AGRONOMIC USE OF PIG SLURRY FOR BROCCOLI PRODUCTION: INVESTIGATION OF PATHOGEN MICROORGANISMS

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ABSTRACT

In the past few years there has been an increasing public concern for the contamination of water, food and air by pathogens residing in manure, the subproducts of manure and bioaerosols.

The objective of this work was to study the microbial load of pig slurry, which is used as an organic fertilizer for broccoli cultivation, and its possible relationship with the microbial content of the crop.

The research was carried out in a 2,300 m^2 plot cultivated with broccoli. This plot is located in the "Centro Integrado de Formación y Experiencias Agrarias (C.I.F.E.A.) in Lorca, province of Murcia (Spain). For a the two years study, the site was fertilized with different quantities of pig slurry. Samples of pig slurry and crops were analyzed for the most representative microorganism (Coliforms, Aerobic Mesophile, moulds and yeasts, etc.) and also for the pathogens microorganisms *Salmonella, Shigella* and *Escherichia coli.*

All the slurry samples analyzed contained high levels of total Coliforms (ranged from 10^3 to 10^5 coliforms/mL pig slurry), but most of the samples did not present faecals coliforms. Three of the eight samples analyzed showed presence of *Salmonella. Shigella* was not found in any of the samples.

The analysis carried out in plant-samples showed total coliforms, but at much lower levels than the samples of pig slurry (ranged from 10^1 to 10^2 coliforms/g of broccoli). However, total coliforms were observed, both in control plants (without pig slurry application) and in crops with pig slurry doses. None of the samples contained food-borne pathogens *Salmonella*, *Shigella* and *E. coli*.

Therefore, could not be found a direct correlation between coliforms and pathogens present in the pig slurry and in the crops.

Keywords: *pig slurry, broccoli, microorganisms, soil.*

INTRODUCTION

The recycling of pig slurry as a natural fertilizer is an important alternative in countries such as Spain. Spain has a Mediterranean climatology an arid and semiarid character and soils with a low content in organic matter (Delgado et al., 2002).

Acea and Carballas (1998) studied the microbiology of soils exposed to forest fire and concluded that the addition of animal or plant residues has been shown to change some characteristics of the soil microbial community, even in unheated soils.

Land application of manure is an important component of livestock production systems. Management options available to livestock producers may be employed to reduce environmental risks. However, the effects of different manure application practices on pathogen persistence are not well documented. Information on the persistence and transmission risk of pathogens in the soil as affected by different manure application rates will allow for more informed management decisions (Gessel et al., 2004).

The objective of this research was to study the microbial load of pig slurry, which is used as an organic fertilizer in broccoli cultivation, and the possible relationship with the microbial load in the crop.

MATERIAL AND METHODS

For this experiment, a rectangular area of 2,300 m^2 was cultivated with broccoli of the variety "Decatlon". This plot is placed in the "Centro Integrado de Formación y Experiencias Agrarias (C.I.F.E.A.)" in Lorca, province of Murcia.

This plot is characterised by an edaphic homogeneity, whose soils are included in the Calcaric Fluvisols unit (F.A.O-I.S.R.I.C-I.S.S.S, 1998), developed on alluvial quaternary calcareous silty sediments. The study area was divided into four parts, each receiving a different amount of pig slurries. The amounts were as follows: part A: blank; part B: 4.86 l/m^2; part C: 11.05 l/m^2; part D: 14.86 l/m^2.

At the beginning of this experience, the application of 4.86 l/m^2 of pig slurries was carried out in parts B, C and D. This addition was previous to broccoli plantation, while the following applications were done later along with the flood irrigation.

One sample was taken from pig slurry in each application, and 80 samples were randomly chosen from the crops, for microbiological analysis. Data presented here include analyses performed on soil samples taken at 0-30 cm prior to slurry application and after each slurry application until the end of the plant cycle. Soil samples at greater depths (30-60 cm) were taken before the initial application of slurry and again after the final harvest.Microbiological analysis for each soil sample, were performed according to the following methodology (APHA, 1998):

- Bacteria, in Tryptic Soy Agar (TSA) agar at 31 ºC during 48-72 h.
- Actinomycetes, in Actinomycetes-agar at 20 ºC during 5-7 days.
- Fungi, in Potato Dextrose Agar (PDA) + oxytetracicline at 20 ºC during 5-7 days.
- Coliforms, in Brilliant Green Bile Lactose Broth (BGBL2%) at 37 ºC during 48 h, with the Most Probable Number (MPN) method.

Microbiological analysis for each pig slurry sample included the microbial groups described previously and the following (APHA, 1998):

- Faecal coliforms: by subculturing positive tubes of total coliforms in BGBL2%, with incubation at 44.5 ºC / 24-48 h.
- *Salmonella* and *Shigella* by classical methods of determining presence / absence in 25 g o mL of sample

Microbiological analysis for each crop sample, included the microbial groups described previously and the following (ICMSF, 2000):

- faecal streptococci (D group of Lancefield): in culture medium kanamycine-esculin-azide broth (KAA) and confirmation in KAA agar.
- Sulfit-reducing clostridia: in sulfit-polymyxin-sulfadiazin agar (SPS), and incubation up to 37 ºC / 24-48 h. in anaerobic conditions.
- *Escherichia coli* by subculturing positive tubes of faecal coliforms in EMB agar and biochemical tests

RESULTS AND DISCUSSION

Microbiological analysis of pig slurries show high levels of microorganisms that are a risk for the contamination of soils. The crops that are being produced in these soils can eventually be contaminated by the microorganisms present in these livestock manures. Table 1 shows average values of microbiological load of pig slurry samples analysed in the first year of study of this research. Results obtained during the second year were similar.

***Table 1**. Microbiological properties from pig slurry.*

MICROORGANISMS	Application 1	Application 2	Application 3
Bacteria (cfu/mL)	$8.00 \cdot 10^6$	$1.23 \cdot 10^7$	$1.30 \cdot 10^7$
Fungi (cfu/mL)	$8.80 \cdot 10^3$	$3.67 \cdot 10^4$	$4.00 \cdot 10^4$
Actinomycetes (cfu/mL)	$1.55 \cdot 10^6$	$9.50 \cdot 10^6$	$2.00 \cdot 10^7$
Coliforms (MPN/mL)	$3.00 \cdot 10^3$	$1.10 \cdot 10^4$	$4.60 \cdot 10^4$
Faecal coliforms (MPN/mL)	< 30	< 30	$4.60 \cdot 10^4$
Salmonella	P	P	A
Shigella	A	A	A

Significant differences on the counts of the different microbial groups were not found after applying the pig slurry on the soil, except for the coliforms, which increased dramatically with the pig slurry dose.

Soil bacteria levels kept constant during the study, with values around 10^6 cfu/g, both the first and the second year. Actynomecetes behaved similarly, also with counts around 10^6 cfu/g, and also fungi, but with values around 10^4 cfu/g.

Coliforms increased with the dose of pig slurry, from less than 30 (MPN/g soil) before any pig slurry was applied to about 1100 MPN/g after the third application. However, after harvesting the crops, the level of coliforms dropped to the original low numbers, below 30 MPN/g.

The analysis carried out in plant-samples showed total coliforms, but at much lower levels than the samples of pig slurry (ranged from 10^1 to 10^2 coliforms/g of broccoli). However, total coliforms were observed, both in control plants (without pig slurry application) and in crops with pig slurry doses. None of the samples contained food-borne pathogens *Salmonella*, *Shigella* and *E. coli*.

This observation leads to the conclusion that enteric bacteria do not survive to the environmental conditions of soil and, hence, autochthonous microflora persists. These results would support the application of pig slurry for cultured soils, because even if there are pathogens in the pig slurry, when harvesting the crops the probability of survival is remote. To hold previous statements, none of the 80 samples collected from broccoli were positive for *Salmonella, Shigella* or *E. coli.*

In summary, soil fertilisation with pig slurries is possible as it improves the properties of soils and the crop production (data not shown of this same investigation) and it does not seem to be any risk of contamination of the harvested crops with pathogens.

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