TOTAL AND FAECAL COLIFORM BACTERIA PERSISTENCE IN A PIG SLURRY AMENDED SOIL

B. Rufete, M.D. Perez-Murcia, A. Perez-Espinosa, R. Moral, J. Moreno-Caselles, C. Paredes
Department of Agrochemistry and Environment, Miguel Hernandez University, EPS-Orihuela, Ctra Beniel Km 3.2, 03312-Orihuela (Alicante), Spain.

ABSTRACT

Pig slurries are a suitable source of nutrients for the soil-plant system, despite this practice could be a pathway for a biological hazard into the soil and the food chain. The aim of this experiment was to study the effect of the application of pig slurry on potential pathogen (total and faecal coliform bacteria) presence in a Typic Xerofluvent soil. The experiment was conducted in field conditions, in the Segura river valley in the South East of Spain. Four treatments, in a fully randomised design with three replicates per treatment, were established in experimental plots of 16 m^2 each one. Fertiliser treatments were: MF, mineral fertiliser (with a complex 16 N-16 P_2O_5-16 K_2O) equivalent to 150 kg N ha^{-1}; PSF, organic fertilisation with swine manure slurry, at two different rates (supplying 150 kg N ha^{-1}, and 210 kg N ha^{-1}), and C, a control treatment without fertilisation. Samplings at 0, 5, 13, 25, 36, 50, 64, 88, 109 and 175 days after the treatment applications were carried out. A high spatial and temporary variability was detected, probably due to the high number of parameters that affect soil microorganisms. The pig slurry amendment induced the highest initial and also persistent presence of total and faecal coliform bacteria. The higher application rate seemed to induce a long-lasting persistence on the total, but especially in the faecal coliform population, in the amended soils compared to the lower one. In most cases, an increase in the pathogen bacteria content was observed in the soils amended with mineral fertiliser compared to control soils. In general, total coliform bacteria were between 2-3 $\log_{10}$ units higher than the faecal ones. Mostly, a decreasing tendency with time was observed in both pathogens in all the treatments.

INTRODUCTION

Faeces, urine, uneaten food and bedding from intensively farmed pigs are usually collected as slurry and stored in lagoons, pits or above-ground tanks until fields conditions are suitable for application to agricultural land (Turner and Burton, 1997). However, animal wastes may contain pathogenic organisms and contribute to agricultural nonpoint source pollution (Reddy et al., 1981). Microbial loads in excess of the levels allowed in human wastes have been found in land-applied liquid animal wastes (Crane et al., 1983). Faecal bacteria can also contaminate streams and groundwater via runoff and leaching from agricultural areas if management is improper (Crane et al., 1983; Meinhardt et al., 1996). Coliforms are usually present in pig slurries and despite different authors reported that the survival time of coliforms in soils are relatively short (1-2 months), a regrowth of these pathogens into the amended soils is possible. Gibbs et al. (1997) concluded that soil amended with biosolids could not be considered free from pathogens for at least one year following amendment. Low temperatures, high humidity and neutral soils generally help the growth and survival of enteric bacteria. The aim of this experiment was to study the effect of the application of pig slurry on potential pathogen (total and faecal coliform bacteria) presence in a Typic Xerofluvent soil with a high intensive agricultural use.

MATERIALS AND METHODS

The experiment was conducted in field conditions, in the Segura river valley in the South

East of Spain. Four treatments, in a fully randomised design with three replicates per treatment, were established in experimental plots of 16 m^2 each one, with a 0.5 m distance between plots. Fertiliser treatments were: MF, mineral fertiliser (with a complex 16 N-16 P_2O_5-16 K_2O) equivalent to 150 kg N ha^{-1}; PSF, organic fertilisation with swine manure slurry, at two different rates (supplying 150 kg N ha^{-1}, and 210 kg N ha^{-1}), and C, a control treatment without fertilisation. Samplings at 0, 5, 13, 25, 36, 50, 64, 88, 109 and 175 days after the treatment applications were carried out. The main characteristics of the soil and the pig slurry applied are shown in table 1.

Table 1. *Main characteristics of the soil and the pig slurry applied.*

Soil		Pig slurry	
pH	8.79	pH (20º C)	7.80
Electrical conductivity (dS m^{-1})	0.21	Electrical conductivity (dS m^{-1})	19.46
Sand (%)	35.2	Suspended solids (mg L^{-1})	10250
Silt (%)	34.9	Redox Potential (Eh) (mV)	- 280
Clay (%)	29.9	Density (g cm^{-3})	1.012
Texture	Clay Loam	Dry matter (g L^{-1})	12.9
Total $CaCO_3$ eq. (%)	36.1	BOD_5 (mg O_2 L^{-1})	5000
Active $CaCO_3$ eq. (%)	10.1	COD (mg O_2 L^{-1})	16613
Organic oxidizable C (g kg^{-1})	8.33	Kjeldahl N (mg L^{-1})	2125
Kjeldahl N (mg kg^{-1})	1190	Ammonium N (NH_4^+-N) (mg L^{-1})	1767

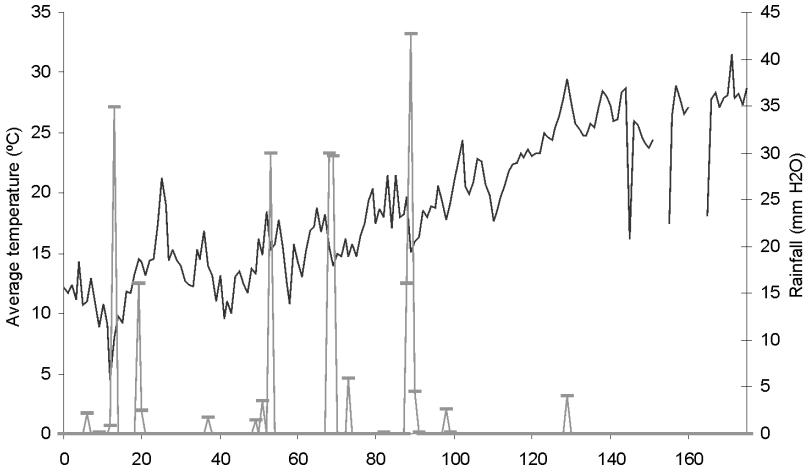

Figure 1. *Average air temperature and rainfall during the field experiment.*

Total and faecal coliforms were determined in soils using the procedure proposed by Estrada et al. (2004). A soil sample of 10 g dry weight was added to 90 mL of solution (peptone water), the resultant 1:10 suspension was homogenized by magnetic stirring and used to make a set of dilutions in peptone water. The dilution levels were chosen to obtain 30-300 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 referred to the dry soil weight of each sample. Each population culture medium used for general enterobacteriaceae was violet-red bile agar with glucose and incubated during 21 ± 3 h at 37 ± 1ºC. For faecal coliforms the temperature of incubation was 44 ± 1ºC.

RESULTS AND DISCUSSION

In figure 1, environmental conditions (air average temperature and rainfall) during the field experiment are shown. The experiment was developing between February and July of 2003, being observed a regular increment of the average temperature with time throughout the experiment. In addition to this, the main rainfall events in the experimental area (SE Spain) usually occur in autumn and in spring, like in our experiment, from day 0 to 90.

Presence and persistence of total and faecal coliforms depending on the treatments are shown in figures 2 and 3. A high spatial and temporary variability was detected, probably due to the high number of parameters that affect soil microorganisms. The pig slurry amendment induced the highest initial and also persistent presence of total and faecal coliform bacteria. The higher application rate seemed to induce a long-lasting persistence on the total but especially in the faecal coliform population, in the amended soils compared to the lower one. In general, total coliform bacteria were between 2-3 $\log_{10}$ units higher than the faecal ones. Mostly, a decreasing tendency with time was observed in both pathogens in all the treatments.

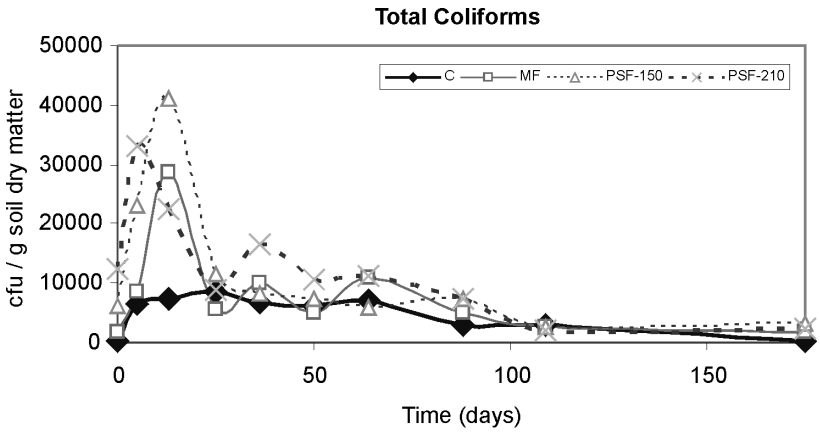

***Figure 2**. Total coliform count in treated soils.*

The survival of bacteria in the soil depends on many parameters, such as temperature, moisture, pH, soil composition and the presence of the other microorganisms. In accordance with the environmental conditions (higher temperature and lower humidity of the soil due to the decrease of the number and quantity of the rainfall events), a stabilisation of the coliform count was observed in all treated soils from day 90 until the end of the experiment. Low temperatures help the survival of bacteria in soil (Gerba et al., 1975), with survival levels tending to decrease with the increases of the temperature. Changes in soil conditions (e.g. temperature and humidity) are expected to affect the structure and the function of microbial communities (Van Gestel et al., 1993). In most cases, an increase in the pathogen bacteria content was observed in the soils amended with mineral fertiliser compared to control soils. This effect was lower than the observed in the pig slurry amended soils.

Persistence of total and faecal coliforms in the experiment decreased with time during the experiment. Zhai et al. (1995) reported a substantial reduction (greater than 99%) of faecal coliform and faecal streptococci concentrations after 2 weeks in a incubation experiment with poultry manure. Estrada et al., (2004) in a field experiment with sewage sludge-amended soils found that 80 days after the application of sludge, the populations of micro-organisms in the soil

were below detections limits for most populations studied. In our experiment the faecal bacteria concentrations were approached detection limits for enumeration from day 109.

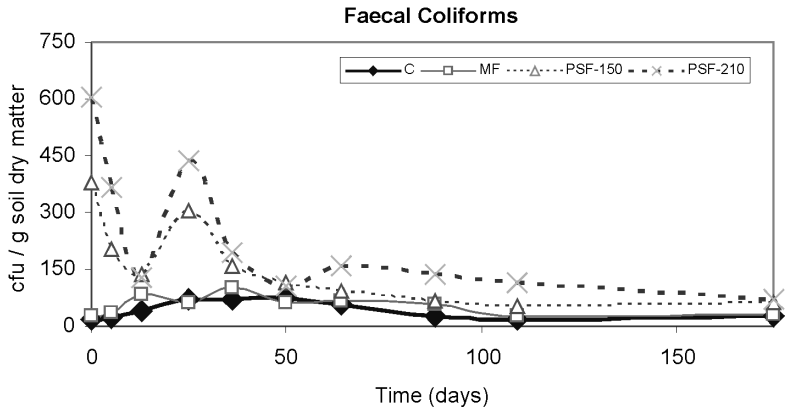

Figure 3. *Faecal coliform count in treated soils.*

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