



The effects of water potential on some active forms of phosphorus in a calcareous soil amended with sewage sludge

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ABSTRACT: Immobilization and mobilization reactions of soil phosphorus depend on biological properties of soil and these soil properties strongly depend on the soil water potential. The objective of this study was to test the effects of water potential on some active forms of soil P. A semiarid soil classified as Calcic Haploxerept was treated with raw sewage sludge at a rate of 20 g kg⁻¹. Water potentials established for soil incubation were: saturation (SA, 0 bar), field capacity (FC, -0.3 bar), and permanent wilting point (PWP, -15 bar). An irrigation treatment was 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 increased soil total P, organic P, available P, microbial P, soluble and easily soluble P contents. The increase of soluble P was relatively higher. The effects of soil moisture, incubation time and their interaction on all active forms of soil P were significant. During 20 days of incubation, available P and soluble P decreased, whereas microbial P, easily soluble P and dicalcium phosphate increased significantly. After that, available P and easily soluble P increased continuously, but microbial P, soluble P and dicalcium phosphate fluctuated during incubation. Microbial P had negative and significant correlations with available P and easily soluble P. Soils incubated in DWC and FC compared to soils incubated in SA and PWP had significantly higher available P, soluble P and easily soluble P contents. However microbial P and dicalcium phosphate were significantly higher in soils incubated in higher water potential. @ JASEM

The use of wastes, such as sewage sludge, in agriculture and for land reclamation is increasingly being identified as an important issue for soil conservation in semi-arid climate zones (Navas et al., 1998; Ros et al., 2003). The influence of sewage sludge on soil physical and chemical properties is well known. 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 of soils (Logan et al., 1997; Navas et al., 1998; Ku[¨]tu[¨]k et al., 2003). Phosphorus (P) is an important macronutrient that constitutes vital molecules such as nucleic acid, phospholipids and sugar phosphates in all living organisms. 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). Bicarbonate-extraction is amongst the most common soil-test P methods. Typically available P represents between 1 and 5% of the total soil P. This operationally defined extraction technique was originally developed for the estimation of plant-available soil P, and is commonly used as an index of soil P status, to determine fertilizer P requirements (Kamprath and Watson, 1980). Phosphorus availability may be the most limiting factor to plant growth in many terrestrial ecosystems (Chapin et al., 1994). For soils rich in CaCO₃, the solubility of P is primarily dominated by the solid phase dicalcium phosphate or chemisorption of P on calcite, with the formation of a surface complex of calcium carbonate-P with a defined chemical composition (Von Wandruszka, 2006). Ion exchange between P and carbonate minerals and the

precipitation of phosphate with calcium significantly limit the P availability in desert soils. Competition of organic anions for adsorption sites on calcite also increases the availability of P in the soil solution (Holford et al., 1990).

Between 30 and 50% of total soil P is composed of organic P, which occurs mainly as phytates, nucleic acids (and their derivatives) and phospholipids (Paul and Clark, 1989). A major constituent of organic P in soil is phytate (inositol hexa- and penta-phosphates), which can account for up to half of the total organic P present. Mineral orthophosphate (PO₄⁻³) is the sole form of P assimilated by microorganisms and plants. Orthophosphate ions are released in soil and litter solutions as a consequence of mineralization of organic phosphorus (Rao et al., 1996). P mineralization is usually positively correlated with residue P concentration and negatively correlated with C/P ratio and lignin concentration or lignin/P ratio (Lupwayi and Haque, 1999; Kwabiah et al., 2003). Changes in both soil moisture and temperature influence microbial activity and thereby affect P mineralization (Kabba and Aulakh, 2004). There is also growing evidence that soil biological properties are very affected by environmental factors and may be potential indicators of ecological stress (Dick and Tabatabai, 1992). Other factors as increases in salinity (Rietz and Haynes, 2003) or decreases in water availability may also reduce microbial activity (Mamilov and Dilly, 2002). Turner and Haygarth (2003), reported that soil drying causes significant changes in water-extractable P, in particular organic P, and that these changes appeared to be related to direct P release from lysed microbial cells. The

response of organically amended soils to wet/dry cycles using incubation experiments have been studied by several researchers (Magid et al., 1999; Mamilov and Dilly, 2002). In most of these studies it was focused on organic matter turnover and N transformations by measuring soil respiration rates, microbial biomass carbon, N mineralization and nitrification (Thomsen et al., 1999; Salamanca et al., 2003; Zaman and Chang, 2004). Bioavailable P from organic manures is mainly in the dissolved form. It occupies only a small fraction of total P and particulate organic P is a major fraction of total P. Although organic forms of phosphorus (P) play an important role in the biological availability of soil P, the concentrations, forms, and dynamics of P in soil are poorly understood. There is not much information about the effects of water potential on P active forms after soil treatment with organic wastes. The objective of this study was to investigate the effects of soil water potential on P mineralization and some active forms of P in a calcareous soil amended with sewage sludge.

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 land in Hamadan, 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.

Soil physical and chemical analyses

Air-dry soil was subsequently crushed and sieved to pass a 2-mm mesh screen for particle-size analysis using the hydrometer method (Gee & Bauder, 1986). Equivalent calcium carbonate (ECC) was measured by back titration procedure (Leoppert. & 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 & Black, 1934). Total nitrogen in all samples was determined by the Kjeldahl method (Hinds & Lowe, 1980). Different forms of soil P including total P, organic P, available P, easily soluble P and dicalcium phosphate were extracted by perchloric-nitric acid, sulfuric acid, 0.5 M NaHCO₃, 1 M NH₄Cl and 0.25 M NaHCO₃ respectively, and determined spectrophotometrically as blue molybdate-phosphate complexes under partial reduction with ascorbic acid (Chang and Jackson, 1957; Jackson, 1958; Sommers and Nelson, 1972; Bowman, 1989; Jiang and Gu, 1989).

Microbiological and biochemical analyses

Fresh soil samples were stored at 4 °C for microbiological analyses. We determined microbial biomass P (MBP) in each sample using CHCl₃ as a biocide, and bicarbonate as an extractant (Brookes et al., 1982; Hedley and Stewart, 1982). The difference between Pi in nonbiocide-treated samples and Pm+Pi in biocide-treated samples was considered to be Pm. Sewage sludge was also analyzed according to those methods.

Incubation procedure

The sampled soil was treated with sewage sludge (SS), at a rate of 20 g kg⁻¹ (dry weight basis). Four levels of irrigation (with deionized water) were established for 90 days. Soil moistures were maintained at: saturation (Sat, 0 bar), field capacity (FC, -0.3 bar), and permanent wilting point (PWP, -15 bar). An irrigation treatment was drying-rewetting cycle (DWC) between -0.3 to -15 bars. After 0, 20, 60 and 90 days of incubation a portion of each soil were taken for analysis. Different forms of soil P including available P, microbial P, soluble P, easily soluble P and dicalcium phosphate were analyzed according to the methods mentioned above. Analysis of soil P forms in DWC treatment carried out at 48 hours after soil rewetting. Soil moisture was near field capacity at this time.

Statistical analyses

Data were statistically analyzed for standard deviation, means were calculated, and Duncan's new multiple range tests were performed to assess the effect of organic amendments and soil water potential on the active forms of phosphorus. The computer programs used for data analysis were Ms-Excel and SAS 6. SPSS 6.0 for windows (spss Inc).

RESULTS

Chemical properties

Table 1 shows some sewage sludge properties used in this study. Sewage sludge EC was relatively high. The addition of sewage sludge to soil increased soil EC and decreased soil pH. Soil treatment with sewage sludge increased soil organic C, total N and P contents significantly (Table 2). The increase of organic C was 1.48 times. 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 P, available P, microbial P, soluble and easily soluble P contents were also increased after addition of sewage sludge to soil. The increase of soluble P was relatively higher. It was 3.51 times of untreated soil. Whereas the increase in easily soluble P was lower. It was only 1.07 times of untreated soil.

Table 3 shows analysis of variance of sewage sludge treated soil available P, microbial P, soluble P, easily

soluble P and dicalcium phosphate contents as affected by soil moisture (SM) and incubation time (IT). Soil moisture, incubation time and their interaction had strongly significant effects on all of those properties ($p < 0.001$). The effects of soil moisture compared to the effects of incubation time (mean squares) on available P, microbial P and soluble P were relatively lower. However the effects of soil moisture compared to the effects of incubation time on easily soluble P and dicalcium phosphate were relatively higher. These may be related to the rate of reactions of different forms of P in soil. The reaction rate of easily soluble P and dicalcium phosphate in soil may be slower than the reaction rate of available P, microbial P and soluble P.

Table 1: Some sewage sludge characteristics applied in soil.

Properties	mean
pH (1:5)	7.50
Electrical conductivity (dS.m ⁻¹)	4.600
Total organic carbon (g.kg ⁻¹)	570.0
Total N (g.kg ⁻¹)	57.30
Total P (g.kg ⁻¹)	30.07
C/N	9.95
C/P	18.57

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

Soil properties	Before treatment	After treatment	Increase ratio
pH (1:5)	7.95	7.64	0.96
EC (dS.m ⁻¹)	0.142	0.232	1.63
Organic C (g.kg ⁻¹)	21.4	31.57	1.48
Total N (g.kg ⁻¹)	3.94	4.64	1.18
Total P (g.kg ⁻¹)	2.03	2.64	1.3
Organic P (g.kg ⁻¹)	0.799	0.885	1.11
Available P (mg.kg ⁻¹)	25.27	36.52	1.45
Microbial P (mg.kg ⁻¹)	21.23	42.55	2
Soluble P (mg.kg ⁻¹)	3.24	11.39	3.51
Easily soluble P (mg.kg ⁻¹)	10.07	10.82	1.07
Dicalcium phosphate (mg.kg ⁻¹)	33.98	37.78	1.11
C/N	5.31	6.8	1.28
C/P	10.54	11.96	1.13
Equivalent calcium carbonate (%)	3.55		
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, soluble P, easily soluble P and dicalcium phosphate as affected by soil moisture (SM) and incubation time (IT) #.

Source of variations	Df	Available P	Microbial P	Soluble P	Easily soluble P	Dicalcium phosphate
SM	3	231.125***	193.838***	96.235***	478.906***	345.126***
IT	3	2355.829***	3242.605***	127.270***	454.620***	320.918***
SM*IT	9	185.471***	8083298***	52.492***	182.602***	170.119***

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

The effects of soil moisture

Table 4 shows the effects of soil moisture on available P, microbial P, soluble P, easily soluble P and dicalcium phosphate contents of the sewage sludge treated soil. Soils incubated in DWC and FC compared to those 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 moistures. Microbial P in soils incubated in different moisture conditions was significantly different. It was significantly higher in soil incubated in SA condition (38.686 mg kg⁻¹). Microbial P in soils incubated in DWC and FC were not significantly different. The lowest microbial P was measured in soil incubated in PWP (28.871 mg kg⁻¹). Soil incubation in different moistures had significantly different soluble P. Soluble P was higher in soil incubated in FC compared to those

incubated in other moistures. The differences between soluble P in soils incubated in DWC, FC were not significant ($p < 0.05$). Soluble P in soil incubated in FC and DWC was significantly higher than in soil incubated in FC and PWP. The lowest soluble P was measured in soil incubated in PWP (3.706 mg kg⁻¹). Easily soluble P in soils incubated in different moisture conditions was significantly different. Same as available P and soluble P, easily soluble P was significantly higher in soil incubated in FC condition (24.415 mg kg⁻¹). Soil incubation in SA decreased easily soluble P significantly. The lowest easily soluble P was measured in soil incubated in SA (9.382 mg kg⁻¹).

Dicalcium phosphate in soils incubated in different moisture conditions was significantly different. Same as microbial P, dicalcium phosphate was higher in soil incubated in SA compared to those incubated in other moistures (43.486 mg kg⁻¹). The lowest

dicalcium phosphate was measured in soil incubated in PWP (30.914 mg kg⁻¹)

Table 4: Available P, microbial P, soluble P, easily soluble P and dicalcium phosphate contents in sewage sludge treated soils incubated in different moistures[#].

Soil moisture ^{##}	Available P (mg.kg ⁻¹)	Microbial P (mg.kg ⁻¹)	Soluble P (mg.kg ⁻¹)	Easily Soluble P (mg.kg ⁻¹)	Dicalcium phosphate (mg.kg ⁻¹)
DWC	47.992 ^a	33.979 ^b	8.969 ^a	18.520 ^b	40.481 ^b
PWP	38.858 ^b	28.871 ^c	3.706 ^c	14.863 ^c	30.914 ^d
FC	47.832 ^a	33.244 ^b	9.550 ^a	24.415 ^a	37.969 ^c
SA	46.931 ^a	38.686 ^a	5.302 ^b	9.382 ^d	43.486 ^a

[#] Values followed by the same letter in each column are not significantly different (P < 0.05).

^{##} 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).

Table 5: Soil available P, microbial P, soluble P, easily soluble P and dicalcium phosphate contents in different incubation times[#].

incubation time (days)	Available P (mg.kg ⁻¹)	Microbial P (mg.kg ⁻¹)	Soluble P (mg.kg ⁻¹)	Easily Soluble P (mg.kg ⁻¹)	Dicalcium phosphate (mg.kg ⁻¹)
0	36.524 ^c	42.552 ^b	11.394 ^a	10.817 ^d	37.776 ^c
20	32.092 ^d	52.142 ^a	4.548 ^c	12.857 ^c	40.254 ^b
60	49.913 ^b	16.946 ^d	7.548 ^b	19.177 ^b	31.303 ^d
90	63.084 ^a	23.141 ^c	4.431 ^c	24.328 ^a	43.516 ^a

[#] Values followed by the same letter in each column are not significantly different (P < 0.05).

Table 6: Pearson correlation coefficients between available P, microbial P, soluble P, easily soluble P and dicalcium phosphate in sewage sludge treated soil (n = 48)[#]

	Available P	Microbial p	Soluble P	Easily soluble P	Dicalcium phosphate
Available P	1				
Microbial p	-0.589 ^{***}	1			
Soluble P	0.144 ^{ns}	-0.047 ^{ns}	1		
Easily soluble P	0.595 ^{***}	-0.576 ^{**}	0.224 ^{ns}	1	
Dicalcium phosphate	0.341 [*]	0.256 ^{ns}	0.005 ^{ns}	-0.0116 ^{ns}	1

[#] correlation coefficients marked by *, ** and *** are significance at P<0.05, P<0.01 and P<0.001, respectively. ns: non significant

The effects of incubation time

Table 5 shows the effects of incubation time on soil available P, microbial P, easily soluble and dicalcium phosphate contents. Soil available P significantly decreased from 36.524 mg kg⁻¹ to 32.092 mg kg⁻¹ in 20 days of incubation in sewage sludge treated soil. It may be related to increase of microbial population and P immobilization. After 20 days of incubation available P increased up to 49.913 and 63.084 mg kg⁻¹ in 60 and 90 days of incubation respectively. The differences between available P in different time of soil incubation were significant (p<0.05). Microbial P was also different in different time of incubation. The highest microbial P was measured after 20 days of incubation. Microbial P significantly increased from 42.552 mg kg⁻¹ to 52.142 mg kg⁻¹ in 20 days of incubation and then decreased to 16.946 mg kg⁻¹ in 60 days of incubation. Microbial P once again

increased to 23.141 mg kg⁻¹ in 90 days of incubation. These changes may be due to temporal variability of microbial populations in soil. Same as available P, soluble P decreased from 11.394 to 4.548 mg kg⁻¹ in 20 days of incubation then it increased up to 7.548 mg kg⁻¹ in 60 days of incubation. Soluble P once again decreased to 4.431 mg kg⁻¹ in 90 days of incubation. The differences between soluble P in soils incubated in 20 and 90 days of incubation were not significant (p<0.05).

The differences between easily soluble P in different time of incubation were significant at 0.05 level. Easily soluble P increased continuously during soil incubation. It rose up from 10.817 mg kg⁻¹ to 12.857, 19.177 and 24.328 mg kg⁻¹ in 20, 60 and 90 days of incubation respectively. It may be a good index for P mineralization in soil. Dicalcium phosphate was also different in different time of incubation. Same as

microbial P, it fluctuated during incubation. Dicalcium phosphate significantly increased from 37.776 mg kg⁻¹ to 40.254 mg kg⁻¹ in 20 days of incubation and then decreased to 31.516 mg kg⁻¹ in 60 days of incubation. Dicalcium once again increased to 43.516 mg kg⁻¹ in 90 days of incubation.

Correlation analysis

Correlation coefficient between soil available P and microbial P was negative and significant at 0.001 level. Whereas, soil available P had positive and significant correlations with easily soluble P ($P < 0.01$) and dicalcium phosphate ($P < 0.05$). However the correlation of microbial P with soluble P was not significant (table 6). Microbial P had negative and insignificant correlations with soluble P. However the negative correlation between microbial P and easily soluble P was significant at 0.01 level. The correlation coefficient between microbial P and dicalcium phosphate was positive but it was not significant. Soil soluble P had positive but insignificant correlations with available and dicalcium phosphate. The correlation between dicalcium phosphate and easily soluble P was also insignificant.

DISCUSSION

This study showed that total P, organic P, available P, microbial P, soluble P, easily soluble P and dicalcium phosphate contents of soil increased by addition of sewage sludge. The increase of soil soluble P and biomass P, 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). It was 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). The results showed that all P forms in soil were severely affected by soil water potential. The highest soil available P were obtained for soils incubated in FC and DWC and the lowest available P was measured in soil incubated in PWP. These finding 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, decreases microbial mobility and restricts substrate and nutrient availability (West et al, 1992; Pulleman and Tietema, 1999). According to Magid et al (1999), microorganisms lose some of their ability to degrade complex substrates during desiccation. In constant moisture regimes, microorganisms was

adapted to water conditions and showed the highest activities. The highest and the lowest microbial P were measured in soils incubated in SA and PWP. However available P and soluble P were relatively higher in soil incubated in drying and rewetting condition. Mikhaa et al (2005) reported that repeated dry-rewetting cycle's, 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. Turner and Haygarth (2003), reported that soil drying causes significant changes in water-extractable P, and that these changes appeared to be related to direct P release from lysed microbial cells. Similar changes were reported for inorganic P in bicarbonate extracts of New Zealand pasture soils, and resin extracts of a range of mainly low organic matter soils (Olsen and Court, 1982).

It was reported that seasonal changes in bicarbonate-extractable inorganic and organic P in sandy Danish soils were far greater than those expected on the basis of biological processes alone. Microbial cell death during the drying and rewetting process is primarily induced by osmotic shock and cell rupture upon rewetting with a solution of low ionic strength. Therefore, the high ionic strength of bicarbonate solution may reduce this effect compared with water extraction (Kieft et al., 1987; Magid and Nielsen, 1992). In this study soluble P compared to other forms of P was relatively high in soil incubated in DWC. Result showed that all studied forms of soil P were affected by incubation time significantly. The first decrease of available P and increase of microbial P in 20 days of incubation may be due to increase of soil microbial population and immobilization of inorganic soil P. Wong et al (1998), reported that available P in sewage sludge treated soil decreased significantly 28 days after soil treatment. Microbial P had negative and significant correlations with available P and easily soluble P. After 20 days of incubation microbial P decreased, whereas available P and easily soluble P increased continuously 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 (Bardgett et al, 1999).

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