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Effect of irrigation on the survival of total coliforms in three semiarid soils after amendment with sewage sludge

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Abstract

Sewage sludges are increasingly used in soil amendment programmes, although not without risk since they contain, among other potential hazards, high concentrations of total coliform bacteria. In this paper we have studied the effect of irrigation on the survival of total coliforms in three semiarid degraded soils with different agricultural practices. Fresh sewage sludge was added at 50 g kg^{-1} soil, and incubated in both the presence and absence of irrigation. The absence of irrigation led to a sharp decrease in the number of total coliforms in all soils, with the bacteria disappearing in 40 days. Irrigation produced a substantial initial increase in the number of coliforms in the three soils, although after 80 days there was none growing in any of the soils. The results showed that there were significant differences in the survival of coliform bacteria due to the presence or absence of irrigation.

1. Introduction

The application of sewage sludge to soil constitutes a modification of many physical, chemical and biological properties of soil (Clapp et al., 1986). Especially important is the effect on the microbiological properties of soil, including the quantities of bacteria and fungi present, as sludge contains between 100 and 1000 times the microorganisms, while soil–sludge has considerable influence on microbial activity (Estrada et al., 2004). Some of the microorganisms present in sewage sludge can be pathogens. In this sense, one of the most dangerous problems that may arise from the addition of sewage sludge to a soil, especially with an agricultural use, is the incorporation of pathogenic organisms (Sequi and Petruzzelli, 1978; Pepper and Gerba, 1989; Vasseur et al., 1996; Gibbs et al., 1997) that are not commonly found in soils. This is the case of total coliform bacteria; this group of bacteria includes some genus of pathogens about which little information exists concerning their survival in soils of agricultural use. Information is even more scarce under semiarid conditions. In such environments, the availability of water in soils can be very scarce and may be a key factor in the loss of coliform bacteria. These microorganisms are extremely sensitive to losses of humidity and this fact implies that the storage and aeration of sludges reduce their numbers (Surampalli et al., 1994). Similarly, the incorporation of sewage sludge into semiarid soils could reduce the number of total coliform due to the loss of moisture in the sewage sludge. The survival of bacteria in soil depends of many parameters such as temperature, moisture, pH, soil composition and the presence of other microorganisms (Wessendorf and Lingens, 1989; Mawdsley et al., 1995).

The objective of this paper was to determine the survival of total coliform bacteria of sewage sludge when incorporated into different agricultural soils, with or without irrigation.

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2. Material and methods

2.1. Soils and sewage sludge

Three soils from different agricultural areas were chosen for experimentation under greenhouse conditions. The soils have different uses and physicochemical characteristics, and were collected from the topsoil (0–25 cm) (Table 1) in southeastern Spain. This is a semiarid zone whose annual rainfall is below 300 mm and annual mean temperature is above 19 °C. Such conditions lead to an important hydric deficit. Soils were used immediately after collection without storage.

Microbial analysis did not detect total coliforms in these soils. The main characteristics, location and agricultural use of the soils, are shown in Table 1.

The sewage sludge used for the experiment was established by anaerobic digestion and came from the wastewater plant at Rincón de León in the city of Alicante. This sludge had been previously characterised by taking monthly samples over a period of 2 years to determine its compositional stability. No significant changes were observed to occur (García-Orenes, 1996); its physicochemical characteristics are summarized in Table 2. The content of total coliform bacteria in the sewage sludge before being added to the soil was 2×10^7 CFU (colony forming units) g^{-1} of sewage sludge (dry weight); this value is similar to values used in other studies on the presence of total coliform bacteria in municipal wastes (Hassen et al., 2001). In this experiment, a certain quantity of dry sludge equivalent to 50 g kg^{-1} soil was added to the three soils, as fresh sewage sludge at 85% moisture (333 g of fresh sludge kg^{-1} soil).

Table 2
Main characteristics of the sewage sludge used in the experiment

Parameter	Sewage sludge
Total organic matter (g/kg)	560
N Kjeldahl (g/kg)	30
EC 1:5 mS/cm	5.2
pH 1:2.5	6.3
P (mg/kg)	1790
K (mg/kg)	2600
Na (mg/kg)	700
Ca (mg/kg)	49400
Mg (mg/kg)	5600
Fe (mg/kg)	9700
Cu (mg/kg)	272
Mn (mg/kg)	115
Zn (mg/kg)	905
B (mg/kg)	79
Cd (mg/kg)	4.1
Cr (mg/kg)	12.5
Hg (mg/kg)	0.9
Ni (mg/kg)	17.8
Pb (mg/kg)	1.9
Total coliform (CFU/g)	2×10^7

2.2. Experimental design

For this study, a 3×2 factorial arrangement representing three sludge–amended soils and two treatments (irrigated or not) were established. Moreover, the effect of time after sludge application on the coliforms survival was assessed through several weekly samplings.

The experiment was carried out under greenhouse conditions, with a temperature between 20 and 30 °C and a relative humidity of 60%, and during 4 months (March-June). A series of 180 containers, each containing about 1 kg of soil, were prepared for each type of soil, 90 with irrigation and 90 without irrigation. In total, 540 destructively sampled containers were prepared for the three soils. The cylindrical containers had a capacity of 1 L and free drainage. The leachate was negligible. Six containers were sampled destructively at each sampling time, with up to 15 samplings. For the irrigation treatment, the water content was controlled by calibrated tensiometers (Irrometer ISRA model ILT) placed in some containers. These containers were chosen according to their position in the greenhouse, and the mixtures were maintained at 25% of field capacity,

Table 1
Main characteristics of the three soils used in the experiment

Parameter	Soil 1	Soil 2	Soil 3
Location	38°11′N–1°5′W	37°57′N–0°59′W	38°40′N–0°45′W
Agricultural use	Unirrigated tree crop	Irrigated horticultural crop	Unirrigated olive tree crop
Soil classification (Soil Survey Staff, 1998)	Xeric Torriorthent	Xeric Torrifluvent	Sandy Typic Xerofluvent
Sand (%)	11.5	24.8	80
Silt (%)	55.2	62.6	15
Clay (%)	33.3	12.6	5
Field capacity (%)	63	38	20
CEC ($cmol^+/kg$)	15.5	13.5	8.3
EC 1:5 mS/cm	0.21	2.37	0.15
pH 1:2.5 (water extracted)	7.9	7.4	8.1
Total organic matter (g/kg)	11.7	2.5	1.5
Total coliform (CFU/g)	<bdl*	<bdl*	<bdl*

bdl*: below detection limit (10 CFU g^{-1}).

which is a typical condition in many Spanish semiarid agricultural areas, by adding the corresponding amounts of distilled water. The tensiometers were checked for accuracy every week.

2.3. Sampling procedures and analysis

Samples were taken weekly, six soil samples per treatment. Sampling ceased when two consecutive samples showed no change in coliform bacteria.

To determine the total coliforms in the sludge amended soils, a soil/peptone water (1:10) extraction was made by stirring continuously for 2 h; subsequently a series of dilutions were made. Finally, samples were spread on petri dishes using Agar-VRBD (Merck) as culture medium, as described by Mossel et al. (1962) and incubated for 21 ± 3 h at a temperature of 37 ± 3 °C. The detection level for this method is roughly 10 CFU g^{-1}.

As a check of the enumeration method, several soil/sludge mixtures were sterilized by autoclaving at 121 °C for 21 min. Then they were inoculated by a known number of cells of a pure culture of *E. coli* (strain k12), provided by the Genetics and Microbiology Department of Alicante University (Colon Valiente, 1990). All cells inoculated were later recovered, indicating that the enumeration procedure is proper. Because the check was done with a different microbial population than was measured in the soil/sludge mixtures, the check cannot ensure that the total coliform values are without bias.

2.4. Statistical analysis of data

The survival of coliforms in sludge-amended soils was analyzed using two-way repeated measures ANOVA. The effects of time (samplings; within subject effects) and soil type and treatment (irrigation or not) were evaluated (between-subject effects) at $P < 0.05$. Sphericity (Mauchly's test, W) was not assumed ($P < 0.05$), and thus, Huynh-Feldt's epsilon was used. To improve normality and homocedasticity, the data of coliforms n_i in soils were transformed by $X_i = \log(n_i + 1)$ prior to the statistical analysis. All statistical analyses were performed using the SPSS 11.5 package.

3. Results and discussion

Results show that either irrigation or its absence is the most important factor on the survival of total coliform bacteria in soils amended with sewage sludge (Table 3). Coliform bacteria from soils without irrigation had a similar behaviour; the coliform counts fell sharply (30 days) in the three soils and the viability of total coliforms disappeared by day 33 of incubation for soil 1 and on day 40 for soils 2 and 3 (Fig. 1).

In the soils subjected to irrigation, an important growth of coliforms was observed in the three types of soils from the beginning of the experiment. The highest growth was observed in soil 1 and the lowest growth observed in soil 2 (Fig. 1). This may be a result of the high salt content in this soil, which would result in greater bacterial lysis. The

Table 3
Results of the two-way repeated measures ANOVA

Source	Type III Sum of squares	df	Mean Squares	*F*	*P*
Between-subjects effects					
Soil type (S)	7.8	2	3.9	1059.9	0.000
Irrigation (I)	242.7	1	242.7	65593.6	0.000
S * I	3.6	2	1.8	491.0	0.000
Within-subjects effects (Huynh-Feldt's epsilon)					
Time (T)	766.3	2.7	281.8	2316.2	0.000
T * Soil	25.7	5.4	4.7	38.8	0.000
T * Irrigation	142.6	2.7	52.4	431.1	0.000
T * S * I	22.0	5.4	4.0	33.3	0.000

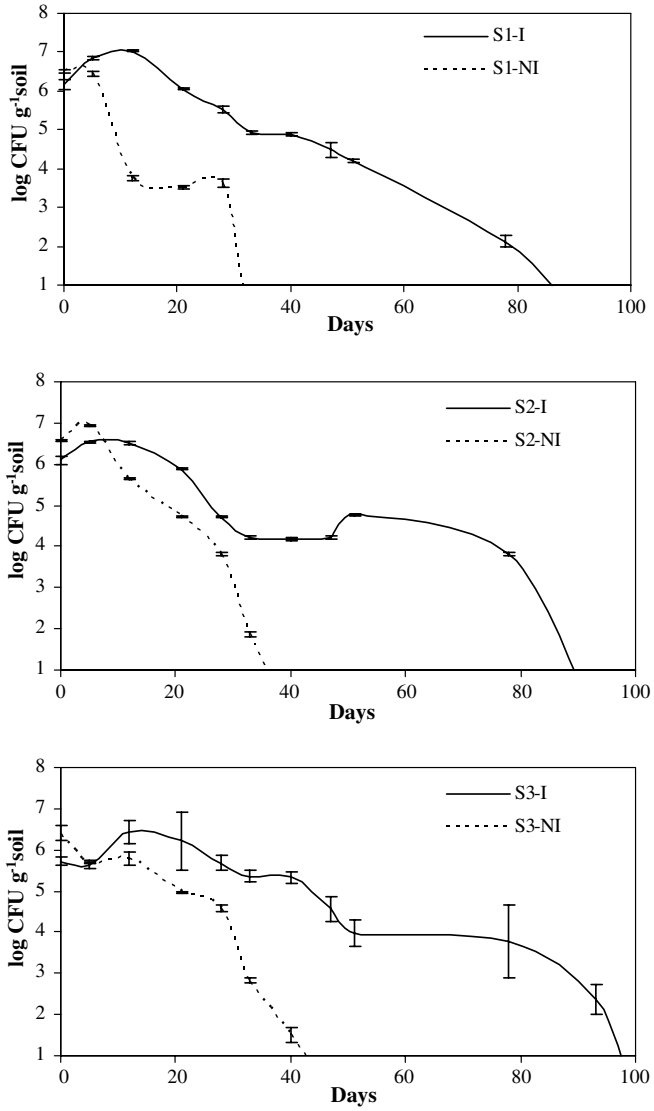

Fig. 1. Evolution of total coliform bacteria (log CFU g^{-1} dry soil) in soils 1 (S1), 2 (S2) and 3 (S3). Irrigated: I; Non-irrigated: NI. Error bars represent standard deviation.

low growth observed in soil 3 may have been due to the texture of this soil. It has a high percentage of sand, which means a very low field capacity, and as a consequence a lower availability of water for the bacteria. It appears that the addition of sewage sludge to these semiarid, irrigated soils does not entail any long-term risk of contamination by total coliform bacteria, since the soils are below 10 CFU g^{-1} of soil in less than 100 days. These results are in accordance with those of Ecosteguy et al. (1993), who studied the viability of total coliform bacteria in one soil amended with sewage sludge, observing a 200-fold decrease in their numbers after the addition of the waste to the soil. Similar results were obtained by Estrada et al. (2004) after 80 days of experimentation in soils treated with different types of sewage sludge, who showed that the population of faecal coliform and *E. coli* were undetectable in some treatments.

The disappearance of coliform bacteria from soil treated with sewage sludge is due to temperature, water availability, pH and the environmental conditions of the soil. This disappearance occurs much more rapidly in the absence of water, since coliform bacteria have no known drought-resistance mechanism unlike most other soil bacteria (Griffin, 1985). This explains the major importance of irrigation noted in this experiment. Under the conditions of this experiment, we have found that the presence of irrigation is the most important factor for the growth of total coliform bacteria. Under these conditions the influence of the type of soil in growth of coliform does not seem very clear. Other works, under field conditions, show that the soil type should be a very important factor to be considered when sewage sludge is applied. Vasseur et al. (1996) found that the presence of total coliform bacteria in a forest soil amended with sewage sludge was influenced by the type of sewage sludge and the forest soil. Other effects such as soil temperature, solar irradiation and competition with other typical soil microorganisms must be studied under field conditions because these factors could lead to different results on the survival of total coliform bacteria after land application of sewage sludge. In addition, the survival of total coliform bacteria will not necessarily describe the survival of pathogenic microorganisms. Consideration of the risks from non-coliform microorganisms is beyond the scope of this research.

4. Conclusions

Our results suggest that there is no permanent risk of coliform contamination when applying sewage sludges to semiarid degraded soils during regeneration programmes. It is important to recognise that this study only considered total coliform bacteria and did not consider specific pathogenic microorganisms. The presence or absence of irrigation is the most important factor for the growth of total coliform bacteria. Under drought conditions, to which they are normally subjected under semiarid conditions, their numbers rapidly decline. In agricultural soils, they also rapidly disappear in drought conditions. However, these are not usually the conditions to which such soils are usually exposed, since they are normally irrigated, in which case the coliform bacteria may be present for up to 100 days. The soil type and use must be taken into account in order to determine if certain crops (depending on the type of soil and use) could be more susceptible than others to be contaminated by coliform bacteria.

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