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Full Length Research Paper

The effect of soil water potential on survival of fecal coliforms in soil treated with organic wastes under laboratory conditions

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Pollution caused by animal wastes has become a great problem in many countries. The objective of this study was to test the effects of water potential on survival of fecal coliforms in a soil treated with 3 manures. A semiarid soil was treated with raw cow and poultry manures (CM and PM) and sewage sludge (SS) at a rate of 20 g kg^{-1} (dry weight basis). Three water potentials established for soil incubation were: Saturation (SAT, 0 bars), field capacity (FC, -0.3 bars), and permanent wilting point (PWP, - 15 bars) . Fourth irrigation treatment was drying-rewetting cycle (DWC) between -0.3 to -15 bars. Colony forming units of *Escherichia coli* and fecal coliforms on EMBA (eosin methylene blue agar) were counted during soil incubation. The population of *E. coli* was higher in the soils treated with CM but the populations of lactose positive and negative coliforms were higher in the soils treated with PM. The populations of *E. coli* and other fecal coliforms were significantly higher in the soils incubated in SAT compared to those in soils incubated in other water potentials especially in the early stages of soil incubation. The populations of fecal coliforms were decreased significantly with increasing time of incubation. Survival of *E. coli* were near 40 days in the soils treated with PM and SS. *E. coli* could survive in the soils treated with CM and incubated in SAT and PWP for more than 90 days may be due to low level of negative interactions in these unsuitable water conditions.

Key words: Organic wastes, soil water potential, *Escherichia coli*, fecal coliforms, survival, incubation.

INTRODUCTION

The use of wastes, such as sewage sludge and animal manures, 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 organic wastes on soil physical and chemical properties is well known. Sewage sludge and animal manures 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 (Navas et al., 1998; Kutuk et al., 2003). The addition of organic wastes has also produced undesirable changes, such as decreases in pH, increases in salinity and increases in heavy metal content (Navas et al., 1998; Veeresh et al., 2003).

Cattle and other farm animals are the main environmental reservoir for *Escherichia coli* O157:H7 and other pathogens which can cause severe gastrointestinal infections in humans (Karmali, 1989). An increasing number of infections have been linked to direct or indirect contact with the environment (Hepburn et al., 2002; Guan and Holley, 2003). *E. coli* and other coliforms are capable of surviving in soil and animal wastes for long periods (Bolton et al., 1999; Maule, 1999) and low numbers of cells (10 to 500) are required to trigger infection in both humans and animals (Chart, 2000). Pathogen coliforms may be introduced to arable and pasture land through application of contaminated animal slurries, manures, abattoir wastes and human sewage

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which are added as either an organic fertilizer or as a means of waste disposal.

Organic matter is important for water retention, the formation and stabilization of aggregates and the formation of microhabitats, all factors with a strong influence on the survival of micro-organisms in the soil (Stotzky, 1989), while Dazzo et al. (1973) proved that the increase in organic matter content in the soil improved the survival of coliforms. Other factors as increases in salinity (Rietz and Haynes, 2003) or decreases in water availability may also reduce microbial activity (Mamilov and Dilly, 2002). *Mycobacterium* survives better in soils with a pH between 6.0 and 7.0 (Ellis and McCalla, 1976) and the best inactivation rates for pathogenic micro-organisms are found in acid soils. *E. coli* and *Listeria monocytogenes* are known to be capable of surviving anaerobic digestion and colonies may grow again after the application of sludge to land in some cases (Gerba et al., 2001).

An important feature characterizing the Mediterranean type climate is the occurrence of a summer drought, with water availability being an important limiting factor in these environments. Several works have reported the response of organically amended soils to wet/dry cycles using incubations assays (Magid et al., 1999; Mamilov and Dilly, 2002). Most of these studies are focused on organic matter turnover and N transformations, showing a decrease of soil respiration rates, microbial biomass carbon (C) and N mineralization and nitrification (Thomsen et al., 1999; Zaman et al., 1999; Salamanca et al., 2003; Zaman and Chang, 2004). However, to our knowledge, there are no studies about the effect of controlled drought on fecal coliforms survival in soil. *E. coli* O157 and other EHEC strains are commonly found in beef and dairy cattle (Elder et al., 2000). On farm monitoring of *E. coli* O157:H7 suggests that shedding occurs episodically (up to 10^5 organisms g^{-1} feces) and can persist for variable periods of time ranging from 1 to 5 months (Shere et al., 1998; Zhao et al., 1995). Several factors influence the survival of pathogens in soil after waste materials are applied. Soil moisture and temperature seem to be the most important of these factors (Crane and Moore, 1986; Sjogren, 1994). Survival of bacterial pathogens in soil increases when the soil is moist and temperatures are warm (Entry et al., 2000). Pathogen survival time in the soil varies from 4 to 160 days (Sjogren, 1994; Abu-Ashour et al., 1994). Obligate parasites usually only live a few minutes outside the host, but many pathogenic organisms can live in groundwater and soil for months (Sorber and Moore, 1987; Entry et al., 2000) . Manure-borne pathogens may enter the soil and travel through vadose zone until they reach ground water (McMurry et al., 1998). Therefore, the study of factors affecting the survival of fecal coliforms is very important. The objective of this work was to assess the potential impact of soil water content on survival of fecal coliforms of a soil amended with different types of organic wastes (sewage sludge, poultry and cow manures).

MATERIALS AND METHODS

Soil and organic waste sampling

The experimental soil was sampled from the top 20-cm layer of an agricultural land in Hamadan, in northwest of Iran, which has a semiarid climate (annual rainfall of 300 mm; annual average temperature 13°C) . Raw sewage sludge was sampled from Serkan Wastewater Plant, which processes domestic wastewater. Raw cow and poultry manures (CM and PM) were collected from the dairy and poultry units of Hamadan.

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 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 and available P were extracted with perchloric- nitric 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; Jackson, 1958).

Microbiological and biochemical analyses

Microbe populations were determined by plate seeding. First, a sample of 1 g dry weight was added to 99 ml of solution ($Na_4P_2O_7$, 0.2% in water), the resultant 1:100 suspension then being homogenized by 15 min shaking and used to make a set of dilutions in distilled water, the dilution levels chosen being those that would give 30 to 300 colonies per plate. The average number of colonies obtained for each dilution was calculated for each sample, population and day of incubation, and then the averages of all the dilution factors used were found and, taking into account the dry soil weight of each sample, each population was expressed as colony-forming units per gram of dry matter. The culture medium used for semi-quantitatively analyzing of fecal coliforms and *E. coli* was Petri dish containing sterilized eosin methylene blue agar (EMBA) (Feng et al., 2002). The Petri dishes were incubated for 48 h at 28°C. They were then examined for the growth of *E. coli*, lactose positive and lactose negative coliforms as evidenced by the metallic green, purple and pinky white colors, respectively.

Heterotrophic bacterial and fungal populations were estimated by plate count method. Soil suspension and dilutions were prepared. Nutrient agar (N. A) rose bengal starch casein nitrate agar (RBSCNA) and modified potato dextrose agar (MPDA) were prepared in laboratory and used for determination of total soil bacterial, actinomycetes and fungi populations respectively. The levels of microorganisms, counted as colony forming units (cfu), were expressed as log10-values per gram dry weight of sample.

Basal respiration was measured as CO_2 evolved in 5 days (Alef and Nannipieri, 1995). Substrate induced respiration (Anderson and Domsch, 1978), was determined in 72 h. The organic wastes (PM, CM and SS) were also analyzed according to those methods.

Table 1. Some characteristics of amendments applied in soil.

Amendment properties	Poultry manure	Cow manure	Sewage sludge
pH (1:5)	7.48 b	8.32 a	7.50 b
Electrical conductivity ($dS.m^{-1}$)	7.00 a	4.97 b	4.60 b
Total organic carbon ($g.kg^{-1}$)	295.0 c	474.0 a	331.0 b
Total N ($g.kg^{-1}$)	36.40 b	18.00 c	57.30 a
Total P ($g.kg^{-1}$)	13.82 b	4.87 c	30.07 a
C/N	8.10 b	26.30 a	5.77 c
C/P	21.34 b	97.33 a	11.00 c
E. coli (cfu g^{-1})	$2.74*10^{4}$ b	$3.45*10^{6}$ a	$6.17*10^{4}$ b
Lac^{+} Coliforms (cfu g^{-1})	$3.46*10^{5}$ b	$5.09*10^{6}$ a	$2.97*10^{5}$ b
Lac^{-} Coliforms (cfu g^{-1})	$4.53*10^{5}$ b	$5.84*10^{6}$ a	$4.87*10^{5}$ b
Bacteria (cfu g^{-1})	$9.02*10^{7}$ b	$1.31*10^{9}$ a	$1.39*10^{8}$ b
Fungi (cfu g^{-1})	$2.67*10^{3}$ b	$6.03*10^{3}$ b	$2.76*10^{6}$ a
Actinomycetes (cfu g^{-1})	$2.05*10^{7}$ b	$4.01*10^{7}$ a	$3.85*10^{7}$ a

Values with different character in each row are significantly different at the 0.05 probability level.

Incubation procedure

A factorial experiment with complete randomized design with three replicates has been done. Soil samples were treated with amendments (PM, CM and SS) at a rate of 20 g kg^{-1} (dry weight basis) separately. Each of amendment (20 g) completely mixed with one kg of soil for incubation. After addition of soil amendments, water potentials of treated soils were equilibrated in 0 and -0.3 bars by hanging water columns and -15 bars by pressure membrane apparatus and their water contents were measured by thermogravimetric (Hillel, 1998; Carter and Gregorich, 2008) . Four levels of irrigation (deionized water) were established for 90 days. Soil water contents were measured gravimetrically and maintained near: (1) saturation (SAT, 0 bar), 2) field capacity (FC, -0.3 bar), and 3) permanent wilting point (PWP, -15 bar) by calculation of the amounts of evaporated water (water loss) and addition of the same amounts of deionized water to soils by spray every 2 days. An irrigation treatment was drying rewetting cycle (DWC) between - 0.3 to -15 bars. Soils were incubated under laboratory condition for 90 days. Laboratory temperature was around 23°C. After 0, 10, 20, 40, 60 and 90 days of incubation, a portion of each soil were 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 was analyzed for *E. coli*, lactose positive and lactose negative coliforms according to the methods previously mentioned.

Statistical analyses

The experiment was considered a completely randomized design as factorial in three replicates. The factors were soil amendments (CM, PM and SS), soil moistures (SA, FC, PWP, DWC), and incubation time (0, 10, 20, 40, 60 and 90 days). 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 amendments, soil water potential and incubation time on the populations of *E. coli*, lactose positive and lactose negative coliforms in soil. The computer programs used for data analysis were Ms-Excel, SAS 6.12 and SPSS 9.0 for windows (spss Inc).

RESULTS

Chemical and biological properties of soil and organic wastes

Table 1 shows some properties of soil amendments used in this study. Cow manure compared to PM and SS had significantly higher pH, OC, C/N and C/P ratios. Sewage sludge had relatively higher total P and N contents. However poultry manure compared to CM and SS had significantly higher EC. The populations of *E. coli*, lactose positive and lactose negative coliforms were significantly higher in CM compared to PM and SS. However the populations of actinomycetes and especially fungi were significantly high in SS may be due to higher resistance of these microorganisms to higher level of heavy metals (Khan and Scullion, 2000; Rajapaksha et al., 2004).

Table 2 shows some properties of soil used in this study. The texture of the soil was sandy loam. It was nonsaline (EC 0.14 dS/m) with relatively low equivalent calcium carbonate (3.55%), organic matter (OC 2.14%) and total nitrogen (TN 0.394%). Soil available P and biomass P were relatively high (25.27 and 21.23 mg kg^{-1}, respectively).

The populations of *E. coli*, lactose positive and lactose negative coliforms in soil were relatively low. The population of *E. coli* in soil was very low. It could not be numbered by plate count method. The populations of lactose positive and lactose negative coliforms in studied soil were $3.11*10^{4}$ and $3.81*10^{4}$ cfu g^{-1}, respectively. The populations of bacteria, fungi and actinomycetes were $4.12*10^{7}$, $1.65*10^{4}$ and $2.72*10^{6}$ cfu g^{-1}, respectively.

Table 3 shows analysis of variance of the populations of *E. coli*, lactose positive coliforms and lactose negative coliforms as affected by soil amendments (AM), soil

Table 2. Some characteristics of soil used in this study.

Soil property	
Sand (%)	63.5
Silt (%)	20.6
Clay (%)	15.9
ECC (%)	3.55
pH (1:5)	7.95
EC ($dS.m^{1}$)	0.14
Organic C ($g.kg^{1}$)	21.4
Total N ($g.kg^{1}$)	3.94
Total P ($g.kg^{1}$)	2.03
C/N	5.43
C/P	10.54
Organic P ($g.kg^{-1}$)	0.799
Biomass P ($mg.kg^{1}$)	21.23
Available P ($mg.kg^{1}$)	25.27
Basal respiration (mg CO_2 d^{-1} g^{-1})	0.22
E. coli (cfu g^{-1})	0.00
Lac^{+} coliforms (cfu g^{-1})	$3.11*10^{4}$
Lac^{-} Coliforms (cfu g^{-1})	$3.81*10^{4}$
Bacteria (cfu g^{-1})	$4.12*0^{7}$
Fungi (cfu g^{-1})	$1.65**10^{4}$
Actinomycetes (cfu g^{-1})	$2.72*10^{6}$

Table 3. Analysis of variance (sum of square) of the populations of *E .coli*, lactose positive coliforms and lactose negative coliforms (cfu g^{-1}) in soil as affected by soil amendment (AM), soil moisture (SM) and incubation time (IT) [#].

Source of variations	*Df*	*E. coli*	*Lac^{+} coliforms*	*Lac^{-} coliforms*
AM	2	$1.7*10^{10}$ **	$1.1*10^{11}$ ns	$2.6*10^{12}$ **
SM	3	$6.0*10^{10}$ **	$3.5*10^{11}$ **	$7.0*10^{12}$ **
IT	5	$39*10^{10}$ **	$12*10^{11}$ **	$13*10^{12}$ **
AM*SM	6	$0.52*10^{10}$ **	$0.21*10^{11}$ ns	$1.0*10^{12}$ **
AM*IT	10	$8.0*10^{10}$ **	$19*10^{11}$ **	$3.0*10^{12}$ **
SM*IT	14	$14*10^{10}$ **	$11*10^{11}$ **	$7.1*10^{12}$ **
AM*SM*IT	28	$2.1*10^{10}$ **	$4.4*10^{11}$ ns	$9.5*10^{12}$ **
Error	138	$1.2*10^{10}$	$25*10^{11}$	$1.4*10^{12}$

Sum of squares marked by *, ** and *** are significant at P < 0.05, P < 0.01 and P < 0.001, respectively. Sum of squares marked by ns are not significant.

moisture (SM), incubation time (IT) and their interactions. Soil amendment, soil moisture, incubation time and their interactions had strongly significant effects on the populations of *E. coli,* lactose positive and lactose negative coliforms ($p < 0.001$). The effects of incubation time (sum squares) compared to the effect of soil moisture and soil amendment on the population of *E. coli,* lactose positive and lactose negative coliforms were relatively higher. The effect of AM*SM interaction on the population of coliforms was the lowest.

The effects of soil amendment

Table 4 shows the effects of soil amendment on the populations of *E. coli*, lactose positive and lactose negative coliforms in the treated soils. Soil treated with CM compared to soils treated with PM and SS had

Table 4. The effect of soil amendment on the populations of *E. coli*, lactose positive coliforms and lactose negative coliforms (cfu g^{-1}) in soils incubated in different moistures[#].

Soil amendment [##]	*E. coli*		*Lac*$^{+}$ *coliforms*		*Lac*$^{-}$ *coliforms*	
	Mean	SD	Mean	SD	Mean	SD
PM	2.17	2.34	5.30	0.19	5.47	0.58
CM	3.71	1.67	5.23	0.34	5.31	0.42
SS	2.40	2.33	5.15	0.11	5.41	0.45

Values with different character in each column are significantly different at the 0.05 probability level. ## PM- poultry manure, CM- cow manure, SS- sewage sludge.

Table 5. The effect of soil water potential on the populations of *E. coli*, lactose positive coliforms and lactose negative coliforms (cfu g^{-1}) in incubated soils treated with different amendments[#].

Soil moisture[##]	*E. coli*		*Lac*$^{+}$ *coliforms*		*Lac*$^{-}$ *coliforms*	
	Mean	SD	Mean	SD	Mean	SD
DWC	2.02	2.22	5.22	0.21	5.65	0.36
PWP	3.21	2.06	5.13	0.24	5.20	0.51
FC	2.63	2.21	5.26	0.22	5.26	0.41
SAT	3.07	2.31	5.29	0.26	5.52	0.53

Values with different character in each column 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), SAT- saturation (0 bar).

Table 6. The effect of incubation time on the populations of *E. coli*, lactose positive coliforms and lactose negative coliforms (cfu g^{-1}) in soils treated with different amendments[#].

Incubation time[##]	*E. coli*		*Lac*$^{+}$ *coliforms*		*Lac*$^{-}$ *coliforms*	
Days	Mean	SD	Mean	SD	Mean	SD
0	4.98	0.24	5.41	0.30	5.43	0.21
10	4.77	0.48	5.33	0.26	5.76	0.39
20	4.36	0.53	5.06	0.25	5.71	0.36
40	1.61	1.95	5.18	0.14	5.56	0.36
60	0.83	1.53	5.19	0.14	5.18	0.46
90	0.53	1.21	5.21	0.16	4.83	0.45

Values with different character in each column are significantly different at the 0.05 probability level.

significantly higher *E. coli* population. However, the populations of lactose positive and lactose negative coliforms in soil treated with PM were significantly higher than that in soil treated with CM. The population of lactose negative coliforms in soil treated with SS was significantly higher than that in soil treated with CM.

The effects of soil moisture

Table 5 shows the effects of soil water potential on the populations of *E. coli*, lactose positive and lactose negative coliforms in the treated soils. Soils incubated in PWP and SAT compared to those incubated in FC and DWC had higher *E. coli* populations may be due to unsuitable condition. Soil incubation in SAT and PWP conditions may reduce the level of negative interactions between *E. coli* and other soil microorganisms. Soil incubated in FC and especially in DWC conditions had significantly lower *E. coli* populations compared to those in other conditions. However, the population of lactose positive coliforms was higher in soils incubated in SAT and FC conditions compared to those in soils incubated in DWC and PWP conditions. The population of lactose negative coliforms was higher in soils incubated in DWC and SAT conditions.

The effects of incubation time

Table 6 shows the effects of incubation time on the populations of *E. coli*, lactose positive and lactose

negative coliforms in the treated soils. The population of *E. coli* decreased continuously from $9.55*10^4$ cfu g^{-1} to 3.39 cfu g^{-1} in 90 days of incubation. It may be related to negative interactions between soil microbial populations and OC mineralization. The decrease of *E. coli* population was significantly higher at early stage of soil incubation. However, the differences between *E. coli* populations during 90 days of soil incubation were significant ($p < 0.05$).

The population of lactose positive coliforms decreased significantly in 20 days of soil incubation. The lowest population of these bacteria obtained in 20 days of soil incubation ($1.15*10^5$ cfu g^{-1}). After that it increased but not significantly (Table 6).

The population of lactose negative coliforms increased (from $2.69*10^5$ to $5.75*10^5$ cfu g^{-1}) in 10 days of soil incubation significantly. After that it decreased continuously. The lowest population of these bacteria obtained in 90 days of soil incubation ($6.76*10^4$ cfu g^{-1}).

The effect of water potential on survival of *E. coli*, lactose positive and lactose negative coliforms in soils treated with PM, CM and SS

The effect of soil water potential on *E. coli* surviving time strongly depended on the type of organic waste applied in soil (Figure 1). The survival of *E. coli* in soil treated with PM was near 40 days. It did not depend on the soil water status. However the population of *E. coli* was obviously high in soil treated with PM and incubated in SAT condition in the early time (0 to 10 days) of soil incubation.

The duration of *E. coli* survival in soil treated with CM was longer than those in soils treated with PM and SS. The survival of *E. coli* strongly depends on the water potential in soil treated with CM. The population of *E. coli* was obviously high in soil treated with CM and incubated in SAT condition in the early stage of soil incubation (0 to 20 days). It decreased in 40, 60 and 90 days of incubation but did not reach zero during 90 days of soil incubation in SAT condition. Surprisingly the decrease of *E. coli* population in soil treated with CM and incubated in PWP condition did not decreased so much during 90 days of soil incubation. It did not reach zero during 90 days of soil incubation in PWP condition. The duration of *E. coli* survival in soil treated with CM and incubated in FC and DWC conditions were 90 and 60 days respectively. Drying and rewetting cycles reduced the survival of *E. coli* in soil treated with CM.

The dependence of *E. coli* survival in soil treated with SS on water potential was relatively low. The duration of *E. coli* survival in soil treated with SS and incubated in SA, FC and DWC conditions were near 40 days. However the population of *E. coli* was obviously higher in soil treated with SS and incubated in SAT condition compared to those in soil incubated in other moisture conditions in the early time (0 to 20 days) of soil incubation. Surprisingly the survival of *E. coli* in soil treated with SS and incubated in PWP condition was (60 days) higher than those in these soils incubated in other moisture condition.

The effect of soil water potential on survival of lactose positive coliforms did not depend on the type of organic waste applied in soil (Figure 2). The population of lactose positive coliforms in soil treated with PM, CM and SS was nearly constant during soil incubation. The changes of lactose positive coliforms populations in soil treated with PM and SS were similar in different moisture contents (Figure 2) . It was higher in soils incubated in FC and especially in SAT in the early stages of soil incubation (0 to 20 days). But it decreased more in soil incubated in SAT condition. The population of lactose positive coliforms in soil treated with CM was significantly higher than those in soils treated with PM and SS at the start of soil incubation may be due to higher population of these bacteria in CM. However CM-borne lactose positive coliforms decreased in soil treated with CM in the early stages of soil incubation (0 to 20 days) obviously. After that, it reached to the numbers in soils treated with PM and SS. Here the population of lactose positive coliforms were also higher in soil incubated SAT condition (Figure 2).

Figure 3 shows the survival of lactose negative coliforms in soils treated with PM, CM and SS and incubated in different water condition. The changes in populations of lactose negative coliforms were higher than those of lactose positive coliforms. However, same as the population of lactose positive coliforms, the population of lactose negative coliforms did not decreased so much during soil incubation. The decrease of these bacteria population same as that of lactose positive coliforms was very lower than the decrease of *E. coli* population during soil incubation. In SAT condition, the population of these bacteria increased in soils treated with PM, CM and SS in early stages of soil incubation but it decreased with increasing incubation time (Figure 3) . In the late stages of soil incubation, the populations of lactose negative coliforms were higher in soil incubated in DWC condition compared to those in soils incubated in other moisture conditions.

Correlation analysis

Table 7 shows correlation coefficient between some soil biological properties and the populations of *E. coli*, lactose positive coliforms and lactose negative coliforms. Correlation coefficients of the population of *E. coli* with the population of lactose positive coliforms, basal respiration and organic carbon content in soil were positive and significant at 0.01 levels. The population of *E. coli* had positive and significant correlation with the population of lactose negative coliforms and substrate

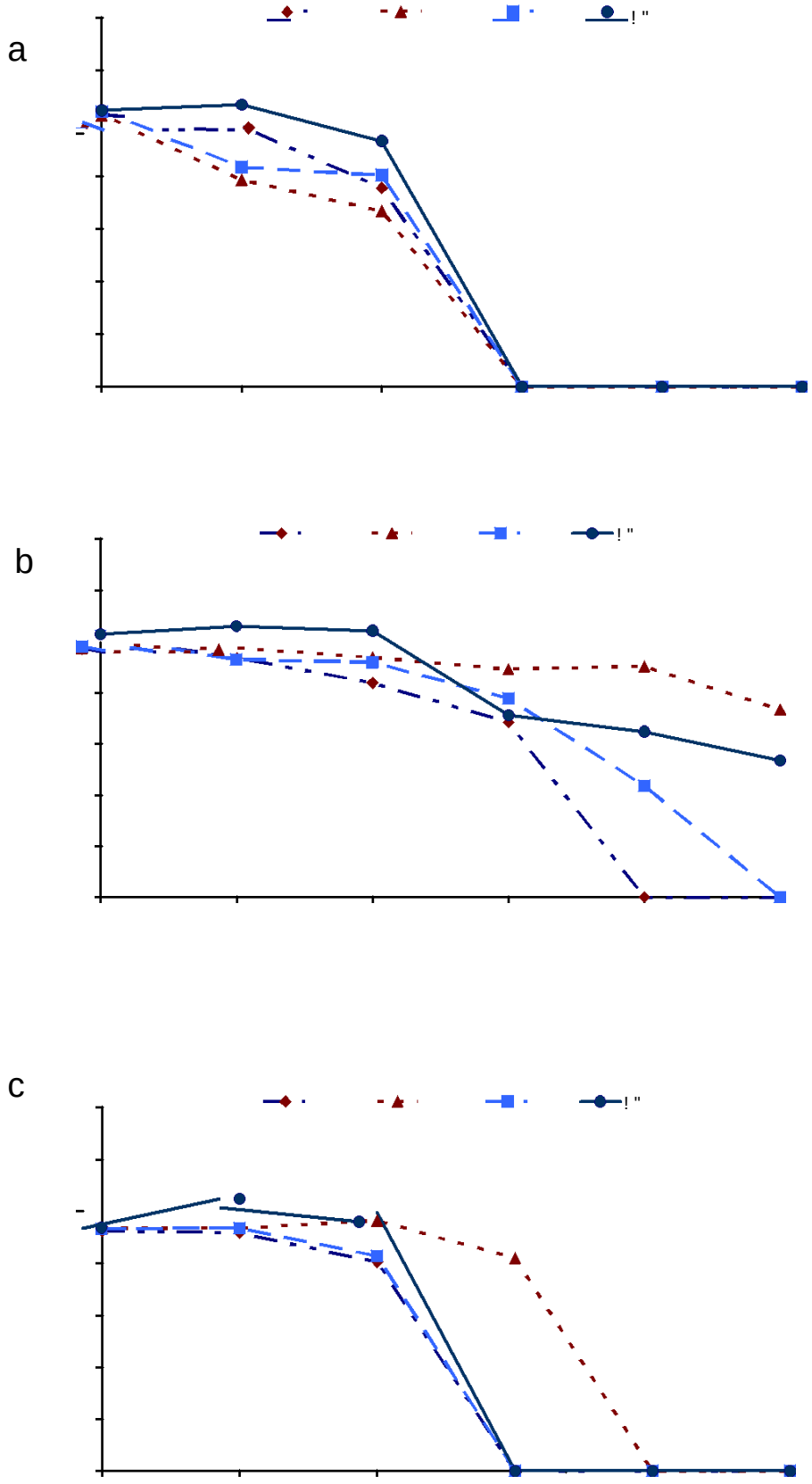

Figure 1. Survival of *E. coli* in soil treated with (a) poultry manure, (b) cow manure and (c) sewage sludge incubated in different water condition.

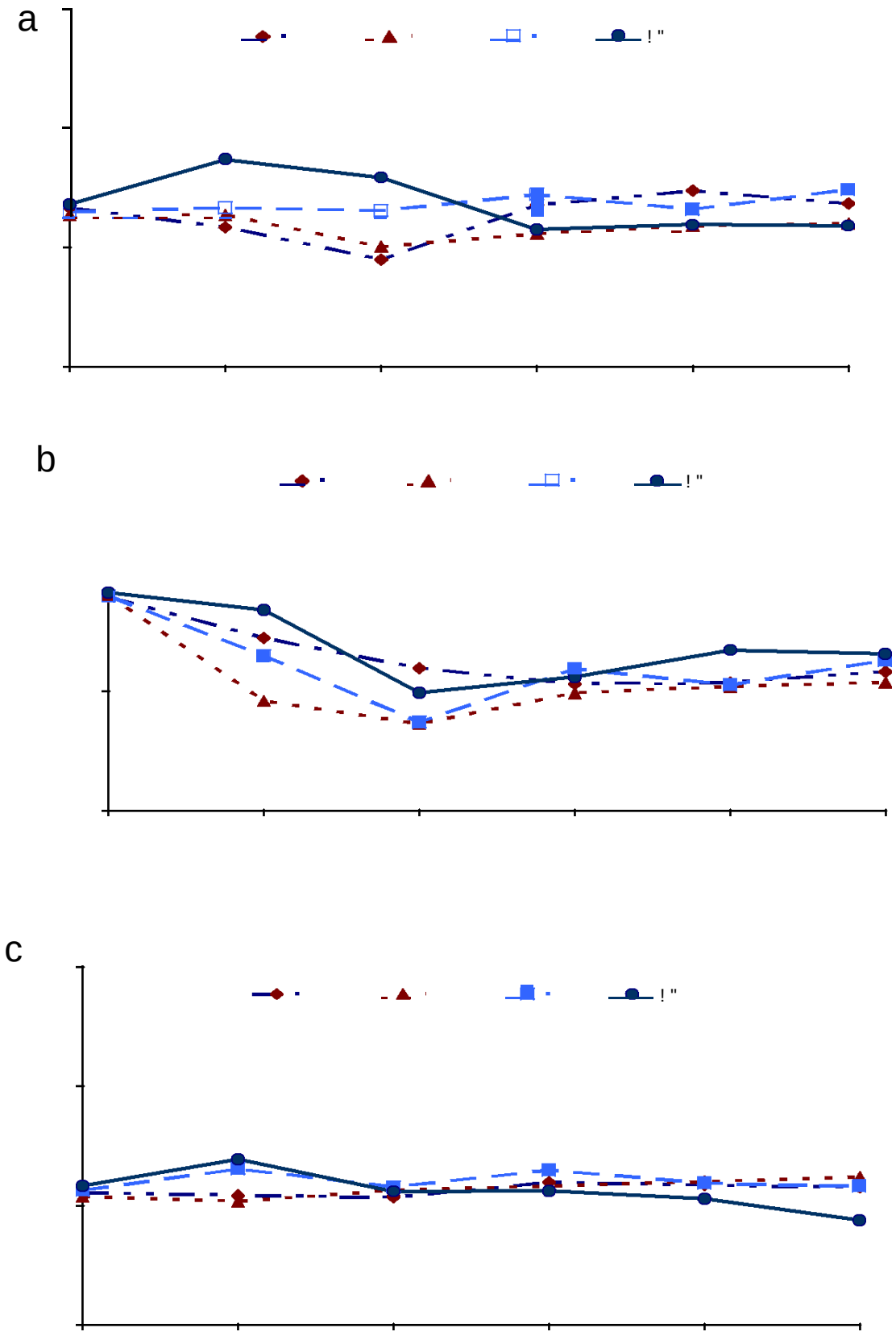

Figure 2. Survival of lactose positive coliforms in soil treated with (a) poultry manure, (b) cow manure and (c) sewage sludge incubated in different water condition.

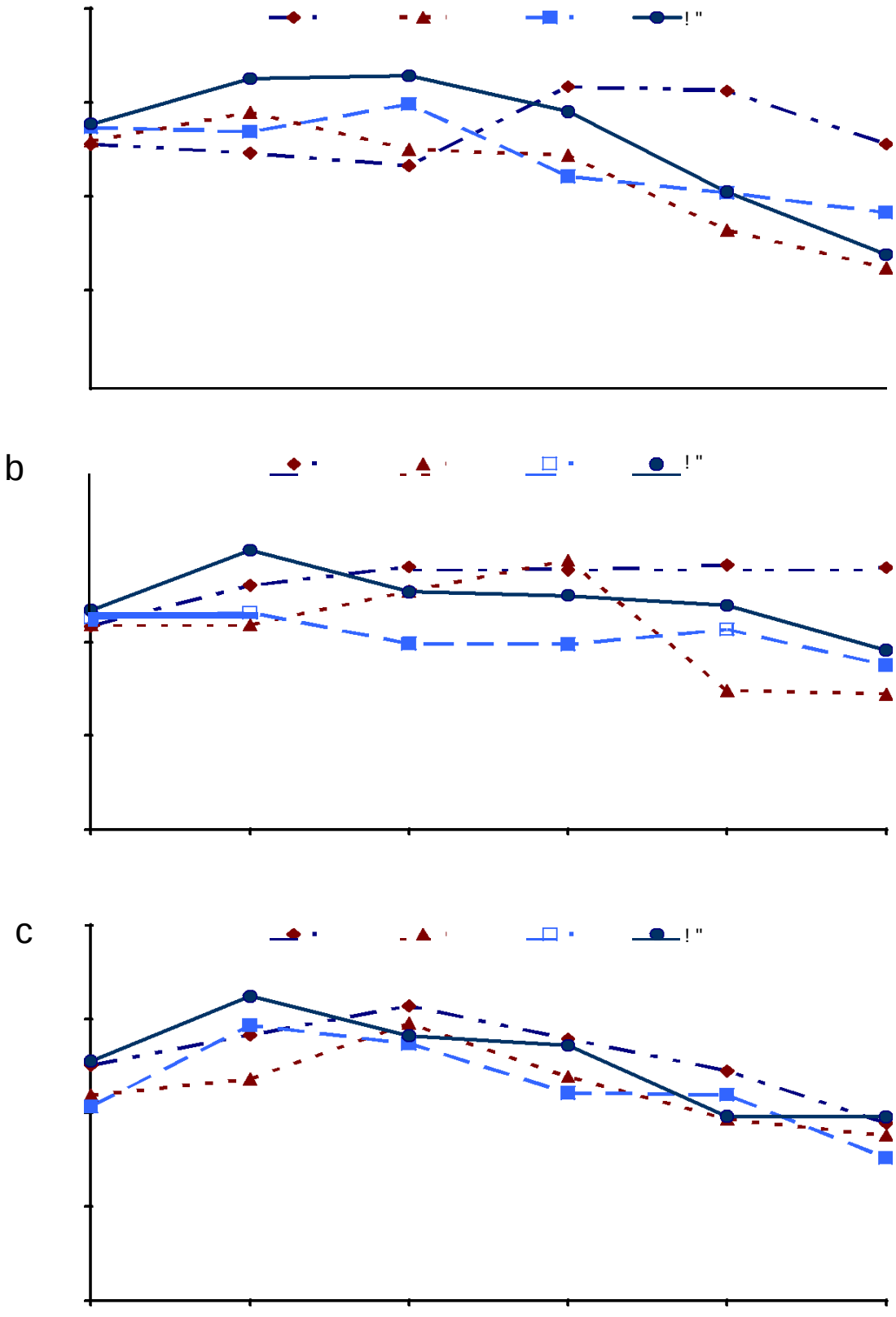

Figure 3. Survival of lactose negative coliforms in soil treated with (a) poultry manure, (b) cow manure and (c) sewage sludge incubated in different water condition.

Table 7. Pearson correlation coefficients of the populations of *E. coli*, lactose positive coliforms and lactose negative coliforms with some biological properties of organic waste treated soil[#].

	E. coli	***Lac$^+$ coliforms***	***Lac$^-$ coliforms***
E. coli population	1		
Lac+ coliforms population	0.53**	1	
Lac- coliforms population	0.31 *	0.22	1
Bacteria population	0.17	0.20	-0.03
Fungi population	-0.20	-0.09	-0.1
Actinomycetes population	0.21	0.1	0.05
Basal respiration	0.49 **	0.46 **	0.22
Substrate induced respiration	0.30 *	0.24	0.39 *
Organic carbon	0.49 **	0.20	0.03

Correlation coefficients marked by *, ** and *** are significance at P < 0.05, P < 0.01 and P < 0.001, respectively.

induced respiration ($p < 0.05$). The population of lactose positive coliforms only had a positive and significant correlation with basal respiration ($p < 0.01$). The correlation of the population of lactose negative coliforms and substrate induced respiration was positive and significant ($p < 0.05$). The correlations between the populations of soil bacteria, fungi and actinomycetes with the populations of *E. coli*, lactose positive coliforms and lactose negative coliforms were not significant.

Conclusion

The result showed that the populations of *E. coli*, lactose positive and lactose negative coliforms were significantly higher in CM compared to PM and SS. However the populations of actinomycetes and especially fungi were significantly high in SS may be due to higher resistance of these microorganisms to higher level of heavy metals (Khan and Scullion, 2000; Rajapaksha et al., 2004). It was reported that dairy cattle in particular have been identified as a principal carrier of pathogenic *E. coli* O157:H7 (Hancock et al., 1994), and *E. coli* O157:H7 resides much longer in manure than in the live animals, and manure-contaminated materials are, therefore, thought to be a source for re-infection of livestock with *E. coli* O157:H7 (Kudva et al., 1998).

Soil treated with CM compared to soils treated with PM and SS had significantly higher *E. coli* population. However, the PM treated soil had relatively higher population of lactose positive and lactose negative coliforms. The duration of *E. coli* survival in soil treated with CM was longer than those in soils treated with PM and SS. Unc and Goss (2006) found that culturability of the indicator organism, *E. coli*, changed with time and was dependent on the type of manure used and its interaction with soil. *E. coli* could be cultured for a longer time from soils with liquid manure additions. Whereas *E. coli* numbers were initially higher from soils treated with solid beef cattle manure, their numbers decreased more rapidly and the duration of their apparent survival was shorter. The effect of soil water potential on the survival of *E. coli* strongly depended on the type of organic waste applied in soil. The survival of *E. coli* in soil treated with PM and SS was near 40 days. It did not depend on the soil water status. However, the survival of *E. coli* strongly depends on the water potential in soil treated with CM. The *E. coli* population in soil treated with CM and incubated in SAT and PWP conditions did not decreased so much during 90 days of soil incubation. It has been reported that pathogen survival time in the soil varies from 4 to 160 days (Sjogren, 1994; Abu-Ashour et al., 1994), and first reflects the organism's ability to respond to nonparasitic and adverse environmental conditions. Obligate parasites usually only live a few minutes outside the host, but many pathogenic organisms can live in groundwater and soil for months (Sorber and Moore, 1987; Entry et al., 2000). Wang et al. (1996) reported that *E. coli* O157:H7 survived in manure for 70 days at 5°C, 56 days at 22°C, and 49 days at 37°C. Cattle feces are known to be relatively higher in moisture content (80 to 85%) and lower in the content of easily biodegradable material than those of grain-fed animals such as poultry or pigs, which makes it difficult to meet the thermal death point for pathogens during the process without appropriate moisture adjustment treatment (Hanajima et al., 2006).

The changes in populations of lactose negative coliforms during soil incubation were higher than those of lactose positive coliforms. In SAT condition, the population of lactose negative coliforms increased in early stages of soil incubation but it decreased with increasing time of incubation. In the late stages of soil incubation the populations of lactose negative coliforms were higher in soil incubated in DWC condition compared to those in soil incubated in other moisture conditions. Reduced survival rates for *Salmonella* under low moisture levels were demonstrated in soil (Zibilske and Weaver, 1978; Chandler and Craven, 1980), and for *E. coli* as well (Tate, 1978; Mubirui et al., 2000). However,

higher population of lactose negative coliforms in soils incubated in DWC condition is in accordance with Bernstein et al. (2007) report. It was reported that that the ability of *Salmonella enterica* serovar Newport to withstand desiccation, as reflected by its long persistence in dry potting medium, is in agreement with a previous laboratory study demonstrating survival of *S. enterica* strains for 46 days following desiccation (Breeuwer et al., 2003). It is possible that *Salmonella* enters a viable but not culturable state, which enables its survival for long periods and allows bacterial re-growth when the supply of water resumed. Similarly, a tolerance to desiccation was previously shown for *Salmonella* serovar Thompson (Brandl and Mandrell 2002) and was suggested to be responsible for its ability to recover efficiently from water stress in the phyllosphere, following hydration. *E. coli* as well was shown to have ability for regrowth when the soil was re-moistened following 14 day in dry soil (Chandler and Craven, 1980).

The effects soil treatments and water potential on the *E. coli* population compared to the other coliforms were higher. The correlation coefficient of the *E. coli* population compared to the populations of the other fecal coliforms with some biological properties of soil was higher. These results show that *E. coli* replies to changes in soil properties better than other coliforms. Survival of *E. coli* were near 40 days in the soils treated with PM and SS. The duration of *E. coli* survival in soil treated with CM strongly depends on the soil water potential. *E. coli* could survive in the soils treated with CM and incubated in SAT and PWP for more than 90 days may be due to low level of negative interactions in these unsuitable water conditions.

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