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Temporal variability of available P, microbial P and some phosphomonoesterase activities in a sewage sludge treated soil: The effect of soil water potential

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Available P and enzyme activities strongly depend on the soil water potential. The objective of this study was to test the effects of water potential on soil available P, microbial biomass P(MBP) and some phosphomonoesterase activities. A semiarid soil classified as Calcic Haploxerept was treated with raw sewage sludge at a rate of 20 g kg^{-1}. Four levels of irrigation (deionized water) were established for 90 days of incubation. Constant water potentials used for soil incubation were: saturation (SA, 0 bar), field capacity (FC, -0.3 bar), and permanent wilting point (PWP, -15 bar) in three treatments. An irrigation treatment was also drying-rewetting cycle (DWC) between -0.3 to -15 bars. After 0, 20, 60 and 90 days of incubation, soils were sampled for analysis. The addition of sewage sludge decreased soil pH and increased soil EC, organic C, total N, organic P, available P, MBP contents and phytase, alkaline and acid phosphatases activities significantly. The effects of soil moisture, incubation time and their interaction on soil available P, MBP and phosphomonoesterase activities were significant at different levels. During 20 days of incubation, available P and phosphatase activities decreased, whereas microbial P and phytase activity increased significantly. Thereafter, only available P increased and phytase activities decreased continuously, but microbial P, alkaline and acid phosphatase activities fluctuated during incubation. Soils incubated in DWC and FC compared to soils incubated in SA and PWP had higher available P contents. Microbial P and phosphomonoesterase activities increased with increasing soil water potentials significantly. The highest (38.7 mg kg^{-1}) and lowest (28.9 mg kg^{-1}) microbial P was measured in soil incubated in SA and PWP respectively. Correlation coefficient between available and microbial P was negative and significant. The activities of alkaline phosphatase, acid phosphatase and phytase were higher and lower in soils incubated in SA and PWP, respectively.

Key words: Soil water potential, available P, microbial P, phosphomonoesterase, incubation.

INTRODUCTION

Many soils in the Mediterranean region have been progressively degraded resulting in a decrease in their fertility (Pascual et al., 1998). Organic wastes have been extensively used in order to improve soil quality. Indeed, organic waste can enhance plant productivity as it contains N, P, etc. Among the different macronutrients contained in sludge, phosphorus is an essential element for plant metabolism since it is present in numerous mole-

cules such as phospholipids or nucleotides. It makes up about 0.2% of plant dry weight. Terrestrial plants generally meet their P requirement by the uptake of soil P in inorganic form (Marschner, 1995).

According to Lima et al. (1996), only a small portion of organic waste total P is in the inorganic form and can be assimilated by plants or microorganisms, while approximately 70% of P is in the organic form. Organic P is a major component of soil P, making up 20-80% of the total P in the surface layers of soil (Dalal, 1977). A major constituent of organic P in soil is phytate (inositol hexa- and pentaphosphates), which can account for up to half of the total organic P present (Dalal, 1977).

To be assimilated, organic P present in organic fraction

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must be previously mineralized into inorganic orthophosphate ions. Mineral orthophosphate is the sole form of P to be assimilated by microorganisms and plants (Rao et al., 1996). This process is catalyzed by phosphatase enzymes, which are found in soil microorganisms, plant roots and in extracellular forms in manures and soils. Among these enzymes, acid and alkaline phosphomonoesterases (E.C. 3.1.3.) and phytase (E.C. 3.1.4.) are considered as the predominant phosphatases in most types of soil and litter (Tabatabai, 1994; Criquet et al., 2004).

Alkaline phosphatase (E.C. 3.1.3.1) is an extra cellular enzyme enabling utilization of phosphomonoesters as the source of inorganic phosphate (Pi) required for the maintenance of cellular metabolism. It is an adaptive enzyme whose biosynthesis is controlled by the concentration of Pi in the medium (Orhanovic et al., 2000)

Phosphatases measured in soils reflect the activity of enzymes bound to soil colloids and humic substances, free phosphatases in the soil solution, and phosphatases associated with living and dead plant or microbial cells (Nannipieri et al., 1990). Phosphatase activity is related to soil and vegetation conditions (Herbien and Neal, 1990), responds to changes in management (Adams, 1992; Clarholm, 1993) and can be related to seasonal changes in soil temperature and moisture (Speir and Cowling, 1991).

Phytate-degrading enzymes, commonly referred to as phytases (myo inositol hexakisphosphate phosphohydrolases), are presumably the primary agents in soil responsible for dephosphorylating InsP6. These enzymes, which originate from a diverse group of organisms including fungi, bacteria and plants (Irving, 1980), catalyze hydrolysis of the phosphate ester bond(s) of InsP6, forming orthophosphate and a series of partially dephosphorylated phosphoric esters of myo-inositol (Mitchell et al., 1997). In some cases hydrolysis may go to completion, yielding myoinositol (Greiner, 2006).

Soil enzyme activities are useful candidate "sensors", since they integrate information both about microbial status and soil physico-chemical conditions (Aon et al., 2001). Variations in phosphatase activity are a good indicator of the biological state of a soil (Pascual et al., 1998). The use of wastes, such as sewage sludge, in agriculture and for land reclamation is increasingly becoming important for soil conservation in semi-arid climate zones (Navas et al., 1998; Ros et al., 2003). One method to reverse such soil degradation involves the addition of organic matter to improve soil quality (Garcıa et al., 2000). Using organic wastes and manures, in arid and semi-arid regions will contribute to enrichment of these soils with organic matter and help to reduce other environmental problems (Kütük et al., 2003). Sewage sludge addition has been shown to produce beneficial changes including increases in organic matter, organic carbon, major nutrients (e.g., N, P), water-holding capacity and porosity (Kütük et al., 2003).

Generally, organic waste application stimulates soil microbial processes by increasing the number of culturable bacteria and fungi, microbial biomass C and N, basal respiration and enzyme activities (Fernandes et al., 2005). Alkaline phosphomonoesterase activity of the manure-treated soil was more than 2- to 4-fold greater than those in soils from the other treatments tested by Parham (2002).

Soil moisture content is one of the most important factors which can affect soil biological and biochemical processes. According to Magid et al. (1999), microorganisms lose some of their ability to degrade complex substrates during desiccation. Harris (1981) reported that microorganism's ability to withstand desiccation was influenced by their cell walls and their growth type. Slow growing soil organisms are less susceptible to drying condition than fast growing soil organisms (Robinson et al., 1965). Mikha et al. (2005) reported that repeated dry-rewetting cycles, did not significantly reduce the size of the microbial biomass.

Therefore, the size of microbial biomass is not a limiting factor for N, C and P mineralization (Franzluebbers et al., 1994). Soil microbial population and enzyme activities strongly depend on the soil moisture contents and the objectives of this study was to assess the temporal variability and to test the soil water potential effects on available P, MBP and some phosphoesterase activities in sewage sludge treated soil.

MATERIALS AND METHODS

Soil and organic waste sampling

The soil classified as Calcic Haploxerept (Soil Survey Staff, 1998), was sampled from the top 20 cm layer of an agricultural soil in Hamedan that was fallowed during the previous year, in northwest of Iran, with semi-arid climate (annual rainfall of 300 mm; annual average temperature 13°C). Raw sewage sludge was sampled from Serkan Wastewater Plant, which processes domestic wastewater. The soil was air dried to do the incubations experiment.

Soil physical and chemical analyses

Air-dried soil was subsequently crushed and sieved to pass a 2 mm mesh screen for particle-size analysis using the hydrometer method (Gee and Bauder, 1986). Equivalent calcium carbonate (ECC) was measured by back titration procedure (Leoppert and Suarez, 1996). Soil pH and electrical conductivity (EC) were measured in a 1:5 soil: water extract after shaking for 30 min (Hesse, 1971). Organic carbon (OC) was analyzed by dichromate oxidation and titration with ferrous ammonium sulfate (Walkley and Black, 1934). Total nitrogen in all samples was determined by the Kjeldahl method (Hinds and Lowe, 1980). Total, organic, and available phosphorus was extracted with percholoric and nitric acid, Sulfuric acid and 0.5 M $NaHCO_3$ (pH 8.5) respectively and determined spectrophotometrically as blue molybdate-phosphate complexes under partial reduction with ascorbic acid (Sommers and Nelson, 1972; Bowman, 1989; Jackson, 1958).

Sewage sludge was also analyzed for pH, electrical conductivity, total organic carbon, total N and total P according to those methods.

Table 1. Some characteristics of the sewage sludge used.

Properties	Mean
pH (1:5)	7.5
Electrical conductivity (dS.m^{-1})	4.6
Total organic carbon (g.kg^{-1})	570.0
Total N (g.kg^{-1})	57.3
Total P (g.kg^{-1})	30.1
C/N	9.9
C/P	18.6

Microbiological and biochemical analyses

Fresh soil samples were stored at 4°C for microbiological analyses. Microbial biomass P (MBP) was determined in each sample using CHCl$_3$ as a biocide and bicarbonate as an extractant (Brookes et al., 1982; Hedley and Stewart, 1982). The difference between P in nonbiocide-treated samples and P in biocide-treated samples was considered to be MBP.

Acid and alkaline phosphatases were analyzed according to the methods of Eivazi and Tabatabai (1977). Phytase activity was assayed by the improved method of Han et al. (1999).

Incubation procedure

The sampled soil was treated with sewage sludge (SS) at a rate of 20 g kg^{-1} (w/w) with three replicates. Four levels of irrigation (deionized water) were established for 90 days of incubation. Constant water potentials used for soil incubation were: saturation (SA, 0 bar), field capacity (FC, -0.3 bar), and permanent wilting point (PWP, -15 bar) in three treatments. An irrigation treatment was also drying-rewetting cycle (DWC) between -0.3 to -15 bars. After 0, 20, 60 and 90 days of incubation a portion of each soil was taken for analysis of soil available P and MBP, phytase, alkaline and acid phosphatases according to the methods mentioned above. Analysis of soil parameters in DWC treatment carried out at 48 h after soil rewetting. Soil moisture was near field capacity at this time.

Statistical analyses

The study was a factorial test with completely randomized design. The effects of soil moisture (SM), incubation time (IT) and their interactions (SM*IT) on available P, MBP and also soil phytase, alkaline and acid phosphatase activities were tested. Data were statistically analyzed for standard deviation, means were calculated and Duncan's new multiple range tests were performed to assess the effect of soil water potential on available and MBP and also soil phytase, alkaline and acid phosphatase activities in a sewage sludge treated soil. The computer programs used for data analysis were Ms-Excel and SAS 6 and SPSS 9.0 for windows (spss Inc).

RESULTS

Selected properties of the sewage sludge used in this study are shown in Table 1. Sewage sludge EC was high. The addition of sewage sludge to soil increased soil EC and decreased soil pH. Also, soil organic C, total N and P contents were increased significantly (Table 2). The in-

crease of organic C was 1.48 times that of the control soil (no sewage sludge addition). Changes in total N content were similar to those obtained for organic C. Total P was increased from 2.03 to 2.64 g kg^{-1}. Soil organic, available and MBP contents were also increased after addition of sewage sludge to soil (Table 2). The increase in MBP was 2 times that of untreated soil. Phytase, alkaline and acid phosphatases activities were increased more than 3 times than that of untreated soil. The increase in phytase activity was higher than those of the other soil properties. It was 13.12 times higher compared to untreated soil.

Table 3 shows analysis of variance of soil available and microbial P contents and alkaline phosphatase, acid phosphatase and phytase activities as affected by soil moisture (SM) and incubation time (IT). Soil moisture, incubation time and their interaction had strongly significant effects (p<0.01) on all of these properties (except acid phosphatase activity). The effect of incubation time on acid phosphatase activity was significant at p<0.05 and the effect of interaction between soil moisture and incubation time on this soil property were not significant.

The effects of soil moisture

Sewage sludge treated soils incubated in DWC and FC compared to sewage sludge treated soils incubated in SA and PWP had higher available P contents. The differences between available P in soils incubated in DWC, FC and SA were not significant (p<0.05). However, available P in soil incubated in PWP was significantly lower than those incubated in other moisture treatments (Table 4).

Microbial P in soils incubated in different moisture conditions was significantly different. It was significantly higher in soil incubated in SA condition (38.7 mg kg^{-1}) and lowest in soil incubated in PWP (28.8 mg kg^{-1}). Microbial P in soils incubated in DWC (33.9 mg kg^{-1}) and FC (33.2 mg kg^{-1}) were not significantly different.

Soil incubation in different moisture obviously changed alkaline phosphates activity. Same as observed for microbial P, alkaline phosphatase activity was higher in soil incubated in SA compared to those incubated in other moisture treatments. Alkaline phosphatase activity in soil incubated in FC was significantly higher that in soil incubated in DWC. The lowest alkaline phosphatase activity was assayed in soil incubated in PWP (4.4 µmol P.N.P g^{-1}h^{-1}).

The effect of soil moisture on acid phosphatase activity was lower than the effect on alkaline phosphates activity. Although acid phosphatase activity in soil incubated in SA was the highest however there were not significant difference between soils incubated in PWP, FC and SA. The lowest acid phosphatase activity was measured in soil incubated in DWC condition (1.8 µmol P.N.P g^{-1}h^{-1}). The ratios of alkaline/acid phosphatase activities were also analyzed in this study. The results showed that alkaline/acid phosphatase ratio was highest in soil incubated in DWC condition (2.6) compared to other conditions The

Table 2. Some soil characteristics before and after treatment with sewage sludge.

Soil properties	Before treatment	After treatment	Increase ratio
pH (1:5)	7.9	7.6	0.9
EC (dS.m^1)	0.1	0.2	1.6
Organic C (g.kg^1)	21.4	31.6	1.5
Total N (g.kg^1)	3.9	4.6	1.2
Total P (g.kg^1)	2.03	2.6	1.3
C/N	5.3	6.8	1.3
C/P	10.5	11.9	1.1
Organic P (g.kg^{-1})	0.8	0.9	1.1
Biomass P (mg.kg^1)	21.2	42.5	2
Available P (mg.kg^1)	25.3	36.5	1.4
Alkaline phosphates act. (µmol P.N.P g^{-1}h^{-1})	1.5	5.3	3.6
Acid phosphatase act. (µmol P.N.P g^{-1}h^{-1})	0.8	3.1	3.9
Phytase activity (µmol P g^{-1}.min^{-1})	0.6	7.8	13.1
ECC (%)	3.5		
Sand (%)	63.5		
Silt (%)	20.6		
Clay (%)	15.9		

Table 3. Analysis of variance (mean square) of sewage sludge treated soil available P, microbial P, alkaline phosphatase, acid phosphatase and phytase activities as affected by soil moisture (SM) and incubation time (IT) [a].

Source of variations	Df	Available P	Microbial P	Alkaline phosphate act.	Acid phosphatase act.	Phytase act.
SM	3	231.1***	193.8***	4.5***	4.3***	342.8***
IT	3	2355.8***	3242.6***	1.0***	1.5*	58.8***
SM*IT	9	185.5***	8083298***	0.2***	0.6	6.4**

[a] Mean squares marked by *, ** and *** are significant at P<0.05, P<0.01 and P<0.001, respectively.

Table 4. Available P, microbial P, alkaline phosphatase, acid phosphatase and phytase activities in sewage sludge treated soils incubated in different moistures[#].

Soil moisture[##]	Available P (mg.kg^{-1})	Microbial P (mg.kg^{-1})	Alkaline phosphates act. (µmol PNP g^{-1}h^{-1})	Acid phosphatase act. (µmol PNP g^{-1}h^{-1})	Alkaline/acid phosphatase ratio	Phytase act. (µmol P g^{-1}min^{-1})
DWC	48.0^a	33.9^b	4.5^c	1.7^b	2.6^a	6.7^c
PWP	38.8^b	28.8^c	4.4^d	2.6^a	1.8^b	4.9^d
FC	47.8^a	33.2^b	5.0^b	2.9^a	1.7^b	11.3^b
SA	46.9^a	38.7^a	5.7^a	3.1^a	1.7^b	16.9^a

[#] Values with different character are significantly different at the 0.05 probability level.
[##] DWC- drying-rewetting cycle (between -0.3 to -15 bar), PWP- permanent wilting point (-15 bar), FC- field capacity (-0.3 bar), SA- saturation (0 bar).

differences between alkaline/acid phos-phatase ratios in soils incubated in PWP, FC and SA were not significant (Table 4).

Phytase activities in soils incubated in different moisture conditions were significantly different. Phytase activity was significantly higher in soil incubated in SA condition (16.9 µmol P g^{-1}min^{-1}) compared to those incubated in other conditions. Phytase activity in soil incubated in FC was significantly different from those assayed in soils incubated in PWP and DWC. The lowest phy-

Table 5. Soil available P, microbial P, alkaline phosphatase, acid phosphatase and phytase activities in different incubation time [#].

Incubation time (days)	Available P (mg.kg^{-1})	Microbial P (mg.kg^{-1})	Alkaline phosphates act. (µmol PNP g[-1]h[-1])	Acid phosphatase act. (µmol PNP g[-1]h[-1])	Alkaline/acid phosphatase ratio	Phytase act. (µmol P g^{-1}min^{-1})
0	36.5[c]	42.5[b]	5.3[a]	3.1[a]	1.9[b]	7.8[d]
20	32.1[d]	52.1[a]	4.7[bc]	2.5[b]	1.9[b]	13.1[a]
60	49.9[b]	16.9[d]	4.7[c]	2.3[b]	2.1[a]	9.7[b]
90	63.1[a]	23.1[c]	4.8[b]	2.5[b]	2.0[a]	9.3[c]

[#] Values with different character are significantly different at the 0.05 probability level.

tase activity (5.0 µmol P g^{-1}min^{-1}) was measured in soil incubated in PWP.

The effects of incubation time

Available P significantly decreased from 36.5 mg kg^{-1} to 32.1 mg kg^{-1} in 20 days of incubation in sewage sludge treated soil (Table 5). It may be related to increase of microbial population and P immobilization. Available P increased to 49.9 and 63.1 mg kg^{-1} at 60 and 90 days of incubation respectively. The differences between available P at different times of soil incubation were significant (p<0.05). Microbial P was also different at different times of incubation. The highest microbial P was measured after 20 days of incubation. Microbial P significantly increased from 42.5 mg kg^{-1} to 52.1 mg kg^{-1} in 20 days of incubation and then decreased to 16.9 mg kg^{-1} at 60 days of incubation and again increased to 23.1 mg kg^{-1} at 90 days of incubation. These changes may be due to temporal variability of microbial populations in soil.

Alkaline phosphatase activity decreased from 5.4 to 4.8 µmol PNP g[-1]h[-1] in 20 days of incubation and to 4.7 and 4.8 µmol PNP g[-1]h[-1] in 60 and 90 days of incubation respectively. The temporal variability of acid phosphatase activity was same as alkaline phosphatase activity. Acid phosphatase activity was significantly higher at start of incubation and thereafter decreased from 3.1 µmol PNP g[-1]h[-1] to 2.3 µmol PNP g[-1]h[-1] at 60 days of incubation and was 2.5 µmol PNP g[-1]h[-1] after 90 days of incubation (Table 5). The ratio of alkaline/acid phosphatase activity increased continuously in 60 days of incubation significantly. Although it decreased from 2.1 in 60 days of incubation to 2.0 in 90 days of incubation but this decrease was not significant.

The differences between phytase activities in different time of incubation were significant at 0.05 level. Same as microbial P, Phytase activity in sewage sludge treated soil increased significantly from 7.8 µmol P g^{-1}min^{-1} to 13.1 µmol P g^{-1}min^{-1} after 20 days of incubation. It may be related to increase of microbial population and activities. After 20 days of incubation phytase activity decreased continuously. It was assayed 9.3 µmol P g^{-1}min^{-1} after 90 days of incubation.

Correlation analysis

Correlation coefficient between soil available P and microbial P was negative and significant at 0.001 level. Soil available P and phosphatases activities had negative correlation coefficient. Whereas, soil available P had positive correlation with phytase activity and the ratio of alkaline/acid phosphatase activity. But these correlations were not significant.

Microbial P had no significant correlations with phosphatases activities. The correlation coefficient between microbial P and the ratio of alkaline/acid phosphatase activity was also not significant. However microbial P had a positive and significant correlation with phytase activity.

Soil alkaline phosphatase activity had positive and significant correlations with acid phosphatase and phytase activities at 0.01 and 0.001 levels respectively. Soil acid phosphatase activity had positive and significant correlations with phytase activity at 0.05 levels.

Correlation coefficient between the ratio of alkaline/acid phosphatase activity and alkaline phosphatase activity was not significant. But the ratio of alkaline/acid phosphatase activity had negative and significant correlations with acid phosphatase and phytase activities at 0.001 and 0.05 levels respectively.

DISCUSSION

This study shows that soil total P, organic P, available P and MBP contents and phosphoesterase activities were increased by addition of sewage sludge to soil. The increase of soil MBP, alkaline and acid phosphatases activities and especially Phytase activity were more obvious. Other researchers also reported that short-term application of organic wastes in soil caused an increase in available P (Lehmann et al., 2005). Jenkinson and Lad (1981) suggested that treatment of soils with animal manure, not only increased the available nutrient in soil, but also affected soil microbial biomass. Addition of organic wastes to soil improved the generation and activities microbial biomass (Martens, 2000). The added organic waste promotes biological and microbial activities, which accelerate the breakdown of organic substances in

the added waste to soil (Agbenin and Goladi, 1998).

Many researchers found the positive effect of the addition organic matter to soil on enzymes activities (Fernandes et al., 2005; Kizilkaya and Bayraki, 2005). The enhanced biological activities in the organic waste treated soil are evidenced by high phosphatase and dehydrogenase activities and microbial C and P. According to Garcia et al. (1993), organic matter of sewage sludge contains high amounts of enzymatic substrates inducing the activity of the different phosphatases in soil. When they are applied to soil, these easily available substrates stimulate enzyme production and consequently microbial growth. Labile organic phosphorus is a substrate of acid phosphates (Thanh Nguyen and Marschner, 2005).

This study showed that soil available P and microbial P were severely affected by soil moisture. The highest and lowest soil available P and especially microbial P were obtained for soils incubated in SA and PWP respectively. These findings may be related to microbial activity and populations. It was reported that soil drying reduces microbial activity and mineralization of organic C, N and P (Pulleman and Tietema, 1999), decreases microbial mobility (Grifin, 1981) and restricts substrate and nutrient availability (Sommers et al., 1981). According to Magid et al. (1999), microorganisms lose some of their ability to degrade complex substrates during desiccation.

However in this study available P was higher in soil incubated in drying and rewetting condition. Mikha et al. (2005) reported that repeated dry-rewetting cycles, did not significantly reduce the size of the microbial biomass. Therefore, the size of microbial biomass was not the limiting factor for N, C and P mineralization (Franzluebbers et al., 1994).

Results presented showed that soil available P and microbial P were affected by incubation time significantly. The first decrease of available P and increase of microbial P may be due to increase of soil microbial population and immobilization of inorganic soil Wong et al. (1998) reported that available P in sewage sludge treated soil decreased significantly 28 days after soil treatment. The addition of organic waste with easily degradable materials to soil results in high microbial biomass due to the stimulation of autochthonous microbial activity in soil and the addition of exogenous microorganisms originating on sludge (Saviozzi et al., 2002). The negative and significant correlation between available P and microbial P observed in this study may be in accordance with Wong et al. (1998) and Saviozzi et al. (2002) reports. After 20 days of incubation, microbial P decreased and available P increased due to microbial autolysis and P mineralization because of the reduction of easily degradable materials (Yan et al., 2000) and the induction of unfavorability of soil for microbial populations (Bardget et al., 1999).

This study showed that soil phosphomonoesterase activities were significantly affected by soil water potential. The highest and the lowest alkaline phosphatase and phytase activities were recorded in sewage sludge treated soil incubated in SA and PWP conditions respectively. These results are in accordance with that of Speir and Cowling (1991). However, Kramer and Green (2000) reported that soil moisture and temperature had limited effects on phosphates activities. It was reported that the role of fungi in alkaline phosphatase production was dominant. Zornoza et al. (2006), studied air-drying and rewetting pre-treatment effects on some soil enzyme activities under Mediterranean conditions and found that acid phosphates showed an increase in activity at the end of incubation time. Higher values of acid phosphatase activity during incubation may be due to changes in environmental conditions with incubation, such as soil moisture, that could favour acid phosphates production, secretion or reactivation.

The study of temporal variability of soil phosphomonoesterase activities showed that in sewage sludge treated soils alkaline and acid phosphatase activities decreased and phytase activity increased with decreasing available P and with increasing microbial P after 20 days of incubation. The correlation between phytase activity and microbial P was positive and significant. However the negative correlation between available P and phosphormonoesterase activity was not significant. There have been reported on the negative and significant relationship between available P and phosphates activity (Moscatelli et al., 2005). This phenomenon can be explained by a competitive inhibition of phosphatases by phosphate ions or by a negative feedback of phosphate ions on PHO genes resulting in a repression of phosphatase synthesis by microorganisms (Oshima et al., 1996). Another explanation could be the availability of C and N substrates, which, as mentioned by Allison and Vitousek (2005), is closely linked to P nutrient mineralization by microorganisms.

However non significant correlation between available P and phosphatase activity in this study may be explained by intending enzyme fractions in soil. A fraction of soil enzymes is associated with living microorganisms, other fractions are associated with non-living cell and particulate matter of the soil matrix and the rest is present in the soil water solution as free enzymes (Burns, 1982). The exogenous enzymes linked to soil colloids (immobilized enzymes), are less susceptible to denaturation (Garcia et al., 1993) and could have an important ecological effect on soil quality, because biochemical activity could remain in soil despite rapid reduction of microbial population.

Garcia et al. (2000) reported that in the calcareous soils with highly stabilized organic matter, the enzymes are mostly protected by soil colloids and generally have a long half-life. This and much of similar work (Jenkinson and Powlson, 1976), supports this idea that the MBP and phytase activity are much more sensitive indicators of changing soil conditions than its acid phosphatase activity, so that the MBP and phytase activity can serve

as early warning of such changes.

In conclusion this study showed that correlation coefficient between available and microbial P was negative and significant in a sewage sludge treated calcareous soil. Phytase, alkaline and acid phosphatases activities were increased more than 3 times after addition of sewage sludge to soil, however the increase of phytase activity were higher than the increase of the other soil properties. Soil microbial P and the activity of alkaline phosphatase, acid phosphatase and phytase were significantly higher in soil incubated in SA and lower in soil incubated in PWP. Although microbial P and phosphomonoesterase activities were higher in soil with higher water potentials but available P was higher in soil with lower soil water potentials.

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