Soil water potential effects on the cellulase activities of soil treated with sewage sludge

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ABSTRACT

To better understand how water stress and availability affect the enzyme activity and microbial communities in soil, we measured the changes of organic carbon (OC), bacteria and fungi populations, and endoglucanase and exoglucanase activities in a semiarid soil treated with air-dried primary sewage sludge at a rate of 20 g/kg. The 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 a 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 OC, endoglucanase and exoglucanase activities significantly. The effects of soil moisture, incubation time and their interactions on OC, and endoglucanase and exoglucanase activities in soil were significant. During 20 days of incubation, OC, endoglucanase and exoglucanase activities decreased significantly. Soils incubated in DWC and FC compared to soils incubated in SA and PWP had lower OC contents due to organic matter mineralization. Organic C, exoglucanase and endoglucanase activities significantly increased with increasing soil water potential. The activities of exoglucanase and endoglucanase in soils incubated in SA were significantly higher than those in soils incubated in PWP.

Keywords: soil water potential; organic carbon; microbial population; endoglucanase and exoglucanase activity; soil incubation

The use of wastes, such as sewage sludge in agriculture and land reclamation is increasingly being identified as an important issue for soil conservation in semi-arid climate zones (Ros et al. 2003). A method to reverse such soil degradation involves the addition of organic matter to improve soil quality. During the treatment of wastewaters (such as sewage) by constructed wetlands, high molecular weight organic pollutants are degraded to low molecular weight nutrients, utilizable by microorganisms.

Current knowledge of soil biochemistry suggests that this mineralization is achieved via the metabolism of microorganisms and the enzyme activity of the soil (Kang et al. 1998). The activity of soil enzyme is sensitive to changes in soil conditions such as temperature, pH, plant exudates and soil water potential. Soil water stress occurs periodically in most soils. Soil prokaryotes live in water films surrounding particles or inside water-filled pores, and are therefore very susceptible to water depletion.

According to Killham et al. (1993), the enzyme activities decreased significantly with decrease in size of the organic soil particles, and increased with a decrease in the size of the mineral soil particles. Enzymes are able to be sorbed and immobilized by clay minerals and humic colloids in soil environments (Duran and Esposito 2000, Safari Sinegani et al. 2005). Adsorption of enzymes can result in inactivation caused by conformational changes and kinetic enhancement due to the increased concentration of enzyme and substrate at the solid-water interface (Duran and Esposito 2000).

In some systems, the mineral particles of soils, such as clays, may interact with enzymes by modifying their catalytic properties or by inhibiting their activity (Quiquampoix et al. 1993). Soil drying causes a rapid and effectively total cessation of microbial activity, which is readily reversible under natural conditions, but whose effects are not yet completely understood. Chemical changes caused by soil drying are generally small, being limited to an increase in water soluble and extractable nutrients such as organic C and P (Wu and Brookes 2005).

Chemical changes in stored samples include increased extractability of humic substances and small changes in cation exchange capacity and pH.

These changes mostly occur within 1–2 months after soil drying. One key example of this is soil drying and rewetting (D/RW), which subjects soil microbes to physiological stresses by decreasing substrate diffusion leading to changes in metabolism (Schimel et al. 2007). Drying and rewetting can also alter soil water potential creating osmotic stress (Halverson et al. 2000), leading to microbial death and cell lysis unless they are able to resist the stress or become dormant until conditions become more favorable (Schimel et al. 2007).

Considering the environmental importance of cellulases, the objectives of this study were to test the effects of soil water potential on OC mineralization, and cellulases activity in a soil treated with sewage sludge.

MATERIALS AND METHODS

Soil and organic waste sampling. The soil was sampled from the top 20-cm layer of an agricultural land in Hamedan, in northwest of Iran, with semi-arid climate (annual rainfall of 300 mm; annual average temperature 13°C). Air dried primary sewage sludge was sampled from Serkan Wastewater Plant, which processes domestic wastewater.

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 P were extracted with perchloric-nitric acid, sulfuric acid and 0.5M $NaHCO_3$ (pH 8.5), respectively, and were determined spectrophotometrically as blue molybdate-phosphate complexes under partial reduction with ascorbic acid (Sommers and Nelson 1972).

Microbiological and biochemical analyses. Endoglucanase (CMCase) and exoglucanase (FPase) activity were analyzed according to the methods of Miller (1959). Heterotrophic bacterial and fungal populations were estimated by plate count method. Soil suspension and dilutions were prepared. Nutrient agar (NA) and modified potato dextrose agar (MPDA) were prepared in a lab and used for determination of total soil bacterial and fungi populations, respectively (Alef and Nannipieri 1995). Sewage sludge was also analyzed according to those methods.

Incubation procedure. A factorial experiment with complete randomized design with three replicates was done. Soil sample was treated with sewage sludge (SS) at a rate of 20 g/kg (dry weight basis). Four levels of irrigation (deionized water) were established for 90 days. Soil moistures were maintained at: (1) saturation (SA, 0 bar); (2) field capacity (FC, –0.3 bar); and (3) permanent wilting point (PWP, –15 bar) and an irrigation treatment was 4) drying-rewetting cycle (DWC) between –0.3 to –15 bars. After 0, 20, 60 and 90 days of incubation in constant temperature (28°C) a portion of each soil was taken for analysis. Analysis of soil parameters in DWC treatment carried out at 48 h after soil rewetting. Soil moisture was near field capacity at this time. Soil OC, bacterial and fungal populations, endoglucanase and exoglucanase activities were analyzed according to the methods mentioned above.

Statistical analyses. Data were statistically analyzed for standard deviation, means were calculated, and the Duncan's new multiple range tests were performed to assess the effect of organic amendments and soil water potential on soil OC, bacteria and fungi populations, endoglucanase and exoglucanase activities. The computer programs used for data analysis were MS-Excel, SAS 6.12 and SPSS 9.0 for Windows (SPSS Inc.).

RESULTS

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. Organic C, bacteria, fungi, endoglucanase and exoglucanase were also increased after addition of sewage sludge to soil. The increase of bacteria was relatively high; it was 9.38 times higher than in untreated soil. On the contrary, the increase in C/P was lower and reached only 1.13 fold of untreated soil. Table 3 shows analysis of variance of OC, bacteria and fungi populations, endoglucanase and exoglucanase activities as affected by soil moisture (M) and incubation time (T). Soil

Table 1. Some sewage sludge characteristics applied in soil

Sewage sludge properties	
pH (1:5)	7.50
Electrical conductivity (dS/m)	4.600
Total organic carbon (g/kg)	570.0
Total N (g/kg)	57.30
Total P (g/kg)	30.07
C/N	9.95
C/P	18.57
Bacteria (CFU/1 g sewage sludge DW*)	9.93×10^8
Fungi (CFU/1 g sewage sludge DW)	1.93×10^6

*dry matter

moisture, incubation time and their interaction had strongly significant effects on all of the soil properties ($P < 0.01$). The effects of soil moisture compared to the effects of incubation time (mean squares) on all of soil properties were relatively lower.

The effects of soil moisture. Table 4 shows the effects of soil moisture on soil OC, bacteria, fungi, endoglucanase and exoglucanase of the sewage sludge-treated soil. Soils incubated in different moistures had significantly different OC. Organic C was the highest in soil incubated in PWP compared to those incubated in other moistures. Organic C in soils incubated in PWP and SA was significantly higher than that in soils incubated in FC and DWC. The lowest OC was measured in soil incubated in FC (1.21%).

Log of bacterial population in soils incubated in different moisture conditions was significantly different. Log of bacterial population was higher in soil incubated in DWC compared to soils incubated in other moistures. Same as bacteria, log of fungal population was significantly higher in soil incubated in DWC condition (7.18). The lowest bacterial and fungal populations were measured in soil incubated in SA (6.69 and 4.41, respectively).

Endoglucanase activity in soils incubated in different moisture conditions was significantly different. Endoglucanase activity was higher in soil incubated in SA compared to that in soils incubated in other moistures (10.22 μmol Gl/1 g soil DW/h). The lowest endoglucanase activity was measured in soil incubated in PWP (3.97 μmol Gl/1 g soil DW/h).

Soils incubated in DWC and SA compared to those incubated in FC and PWP had higher exoglucanase activity. The differences between exoglucanase activity in soils incubated in DWC and SA were not significant ($P < 0.05$). However, exoglucanase activity in soil incubated in PWP

Table 2. Some soil characteristics before and after treatment (0 days) with sewage sludge

Soil properties	Before treatment	After treatment	Increase ratio
pH (1:5)	7.95	7.64	0.96
EC (dS/m)	0.142	0.232	1.63
Organic C (g/kg)	21.4	31.57	1.48
Total N (g/kg)	3.94	4.64	1.18
Total P (g/kg)	2.03	2.64	1.3
C/N	5.31	6.8	1.28
C/P	10.54	11.96	1.13
Endoglucanase (μmol Gl/1 g soil DW/h)	4.19	12.41	2.96
Exoglucanase (μmol Gl/1 g soil DW/h)	0.29	2.72	9.38
Bacteria (CFU/1 g soil DW)	3.14×10^6	2.24×10^7	7.13
Fungi (CFU/1 g soil DW)	3.53×10^4	7.40×10^4	2.10
Equivalent calcium carbonate (%)	3.55	–	–
Sand (%)	63.5	–	–
Silt (%)	20.6	–	–
Clay (%)	15.9	–	–

*dry matter

Table 3. Analysis of variance (mean square) of sewage sludge treated soil (organic C, bacteria, fungi, endoglucanase and exoglucanase) affected by soil moisture and incubation time

Source of variations	*df*^{a}	Organic C	Bacteria	Fungi	Endoglucanase	Exoglucanase
Moisture treatment (M)	3	1.46**	0.55**	0.46**	85.59**	4.66**
Time (T)	3	1.77**	1.10**	0.74**	139.89**	5.63**
M × T	9	0.25**	0.23**	0.18**	10.99**	4.61**

#mean squares marked by **are significantly different at $P < 0.01$

was significantly lower than that incubated in other moistures.

The effects of incubation time. Soil OC, bacterial population, endoglucanase and exoglucanase activities significantly decreased in 20 days of incubation. Soil fungal population increased in 20 days of incubation (Table 5).

The differences between organic C in different time of incubation were significant at 0.05 level. Organic C decreased continuously during soil incubation. It decreased from 3.16% to 1.80, 1.28 and 1.13% in 20, 60 and 90 days of incubation, respectively.

After 20 days of incubation log of bacterial abundance increased up to 6.94 and 7.09 in 60 and 90 days, respectively. The differences between bacterial abundance in different time of soil incubation were significant ($P < 0.05$).

Log of fungal abundance decreased from 4.84 in 20 days to 4.27 in 60 days of incubation then it increased up to 4.49 in 90 days of incubation, showing usual fluctuation in soil.

Endoglucanase activity decreased from 12.40 to 5.21 μmol Gl/1 g soil DW/h and then to 5.14 μmol Gl/1 g soil DW/h in 20 and 60 days of incubation, respectively; it increased up to 8.09 μmol Gl/1 g soil DW/h in 90 days of incubation. The differences between endoglucanase activities were not significant ($P < 0.05$) in soil in 20 and 60 days of incubation.

Exoglucanase activity significantly decreased from 2.70 to 1.05 μmol Gl/1 g soil DW/h in 20 days of incubation and then increased to 2.11 μmol Gl/1 g soil DW/h in 60 days of incubation and once again decreased to 2.09 μmol Gl/1 g soil DW/h in 90 days of incubation. The differences between exoglucanase activities were not significant ($P < 0.05$) in soils incubated in 60 and 90 days of incubation.

The interaction of soil moisture and incubation time. Figure 1 shows soil OC mineralization in different water potentials. Organic C decreases in soils incubated in SA and PWP were lower than those in soils incubated in FC and DWC in 20 days of incubation. After 20 days of soil incubation OC decreased in soils incubated in SA, FC and DWC conditions continuously. But the decrease of OC was markedly low in soil incubated in PWP.

Figures 2 and 3 show that the changes of endoglucanase and exoglucanase activities were not similar in soils incubated in different water potential. The endoglucanase activities decreases were more obvious in 20 days of incubation, especially in soils incubated in drier condition. After

Table 4. Means and standard deviations (SD) of soil organic C (%), bacterial and fungal populations (log of CFU/1 g soil DW) and endoglucanase and exoglucanase activities (μmol Gl/1 g soil DW/h) in sewage sludge treated soils incubated in different moistures

Soil moisture$^{\#\#}$	Organic C		Bacteria		Fungi		Endoglucanase		Exoglucanase	
	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean
DWC	0.18	1.32^{c}	0.5	7.18^{a}	0.25	4.85^{a}	2.75	7.88^{c}	0.76	2.38^{a}
PWP	0.12	1.99^{a}	0.32	6.78^{b}	0.27	4.47^{c}	1.19	3.97^{d}	0.73	1.09^{c}
FC	0.51	1.21^{d}	0.33	6.80^{b}	0.24	4.58^{b}	2.58	8.76^{b}	0.52	2.05^{b}
SA	0.63	1.59^{b}	0.15	6.69^{c}	0.18	4.34^{d}	2.04	10.22^{a}	0.66	2.43^{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. Means and standard deviations (SD) of soil organic C (%), log of bacterial and fungal populations (log of CFU/1 g soil DW), endoglucanase and exoglucanase activity (μmol Gl/1 g soil DW/h) in different incubation times (averages of all soil water regimes)

Incubation time (days)[#]	Organic C		Bacteria		Fungi		Endoglucanase		Exoglucanase	
	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean
0	0.28	3.16^a	0.51	7.01^b	0.15	4.81^a	2.16	12.40^a	0.64	2.70^a
20	0.36	1.80^b	0.09	6.42^d	0.09	4.84^a	1.03	5.21^c	0.48	1.05^c
60	0.41	1.28^c	0.20	6.94^c	0.49	4.27^c	1.35	5.14^c	0.85	2.11^b
90	0.49	1.13^d	0.14	7.09^a	0.08	4.49^b	2.09	8.09^b	0.65	2.09^b

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

that the endoglucanase activity increased in soils incubated in FC and DWC. The decrease of endoglucanase activity continued in soil incubated in SA with lower slop compared to those in other soils. However it increased in this soil after 60 days of incubation markedly. The increase of exoglucanase activity in soil incubated in SA occurred in 20 days. This increase in soil incubated in FC was relatively lower. After 90 days of incubation, soils with higher water potential had higher endoglucanase and exoglucanase activities.

DISCUSSION

This study showed that organic C, bacteria, fungi, endoglucanase and exoglucanase activities in soil increased by addition of sewage sludge. The increase of soil endoglucanase, bacteria and especially exoglucanase were more obvious. It was suggested that the treatment of soils with organic wastes not only increased the available nutrient in soil but also affected soil microbial biomass. Indeed, changes in the composition of microbial communities as a result of incorporating inorganic or organic amendments were observed (Marschner 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). The results showed that organic C content was affected by incubation time significantly. Soil organic C mineralization rate was affected by soil moisture significantly. Organic C mineralization rate was slow in soil incubated in SA and PWP conditions. They may be due to unsuitable aeration in SA and water supply in PWP (Skopp et al. 1990). The high rate of OC mineralization in SA compared to PWP may be due to higher enzyme activity. Organic C

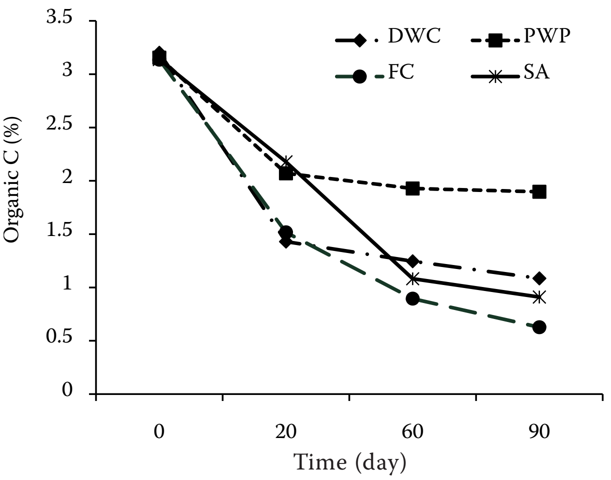

Figure 1. Organic carbon mineralization in soil treated with sewage sludge and incubated in different water potentials

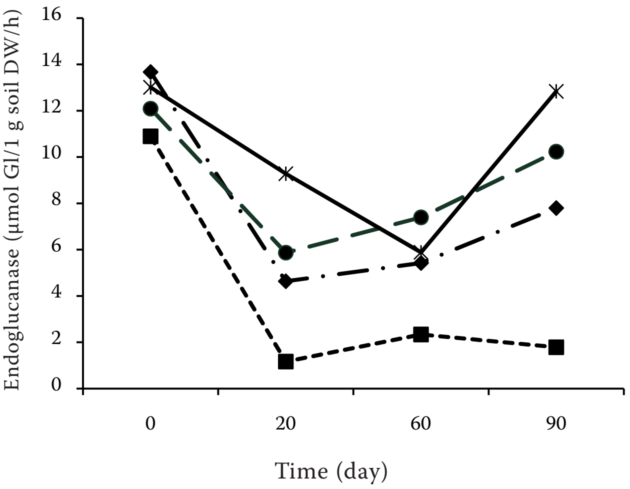

Figure 2. Endoglucanase activity in soil treated with sewage sludge and incubated in different water potentials

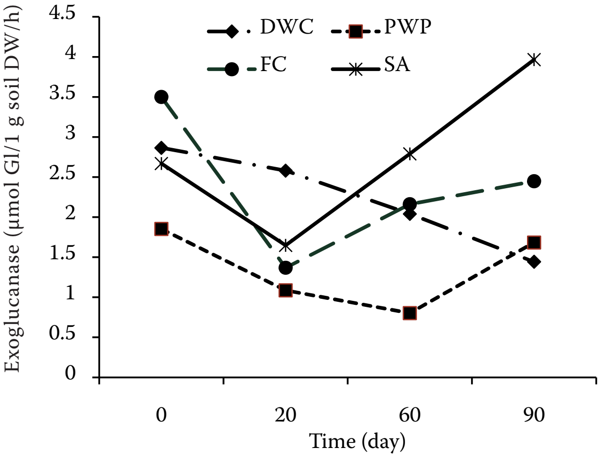

Figure 3. Exoglucanase activity in soil treated with sewage sludge and incubated in different water potentials

in soil incubated in DWC condition decreased with higher rate in 20 days and then lower rate than FC in 60 and 90 days. It shows that repeated DW cycle reduces the organic C mineralization. A reduction in C mineralization with repeated DW cycles could be due to the changes in the physiological state of the decomposer community, making them less susceptible to desiccation. Rapid increases in C mineralization rates in FC could be related to the release of readily degraded organic compounds (resulting from microbial death), which, in turn, would be used by the surviving soil microorganisms upon rewetting (Van Gestel et al. 1993). However, it was suggested that soil microbial C was more sensitive than organic C to changes in soil moisture conditions (Powlson and Jenkinson 1976).

The most of bacteria and especially fungi are aerobic microorganisms that cannot live in anaerobic condition. Furthermore DWC may increase the reproduction of soil microorganisms. Decrease of bacterial (to 20 days) and fungal population (to 60 days) showed the sensitivity of these microorganisms sourced by manures, while the subsequent increase could be due to changes in microbial diversity. Bacterial and fungal populations in soil incubated in SA condition were lower than those in other moisture treatment especially in the end of incubation time. Bacterial and mainly fungal populations were lower in soil incubated in SA and PWP conditions; it may be due to unsuitable aeration in SA and water supply in PWP that inhabited the microbial activity and were stored in dormant forms (endospores, spores and cysts).

Cellulase activities were significantly increased by the addition of sewage sludge to soil. Many researchers reported a positive effect of the addition organic matter to soil on enzymes activities (Fernandes et al. 2005).

Soil incubation in different water potentials had a significant effect on endoglucanase and exoglucanase activities. The highest and lowest endoglucanase and exoglucanase activities were obtained for soils incubated in SA and PWP, respectively. Higher activity of enzymes in soil incubated in SA condition was not in agreement with lower abundance and activities of microorganisms in the SA. The ability of anaerobic microorganisms in cellulase production is lower than that of aerobic microorganisms. Therefore the higher enzymes activities in SA could not result from higher enzyme production in SA condition; they may be related to higher enzymes stability in SA. Unsuitable aeration may be an inhibiting factor in destruction of enzyme proteins. Enzymes can either be free, particularly in aerobic microorganisms, or grouped in a multi-component enzyme complex, such as in anaerobic cellulolytic bacteria (Bayer et al. 1998). The cellulase activities were increased in the end of incubation. This increase may be related to two processes: (1) the increase of cellulase producers as a result of changes in soil microbial communities; and (2) adsorptions and immobilization of enzymes on soil colloids and organic matter (Safari Sinegani et al. 2005). Burns (1982) suggested that a fraction of soil enzymes is associated with living microorganisms, other fractions are associated with non-living, and particulate matter of the soil matrix and the rest is present in the soil water solution (free enzymes). Enzymes are able to be sorbed and immobilized by clay minerals and humic colloids in soil environments (Safari Sinegani et al. 2005). The exogenous and immobilized enzymes are less susceptible to denaturation (Garcia et al. 1993), and can have an important ecological effect on soil quality, because biochemical activity could remain in soil despite rapid reduction of microbial population, thereby playing a very important role in the decomposition of organic matter in soil.

Although bacterial and especially fungal populations in soil incubated in SA condition were lower than those in other moisture treatments, the highest endoglucanase and exoglucanase activities were obtained for soil incubated in SA condition. It may be concluded that the dependence of cellulase activity in soil on the protein denaturation and degradation in soil was higher compared to its microbial production. It strongly depends on the protein denaturation and degradation in soil. Thus cellulase activity in soils significantly depends on the soil water potential (dryness) as an important factor in cellulose adsorption, immobilization and inactivation processes. This finding need to be studied more.

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