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Adsorption of Fecal Coliforms, *Escherichia coli*, in Soils of Sarawak

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ABSTRACT

In this study, fecal bacterial adsorption in three different soils (clay loam, silt loam, sandy loam) was investigated. A wild strain of *Escherichia coli* (*E. coli*) isolated from animal wastewater was used in the study. For adsorption kinetics study, adhesion of bacterial cells to soil particles was found to be immediate. Percent adsorption was found to be dependent on the initial concentration of *E. coli*. Results indicate that at low *E. coli* concentrations (below 10^3 cfu/ml), the percent adsorption was very much less in sandy loam than in silt loam and clay loam. However, as the bacterial concentration increased, all the soils tested displayed higher bacteria adsorption with maximum values of 99.9% for clay loam, 99.9% for silt loam and 99.7% for sandy loam. Soil with higher clay content (35.2 % and lower pH (pH 4.5)) had significantly higher capacity for adsorbing *E. coli*. Furthermore, *E. coli* concentration of 10^6 cfu/ml in suspension was found to decrease by 2 orders of magnitude in all the three soils. Therefore, land treatment of wastewater from animal farm lagoons should be considered to reduce microbial contamination of rivers.

Keywords: Adsorption, fecal coliforms, *E. coli*, soils, animal waste

INTRODUCTION

Animal waste is known to be a potential source of bacterial pathogens (Pell 1997). Direct discharge of animal wastewater or lagoon effluent could result in microbial contamination of surface water. Land treatment or utilization provides a viable option in the treatment of animal waste since animal waste is a good source of plant nutrient (Fuller and Warrick 1985). However, knowledge on interaction of bacteria with different types of soils especially in quantitative terms is far from complete. Therefore, further studies need to be conducted to investigate the adsorption of bacteria in different soils, thus avoiding those that are not capable of adsorbing fecal bacteria at waste disposal sites. Due to the difficulties involved in the identification of specific disease-causing bacteria or virus, *Escherchia coli* is widely used as an indicator of fecal pollution. Adsorption of *E. coli* in soil is a complex process. It depends on the surface charge of the organism and soil particles, and the soil solution. According to Marshall (1971), clay and organic matter are key soil components responsible for the adsorption of bacteria.

TABLE 1
Characteristics of the three soils used in the study

Clay (%)	Fine Silt (%)	Medium Silt (%)	Coarse Silt (%)	Sand (%)	Texture	Organic Matter (%)	pH
35.2	10.2	17.6	8.4	28.6	Clay Loam	15.5	4.5
18.6	8.3	47.7	13.0	12.4	Silt Loam	13.3	5.7
6.8	3.6	19.1	11.5	59.0	Sandy Loam	7.3	3.8

Most studies report that microbial adsorption in soil increases with increasing clay content (Ling *et al.* 2002; Weaver *et al.* 1978). Ling *et al.* (2002) studied the adsorption of *E. coli* on two soils of Louisiana and reported that Commerce Clay Loam (35% clay) adsorbed significantly more *E. coli* than Tangi Silt Loam (12% clay). Weaver *et al.* (1978) studied adsorption of bacteria on four different soils of Texas and found that bacterial adsorption increased with increasing clay content. The objectives of this study were to determine the adsorption kinetics of *E. coli* in a soil-water system and to compare the adsorption rates of *E. coli* in three different soils in Sarawak.

MATERIALS AND METHODS

A total of 3 different soil samples were collected around Kuching, Sarawak. Soil pH was determined using a pH meter, while particle-size analysis was carried out using the pipette method (Anon 1992). Total organic matter of the soils was determined by the loss-on-ignition method (Nelson and Sommers 1996).

Escherichia coli was isolated from fresh pig farm wastewater. Identification of the isolates was confirmed by Gram reaction tests and API 20E diagnostic kit (BioMerieux, France). Adsorption studies of *E. coli* on different soils at room temperature (25°C) were carried out according to the weak adsorption method employed by Ling *et al.* (2002). Six milliliters of *E. coli* suspension were shaken with 6 g soil in a 50 ml sterile standard centrifuge tube using EYECA MMS multi-shaker (Rikakikai Co. Ltd., Tokyo). The mixture was then centrifuged at 50G for 3 minutes in EBA-21 centrifuge (Hettich Zentrifugen, Germany). Stokes' Law was applied to determine the G-force required to pellet clay particles of 1 μm effective diameter. For the adsorption kinetics study, sampling was done at 5, 15, 30, 60 and 120 minutes. Bacterial concentration was determined by standard pour plate method. Cultures were grown on EMB media at 37°C for 24 hours. For the batch adsorption studies, different initial concentrations of bacteria ranging from 10^2 to 10^{11} cells per millilitre were used. All the experiments were carried out in triplicates. Computation of adsorption was conducted according to Ling *et al.* (2002). Data were statistically analyzed using SPSS 11.0 (SPSS Inc., USA).

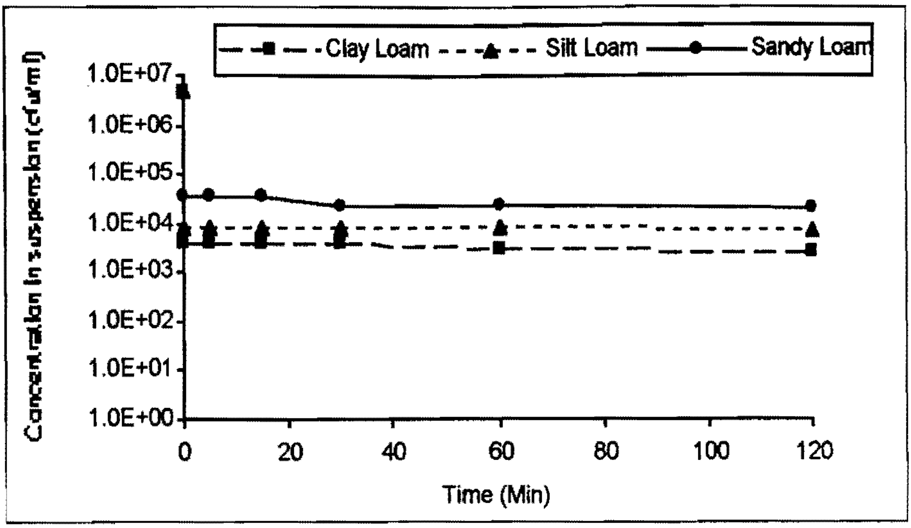


Fig. 1. The depletion of Escherichia coli from suspension in the 3 soils, with initial bacterial concentrations of 4.50 x 10^6 cfu/ml

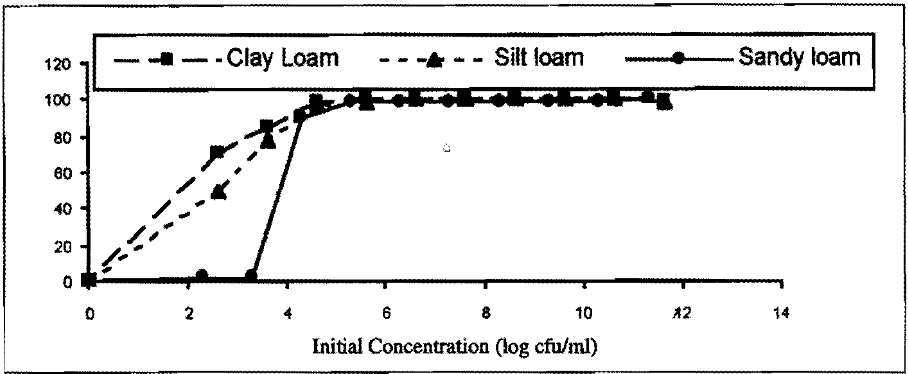
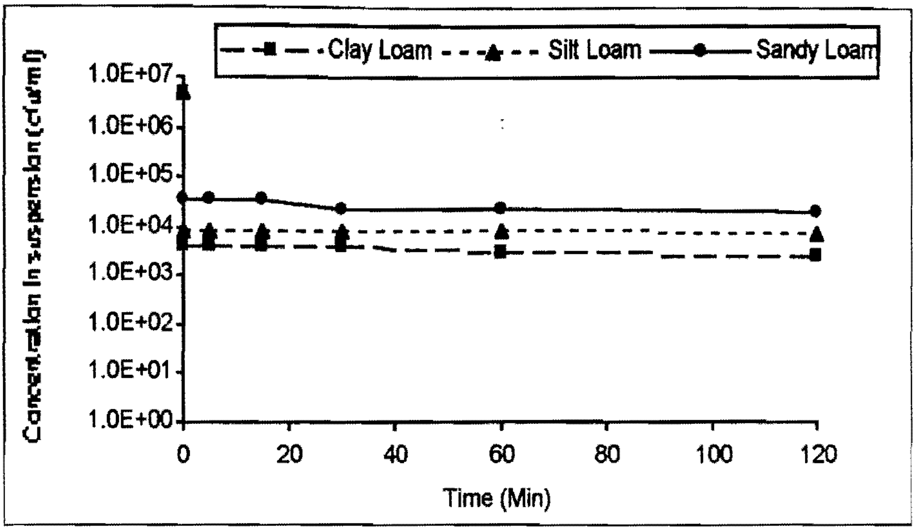


Fig. 2. The absption of E. coli at different initial E. coli concentrations in clay loam, silt loam and sandy loam

RESULTS AND DISCUSSION

Characteristics of the soils used in the study are presented in Table 1. The clay content of the soils ranged from 6.8% to 35.2% and the organic matter ranged from 7.3% to 15.5%. All the soils used in the adsorption studies are acidic, with pH 5.7 or less (Table 1).

The bacteria depletion curve, that is, the number of bacterial cells remaining in supernatant as a function of time is shown in *Fig. 1*. Immediately after inoculation, *E. coli* concentration in the supernatant dropped sharply. The greatest decrease was observed in the clay loam and the least in the sandy loam. In all the soils, the



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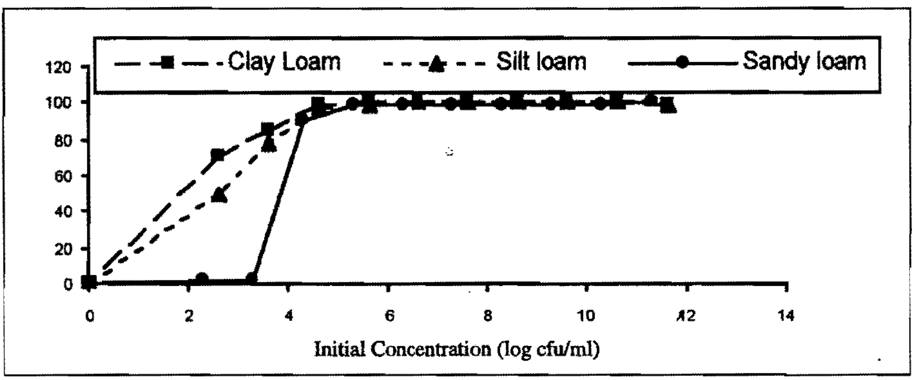

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TABLE 2
Concentrations of *Escherichia coli* in supernatant after 3 seconds at 25°C, with different initial bacteria concentrations from 10^2 to 10^{10} cfu/ml in 3 different soils

Initial *E. coli* concentration (cfu/ml)			Final *E. coli* concentration (cfu/ml)		
Clay loam	Silt loam	Sandy loam	Clay loam	Silt loam	Sandy loam
4.50×10^2	3.30×10^2	1.15×10^2	1.31×10^2	1.97×10^2	1.13×10^2
4.50×10^4	3.30×10^4	1.15×10^4	7.50×10^2	8.50×10^2	9.70×10^2
4.50×10^6	3.30×10^6	1.15×10^6	3.60×10^3	5.90×10^3	7.70×10^3
4.50×10^8	3.30×10^8	1.15×10^8	2.49×10^5	3.80×10^5	4.50×10^5
4.50×10^{10}	3.30×10^{10}	1.15×10^{10}	4.10×10^7	3.30×10^7	4.50×10^7

decrease was about two orders of magnitude from the initial 10^6 cfu/ml. The sharp decrease of *E. coli* concentration in suspension initially was followed by a slight decrease within the 120-minute experimental period. There was no significant drop in concentration of *E. coli* after the initial drop ($P = 0.856$) for the three soils. This indicates that the equilibrium of *E. coli* in water and soil particles is achieved immediately. Hattori (1970) also reported immediate adsorption and proposed that adsorption between bacteria and clay particles proceeds in two steps, a rapid followed by a slow rate. At equilibrium, the concentrations of *E. coli* in the supernatant of the three soils are in the order following order : sandy loam>silt loam>clay loam. This shows that soil of higher clay content adsorbs more *E. coli* compared with soil of lower clay content. Weaver *et al.* (1978) have reported a similar result.

The effect of bacterial inoculum concentration on adsorption is shown in *Fig. 2*. At concentrations below 10^3 cfu/ml, *E. coli* adsorbed was very much lower in sandy loam (below 3%) compared with that in silt loam and clay loam. This is most likely due to the lower clay content in sandy loam compared to clay loam and silt loam. For sandy loam, between 10^3 and 10^4 cfu/ml, there was a sharp increase in adsorption. This seems to indicate the occurrence of multi-layer adsorption. Percent adsorption of *E. coli* in clay loam and silt loam increased linearly with the increase in initial bacteria concentrations until 10^4 cfu/ml. Above 10^6 cfu/ml, all the 3 soils tested in the study displayed high bacteria absorption of over 99%. The maximum mean percent adsorption was found to be 99.94, 99.92 and 99.68% for clay loam, silt loam and sandy loam, respectively.

The concentration of *E. coli* in supernatant with different initial concentrations from 10^2 to 10^{10} cfu/ml is shown in Table 2. For concentrations of 10^4-10^6 cfu/ml, after adsorption, the concentration in supernatant was reduced by two orders of magnitude. For initial concentrations of 10^8-10^{10} cfu/ml, the concentration in supernatant was reduced by three orders of magnitude. This indicates that soils could play a role in reducing *E. coli* concentration in wastewater.

Comparing adsorption results in the present study with those of the studies of Weaver *et al.* (1978) and Ling *et al.* (2002), for a soil of 35% clay content and initial *E. coli* concentration of 10^6 cfu/ml, the clay loam tested in the present study had significantly higher adsorption capacity than the Commerce clay loam and San Angelo sandy clay loam. High bacterial absorption in the present study may be due to the higher organic matter content and lower pH of the soils. Commerce clay loam (Ling *et al.* 2002) has far lower organic matter content (<1%) and also higher pH (pH 5.1) than the soil of this study. Even though Tangi silt loam (Ling *et al.* 2002) has higher clay content (12%) than silt loam of this study, it only adsorbed 38% of the *E. coli*. This is most likely due to the much lower organic matter content (<1%) and also higher pH of Tangi soil. According to Daniels (1980), optimum adsorption of bacterial cells to soil particles generally occurs in low pH conditions. According to Hattori (1970), bacteria-clay complex is more stable in acid condition as compared to alkaline conditions.

CONCLUSION

Sorption equilibrium of *E. coli* cells between water and soil particles was found to be immediate. All the soils tested displayed high *E. coli* removal from suspension. However, the effectiveness of bacterial removal in soil systems is dependent upon the initial concentration and soil properties. Soil with higher clay content showed a higher capacity for adsorbing *E. coli*. The reduction of *E. coli* numbers in suspension by more than 99% obtained in this study shows that soils could play an important role in the reduction of coliform bacteria from wastewater. Adsorption of different types of pathogenic bacteria on different tropical soils should be further investigated.

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