 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular 939. U.S. Government Printing Office, Washington USA.
- Olsen, S.R. and L.E. Sommers, 1982. Phosphorus. In: *Methods of Soil Analysis*. Page A.L., Miller, R.H. and D.R. Keeney (Eds.), 2nd Edn. Agronomy Series No. 9, Part 2, Soil Science Society of America, Inc., Madison, WI, pp: 403-430.
- Pagliai, M. and M. De-Nobili, 1993. Relationships between soil porosity, root development and soil enzyme activity in cultivated soils. *Geoderma*, 56: 243-256.
- Pascual, J.A., J.L. Moreno, T. Hernández and C. García, 2002. Persistence of immobilized and total urease and phosphatase activities in a soil amended with organic wastes. *Bioresour. Technol.*, 82: 73-78.

- Peech, M., 1965. Hydrogen-ion activity, In: Methods of Soil Analysis. Black, C.A., D.D. Evans, J.L., White, L.E. Ensminger and F.E. Clark (Eds.), Part 2, Chemical and Microbiological Properties, Agronomy 9, ASA, Madison, Wisconsin, USA, pp: 914-925.
- Peterson, G.W. and R.B. Corey, 1966. A modified Chang and Jackson procedure for routine fractionation of inorganic soil phosphates. *Soil Sci. Soc. Amer. Proc.*, 30: 563- 565.
- Rowell, D.L., 1996. *Soil Science: Methods and Applications*. Longman, UK, pp: 350.
- Saunders, W.M.H. and E.G. Williams, 1955. Observations on the determination of total organic phosphorus in soil. *J. Soil Sci.*, 6: 254-267.
- Sing, R.N., H. Sinha and B.P. Sahi, 1968. Fractionation of soil inorganic phosphorus. *J. Indian Soil Sci. Soc.*, 16: 371-375.
- Soil Survey Staff, 1993. *Soil Survey Manual*. USDA Handbook No:18, Washington, USA.
- Speir, T.W. and D.J. Ross, 1978. Soil Phosphatase and Sulphatase. In: *Soil Enzymes*. Burns, R.G. (Ed.), Academic Press London, pp: 197-250.
- Trasar-Cepeda, C., M.C. Leiros and F. Gil-Sotres, 2000. Biochemical properties of acid soils under climax vegetation (*Atlantic oakwood*) in an area of the European temperate-humid zone (Galacia, NW Spain): Specific parameters. *Soil Biol. Biochem.*, 33: 913-919.
- Tripathi, B.R., H.L.S. Tandon and E.H. Tyner, 1970. Native inorganic phosphorus forms and their relation to some chemical indices of phosphate availability for soils of Agra district. *J. Indian Soil Sci. Soc.*, 109: 93-101.
- Udo, E.J. and J.A. Ogunwale, 1977. Phosphorus fraction in selected Nigerian soils. *Soil Sci. Soc. Am. J.*, 41: 1141-1146.
- Uriyo, A.P. and A. Kasseba, 1973. Phosphate fraction in some Tanzania soils. *Geoderma.*, 10: 181-192.
- Woolhouse, H.W., 1969. Differences in the Properties of the Acid Phosphatase of Plant Roots and Their Significance in the Evolution of Edaphic Ecosystem. In: *Ecological Aspects of the Mineral Nutrition of Plants*. Rovison, I.H. (Ed.), Blackwell Scientific Publications, Oxford England, pp: 357-380.
- Yakovchenko, V., L.J. Sikora and D.D. Kaufman, 1996. A biologically based indicator of soil quality. *Biol. Fertil. Soils*, 21: 245-251.