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Soil Textural Inactivation of Residual Faecal Indicator Organisms in Bio Slurry Used for Carrot Production

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ABSTRACT

A screen house pot study using bio-slurry at the rate of 7.8 t N / ha was conducted at Makerere University Agricultural Research Institute (MAURIK) Kabanyolo, Uganda. This was monitored using fecal indicator organisms (coliforms, *Escherichia coli* and *Enterococci*) in loamy sand, sandy loam and sandy clay loam texture obtained at the study site within 5litres pots under a Complete Randomized Design under a screenhouse. The different textures significantly ($p<0.05$) reduced the fecal indicator organisms. Conditions within the loamy sand texture reduced the pathogenic microorganisms within 90 days due to its high sand content. In this soil texture, *E. coli* and enterococci were reduced to undetectable levels within 90 days unlike the coliforms.

Keyword: Fecal, Bio slurry, coliforms, *Escherichia. coli*, *Enterococci*

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INTRODUCTION

The increasing use of biogas for fuel has given rise to the levels of bio-slurry as a by-product. If not, properly handled bio-slurry can be a nuisance to the environment due to its residual pathogens (Neve et al., 2012) and high nutrient loads that could end up causing pollution (Ni et al., 2012). However, in sub-Saharan Africa, where low soil fertility is a major challenge to crop production (FAO 2017), bio-slurry can be used as an organic fertilizer. This will reduce expenses in purchasing fertilizers, which most small-scale farmers who dominate crop production in sub-Saharan Africa cannot afford (Mwirigi et al., 2014). However, it could contain residual enteric pathogens that survived the process of digestion through biodigesters making it non-sanitized. Bio slurry could contain residual protozoa, bacteria, fungi, and viruses which can cause diseases to humans (Nelson and Murray, 2008). The major species of concern are enteric zoonotic pathogens, such as *Salmonella sp, Listeria spp, Escherichia coli, Bacillus spp, Campylobacter spp, Mycobacteria spp, Clostridia spp, Brucella spp and Yersinia spp* (Sobsey et al., 2006). These pathogens pose a risk to humans through contaminated food and water. The risk is higher where food is likely to be eaten raw, for example, some vegetables. Therefore, it is necessary that residual pathogens within the applied bio-slurry do not end up in the harvested food.

Studies have shown that residual pathogens are inactivated in soil when subjected to environmental stress such as moisture and soil properties such as texture, but the effect is inconsistent (Cools et al., 2001). Soils with high sand texture are considered to contribute the most to the reduction of enteric microorganisms than clay soil types because they are

more susceptible to moisture stress (Fenlon et al., 2000). Clay rather provides more micro habitants from the availability of small pore spaces that protect them from predation and these provide adequate levels of water and nutrients (Cools et al., 2001). It has not been clearly established which soil textural range enhances pathogens to die off. It is important to know the soil textural range that reduces the pathogens by the maximum amount causing less contamination as this will act as a benchmark for controlling the spread of diseases and cycling of pathogens in the environment. Therefore, this study aimed to establish the effect of texture on the inactivation of residual pathogens in bio-slurry for reduced contamination of agricultural produce using residual fecal indicator organisms (FIOs), coliforms, *E. coli*, and *Enterococci*. These are internationally recommended fecal indicator organisms because they are good predictors of the presence and density of other species of human enteric bacteria and pathogens (Standing Committee of analyst, 2003).

METHODOLOGY

Site description

The study was done at Makerere Agricultural Research Institute Kabanyolo (MUARIK) in Central Uganda. MUARIK is in Central Uganda at $32^0$37' East and $0^0$28', at approximately 1,200 m above sea level. The rainfall pattern at MAURIK is bimodal with a mean value of 1,250 mm with peaks in April and November. Mean maximum temperatures vary from 28.5 ^{0}C in January to 26.0 ˚C in July, and minimum temperatures, 17.4 ^{0}C in April to 15.9 ^{0}C in July and August.

Treatments and research design

A screen house experiment was conducted with soils that were obtained from various locations of MAURIK; (i) sandy loam (ii) loamy sand and (iii) sandy clay loam as treatments. 4.5 kg weight of each of the soil textural classes from the same depth was added to 5liter buckets. This study was set up following a Complete Randomized Design (CRD) with three replicates. The buckets were randomly placed in the screen house as a set of 10 pots per replicate, placed in rows at 2 m apart and 60 cm between the buckets within the row. The microbial load of bio-slurry before being applied was determined and the load was above the standards of waste disposal according to WHO (2012). It was applied to the soil surface of each bucket before sowing at a rate of 7.8 t N ha^{-1}.

Carrots seeds were then directly sowed in the pots and thinned to four plants per bucket 1cm apart at two weeks. Soil water content was set to 7 mg in all pots. The pots were weighed at the start of the experiment and during the experiment and adjusted with deionized water three times a week every after two days. The pots were also kept free of weeds by continuously hand weeding to ensure clean pots with no pesticide used. Daily soil temperature was determined in the morning at 6 am and evening at 6 pm during the entire experiment.

Four soil samples of the soils to be used were obtained at a depth of 0-15 cm from the sites where the soil used for this experiment was obtained. The four samples were mixed thoroughly and a composite sample of one kilogram was obtained using the quarter sampling method. The composite sample was air-dried, sieved using a 2mm sieve and then subjected to chemical properties and microbial analyses; soil texture, pH, soil organic matter (SOM), total N, available P available and K. The methods used for physiochemical analysis for organic carbon (Walkley and Black method), texture (hydrometer method procedure of Bouyoucos), available phosphorus (colorimetry method), total nitrogen (Kjeldehal method), exchangeable Ca and Mg (Neutral ammonium acetate method) as described in (Okalebo et al., 2002). The microbial parameters were analyzed by obtaining a 10g soil sample and placed in the filter bag to which 90 ml sterile water was added. Further ten-fold dilutions were prepared to obtain readable counts of Coliforms*, E. coli* and *Enterococc*i which were enumerated using Idexx Quanti-Tray the Colilert and Enterolert most probable number (MPN) method described by Idexx, UK. The soil analysis results for the tested parameters are shown in Table 1.

Data collection

Sampling for pathogen changes was done at the 4^{th}, 8^{th} and 12^{th} weeks (30, 60 and 90 days) after planting. At week 4, the soil closest (0.01cm) to the roots was sampled and at weeks 8 and 12, five carrots were randomly sampled from the middle three pots and placed in sterile filter bags. The pathogen (*E. coli*, *Enterococci,* and coliforms) population was then determined by rinsing the soil off the carrots in the filter bag with 100 ml of sterile water then 1: 2 (V/V) dilutions were performed. This was done by adding 50 ml of the rinse to 50 ml of sterile water and shaken (25 revolutions per minute (rpm); 30 seconds). 1 ml is then measured and added to 100 ml of the second dilution to perform a third dilution making 1: 2000 (V/V). This is then analyzed using Idexx Quanti-Tray the Colilert and Enterolert most probable number (MPN) method described by Idexx, UK. However, for the 4^{th}-week sample, 10g of soil close to the carrots was diluted with 90 ml of water in the filter bag giving 1:9 (V/V). Further ten-fold dilutions were prepared as necessary to obtain readable counts of *E. coli* and *Enterococci* enumerated by the methods described above.

Data analysis

All FIOs counts were log_{10}- transformed to normalize

Table 1: Biological, physical, and chemical characteristics of the soil used.

Parameter	Textural class		Sandy Loam	Loamy Sand	Sandy clay loam
	pH		6.2	5.9	4.5
	OM	%	2.16	1.02	2.99
	N	%	0.19	0.08	0.24
	P	mg/ kg	62.3	4.2	16.25
	K	c moles/kg	1.3	0.21	0.65
	Na	c moles/kg	0.1	0.08	0.19
	Ca	c moles/kg	8.4	5.3	3.3
	Mg	c moles/kg	2.4	1.1	1.4
	Sand	%	64	78	54
	clay	%	10	5	32
	Silt	%	26	17	14

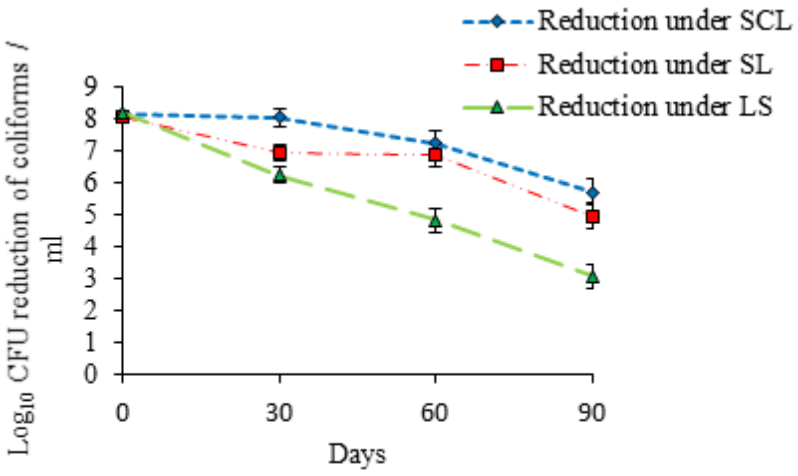

Figure 1: Reduction of coliforms under different soil texture.

the data. The data were then analyzed for variance by soil type on FIOs counts using GenStat 12th edition. A multi-conditional analysis of variance was also done to establish significance among the treatments. The significant means were separated using the least significant differences (LSD) at 5% significance.

RESULTS

The soil used in the study had pH values of 6.2, 5.9 and 4.5, nitrogen 0.19, 0.08 and 0.24, respectively. Organic matter was low ranging between 1.0 and 3.0 % in all soil types. Apparently, these soils had a population of indicator pathogens already native in soils and these combined with residual in bio-slurry the *E. coli* in sandy loam, loamy sand and sandy clay loam were: 8.2, 8.4, 8.3 coliforms, 6.7, 5.7, 5.4, and *Enterococci* 7.4, 7.4,7.8, respectively (Table 1).

Reduction of FIOs under different soil textural classes

The coliforms significantly reduced in soils after 90 days bio-slurry application. The reduction was significantly ($P<0.05$) more under loamy sand (LS) compared to sandy loam (SL) and sandy clay loam (SCL) soil textures. The least significant decline was observed under the SCL textural class (Figure 1). The least population of coliforms was still within the detectable range at 1.1×10^3 CFU/ml. Generally, the population of *E. coli* significantly ($p<0.05$) reduced to undetectable levels 90 days after application of bio-slurry in all the soil textural classes. It declined significantly faster in LS

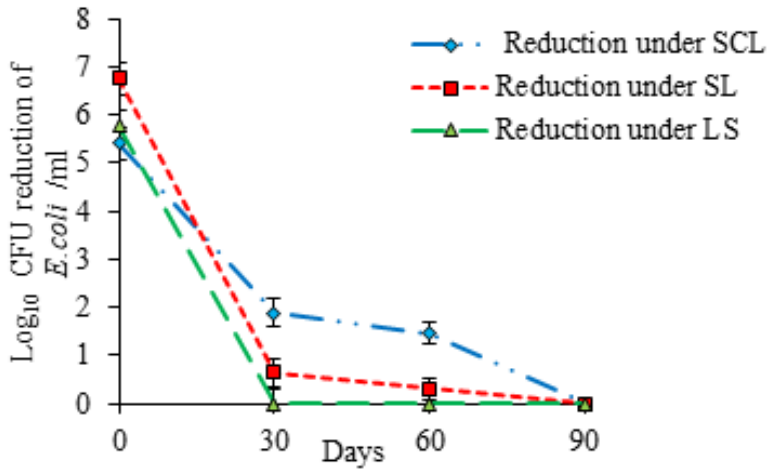

Figure 2: Reduction of *E. coli* under different soil texture.

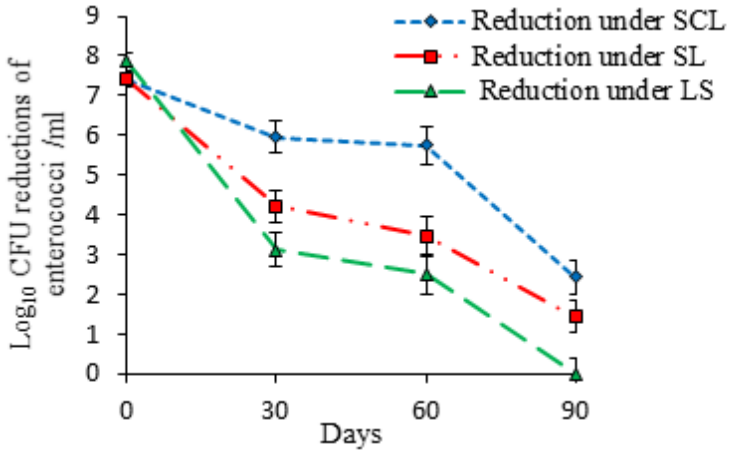

Figure 3: Reduction of enterococci under different soil texture.

textural class than the rest from 5.7 x 10^5 CFU/ ml to undetectable levels within 30 days after applying bio-slurry (Figure 2). This was closely followed by a similar reduction trend under the SL textural class. The least significant rate of reduction was obtained when bio-slurry was applied to SCL textural class. It should be noted that there were undetectable population carrots harvested on the 90th day. The *Enterococci* significantly ($P<0.05$) reduced most under the LS soil textural class compared to SL and SCL. At harvesting time (90 days after planting), LS had a reduced population of enterococcus to undetectable levels unlike SL and SCL (Figure 3). On the other hand, SCL maintained a significantly ($P>0.05$) higher population of *Enterococcus* experiencing the least reduction.

DISCUSSION

The soil characteristics that affect microbial diversity are pH, organic matter, nutrients moisture and texture (Mubiru et al., 2000; Franz et al., 2011). The pH range of 4.5-6.5 is suitable for these pathogenic bacteria and could have had less impact on their reduction. Although the soil organic matter and nutrients were low, the microorganisms should have benefited from the organic matter and nutrients contained in the bio-slurry. Similarly, moisture from the bio-slurry and water used to maintain the experiment provided sufficient moisture for the microorganisms. Therefore, the main factor explaining variability observed in these FIOs trends is texture. Organisms have charged bodies (Unc and Goss, 2004) that attract them to the charge on clay being easily fed on by macrophages thus increasing their deactivation.

Coliforms' significant survival compared to *E. coli* and *Enterococci* under the three soils textural classes was attributed to initial population levels of coliforms in both the bio-slurry (before being applied to the soil) and the soil. There was higher population of coliforms at the

start than *E. coli* and *Enterococci*. The soil was collected from MAURIK with an animal farm being the source of these pathogens. Therefore, the rate of inactivation of high coliforms population is expected to be less than that of *Enterococci* and *E. coli*. Furthermore, coliforms are positively charged microorganisms and positively charged microorganisms are adsorbed onto the soil particles especially with clays due to their divalent cations (Ling et al., 2002). This protects them from the water forces and predation by other microorganisms. However, coliforms later reduced though at a slow rate due to the presence of bio-slurry which provided organic matter. The presence of organic matter in the soil considerably decreases the number of bacteria attached to soil particles by competing with bacteria for the same attachment sites (Guber et al., 2007). Additionally, it could also have been due to microorganisms competing within themselves for attachment sites on the soil.

Within the three different soil textural classes, coliforms survived more in SCL, and SL compared to LS. This is because LS contains less silt (17%) and clay (5%) as coliforms are more associated with clay and silt fractions than with sand particles (Ling et al., 2002). Additionally, majority of microorganisms inhabit the clay fractions of the soil, and this is attributed to the presence of manure, increasing survival in soils with high clay particles (Marschner and Kalbitz, 2003). Therefore, LS texture with more sand (78%) than silt (17%) and clay (5%) fractions enabled the deactivation of coliforms the more.

The faster reduction of *E. coli* population in comparison with *Enterococci* and coliforms could be attributed to their lower initial levels in both the bio-slurry and soil at the beginning of the experiment. The level of initial population of the residual organisms influences its rate of degradation. Low initial population of the indicator organisms with environmental stress and or soil parameters like texture leads to faster reduction. Some fecal coliforms share some characteristics like feeding genre with *E. coli* and hence their ability to compete for the same resources. Besides *E. coli* having a net negative rate of growth in the secondary habitat like soil, its survival is also affected by various stress influences such as variation in soil texture, pH (Johannessen et al., 2005), low organic matter content (Franz et al., 2008), and predation.

It was noted that *E. coli* decreased to undetectable levels under all soil textural classes, suggesting that it exhausted its required bioavailable nutrients supporting its growth and survival. This indicates that soil is not a suitable habitat for *E. coli* and there is less risk of spreading from bio-slurry applied on vegetables. Tentatively, *E. coli* could have become dormant (non-cultivable but viable) during starvation conditions, where it is metabolically active but cannot be cultured in the laboratory (Semenov et al., 2007). Furthermore, *E. coli* in the different textural classes of LS, SL and SCL significantly declined at different rates but surviving more in SCL having 54% sand than SL and LS with 64 and 75% sand, respectively. This difference in survival times in the different soil textures could be due to differing soil abiotic factors like textural effects in soils like aeration and moisture retention (Guan and Holley, 2003). It was noted that *E. coli* reduced most under loamy sand compared to sandy loam and sand clay loam because LS had more sand content, which drains easily thereby creating moisture stress and inactivating the *E. coli* in it. Similarly, loamy sandy soils had more sand, but less silt and clay particles compared to the other soil types. Soils with high sand particles contribute to the low survival of *E. coli* because they do not allow adherence of the organism on them. They also hinder microbial activities through the provision of less micro habitants from small pore spaces that protect them against predation (Nicholson et al., 2005). On the other hand, high clay, and silt particles in the SCL soil increased the persistence and activity of the organisms in this soil type (Cools et al., 2001).

Enterococci survived longer than *E. coli* in different soil conditions because enterococci have greater survival abilities than gram-negative *E. coli* (Bale et al., 1993). *Enterococci* could also be resistant to the lytic effects of bacteriophages and protozoan (Byappanahalli et al., 2012). This study is consistent with Cools et al., (2001) who reported a faster reduction of *E. coli* than *Enterococci* in sandy than clayey soils following application of piggery bio-slurry. Therefore, the application of bio-slurry in the soil posed less risk of spreading pathogens as they were reduced to an insignificant level by the time of harvesting carrots.

CONCLUSION

Soil texture is an important parameter to consider while applying bio-slurry with residual pathogens in the gardens. Conditions within more sand texture above 75% sand reduce some FIOs within 90 days. The *E. coli* and *Enterococci* reduced to undetectable within 90 days unlike the coliforms. This indicates that the other classes of coliforms other than *E. coli* remain a major challenge especially when bio-slurry is applied to vegetable gardens. Secondly, the last dose of bio-slurry should be applied at least 90 days before harvesting a vegetable or at planting for carrots.

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