Research article

Changes in *Escherichia coli* Efflux from Farmland by Surface Runoff and Percolation under Different Application Methods of Manure

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Abstract The application of cattle manure in farmlands was considered as a proper treatment from a viewpoint of organic agriculture. However, pathogenic bacteria known as *E. coli* in immature fermented manure happen to spread out from farmlands. So, the objective of this study is to investigate *E. coli* efflux under different application methods of cattle manure. Slope modeling experiment was conducted under an artificial rainfall simulator. Slope plots were filled with soil then fresh cow dung and 2 weeks fermented manures were applied into the plots. Cow dung and manure were applied with 2 application methods: the broadcasting method and the incorporating method. Based on the experimental results, it was found that *E. coli* efflux increased with soil loss through surface runoff. Moreover, it showed that the amounts of *E. coli* efflux were higher than that of input in either plot broadcasted or incorporated with cow dung. In addition, there were tendencies that *E. coli* had been transported by percolation although it was smaller in number than that by surface runoff.

Keywords *Escherichia coli*, total suspended solid, soil erosion, cow dung, manure

INTRODUCTION

Large amount of dung has been produced from cattle farms. So, making manure was considered as one of the proper treatments for utilizing cattle manure from a viewpoint of organic agriculture. However, most of manures produced from cattle farms may include immature fermented manure. The pathogenic bacteria known as *E. coli* may survive and remain in immature fermented manure (Chun-Ming et al., 2005). It was reported by Islam et al. (2005) that *E. coli* applied through immature fermented manure survived in longer days at farmlands. Also, some reports mentioned that the efflux of *E. coli* occurs through surface runoff from grazed land and farmland where cattle manures were applied (Mishra et al., 2007; Yagura et al., 2006) and large amounts of *E. coli* were transported along with soil erosion from farmland where fresh dung was applied (Ishikawa and Mihara, 2010). In addition, *E. coli* efflux remarkably influences environmental pollution in watersheds as well as contamination of potable water source affecting human health (Tamura et al., 2006; Mishina et al., 2007). However, only few studies has been carried out concerning the efflux of *E. coli* from farmlands where immature fermented manures were applied.

In this study, the objectives were to investigate the relationship between the efflux of *E. coli* and total suspended solids under broadcasting and incorporating methods of manures, to evaluate the difference between *E. coli* efflux through surface runoff and that through percolation, and to compare the efflux of *E. coli* with the input amounts of *E. coli*.

METHODOLOGY

Cow dung and manure used in this experiment were collected from Fuji Farm, a cattle farm of Tokyo University of Agriculture located in Shizuoka Prefecture. There were two types of cow dung collected for the experiments, the fresh cow dung (Fig. 1a) and the 2 weeks fermented manure (Fig. 1b) with water contents at 84 and 72%, respectively. For sanitary reasons, collected cow dungs were kept in a close container under anaerobic condition. Organic matters were 89% in fresh cow dung and 87% in 2 weeks fermented manure as shown in Table 1. The colony forming unit (cfu) of *E. coli* was 19×10^6 cfu/g in fresh cow dung and 21×10^3 cfu/g in 2 weeks fermented manure.

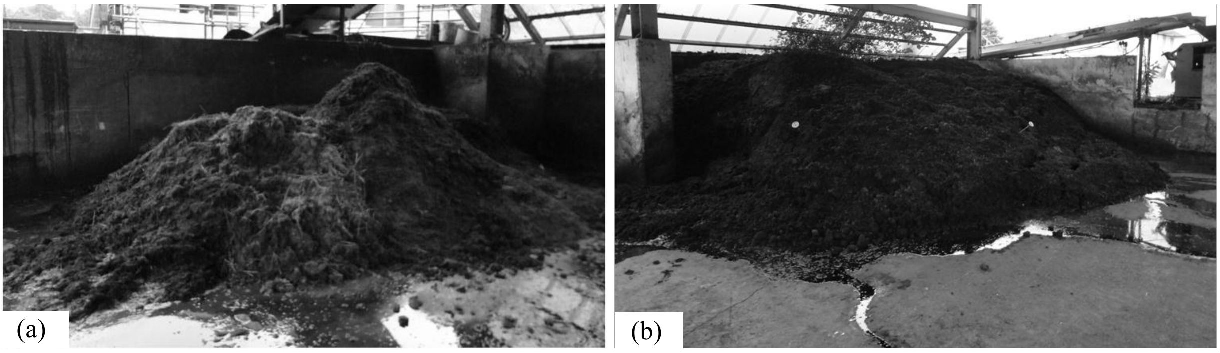

Fig. 1 Piles of fresh cow dung (a) and 2 weeks fermented manure (b) at Fuji Farm

Table 1 Properties of cow dung and fermented manure

	Period of fermentation	*Escherichia coli* (cfu/g)	Water content (%)	Organic matter (%)
Cow dung	0 days	19×10^6	84	89
Manure	2 weeks	21×10^3	72	87

Stainless slope model plots of 130 cm long and 11 cm wide were filled up with light clay soil, andosol, at 5 cm deep (Table 2). The plots were set up at 8 degrees in slope then cow dung and 2 weeks fermented manure were applied. The amounts of cow dung and manure applied into each plot were 1000 g/m^2 (10 t/ha) in dry mass. Both of fresh cow dung and fermented manure were applied by broadcasting method and incorporating method into each designated slope model plot. Broadcasting method is spreading fresh cow dung or fermented manure on soil surfaces, while incorporating method is mixing fresh cow dung or fermented manure with soil. The prepared slope model plots were kept to make soil the 24 hour field capacity moisture before starting the rainfall simulation.

Table 2 Physical properties of soil

Specific gravity	Particle size distribution (%)					Soil texture
	Gravel	Coarse sand	Fine sand	Silt	Clay	
2.68	2.0	12.3	27.8	30.2	27.7	LiC

Table 3 Chemical and biological properties of soil

Ignition loss (%)	Total nitrogen (10^{-5} kg/kg)	Total phosphorus (10^{-5} kg/kg)	*Escherichia coli* (cfu/g)
13.41	226.31	30.36	0

Artificial rainfall simulation was carried out at rainfall intensity of 60 mm/hr for 2 hours. Suspended water samples through surface runoff were collected from each plot (Fig. 2). Suspended water samplings were done every 15 minutes for the first one hour and every 30 minutes for the second one hour of the simulation. Percolation water was sampled only once after 24 hours passed from the end of simulation.

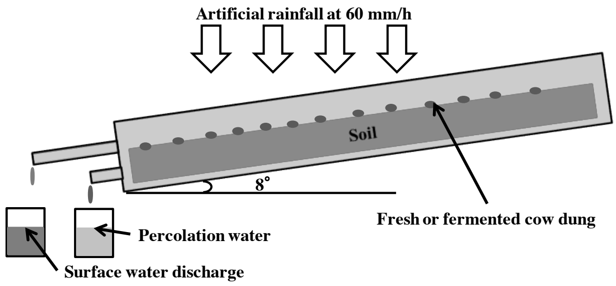

Fig. 2 Slope model plot and suspended water collected by surface runoff and percolation water

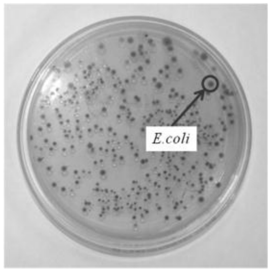

Fig. 3 Propagated *E. coli* by culture media in petri dish

The colonies of *E. coli* in the collected suspended water samples were evaluated through laboratory analysis. The analysis was carried out by bacteria culture medium XM-G as shown in Fig. 3. The mass of solids in surface runoff and in percolation water were also measured by oven drying method.

RESULTS AND DISCUSSION

The relationship between *E. coli* efflux and total suspended solids loss from the plot applied fresh cow dung was summarized in Fig.4. It was observed that *E. coli* efflux significantly increased with total suspended solids in surface runoff from both application methods. A confidence interval at 99% was also observed in surface runoff from the plots broadcasted and incorporated with cow dung. However, *E. coli* efflux tended to be very low in correlation with suspended solid losses for percolation water.

The relationship between *E. coli* efflux and suspended solids loss in the plots applied 2 weeks fermented manure was shown in Fig. 5. There was a tendency that *E. coli* efflux increased with suspended solids losses in surface runoff. It was also observed that *E. coli* efflux was very low in broadcasted plots comparing to that in incorporated plots. The tendency of *E. coli* efflux with suspended solids loss was very low in percolation water. Therefore, it was considered that *E. coli* efflux had a remarkable tendency to be transported along with suspended solids loss for surface runoff, but that tendency was not clear for percolation water.

Additionally, Fig. 6 shows the total input and efflux of *E. coli* through surface runoff or percolation from the plots applied cow dung. The experimental results of fresh cow dung indicated that the number of *E. coli* efflux was higher than that of input in both broadcasting and incorporating methods. However, the significant difference was not observed between the input and the efflux of *E. coli*. In the plots applied 2 weeks fermented manure, it was observed that *E. coli* efflux was higher than that of input in incorporation methods as shown in Fig. 7. Although a significant difference was not observed, the number of *E. coli* efflux from incorporated plot was higher than that from broadcasted plot. The tendency of *E. coli* efflux being higher than that of *E. coli* input was considered that *E. coli* may be facultative anaerobic pathogens which may propagate for a certain period after applying into an open land. Hence the condition before applying *E. coli* was an anaerobic then it changed to aerobic condition after applying.

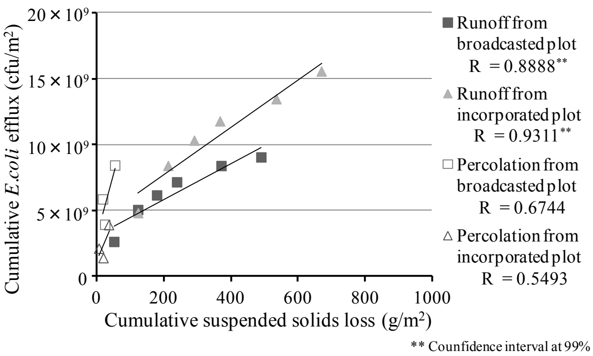

Fig. 4 Relationship between *E. coli* efflux and suspended solids loss from plots where fresh cow dung was applied

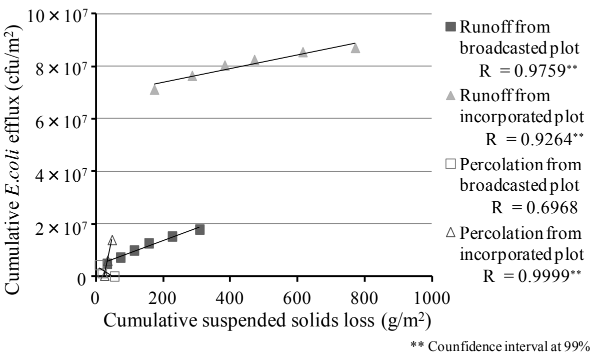

Fig. 5 Relationship between *E. coli* efflux and suspended solids loss from plots where 2 weeks fermented manure was applied

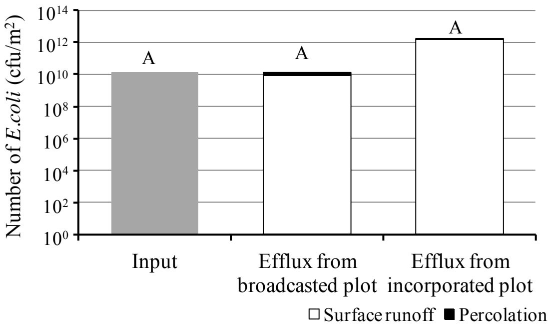

Fig. 6 Number of *E. coli* input in fresh cow dung and *E. coli* efflux through surface runoff and percolation

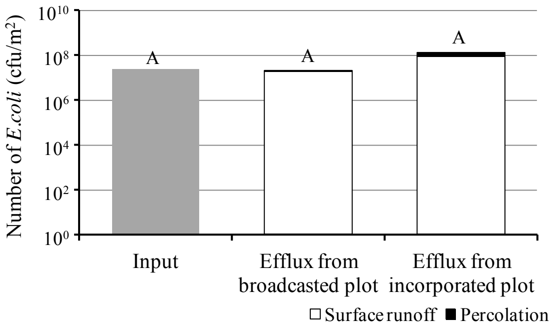

Fig. 7 Number of *E. coli* input in 2 weeks fermented manure and *E. coli* efflux through surface runoff and percolation

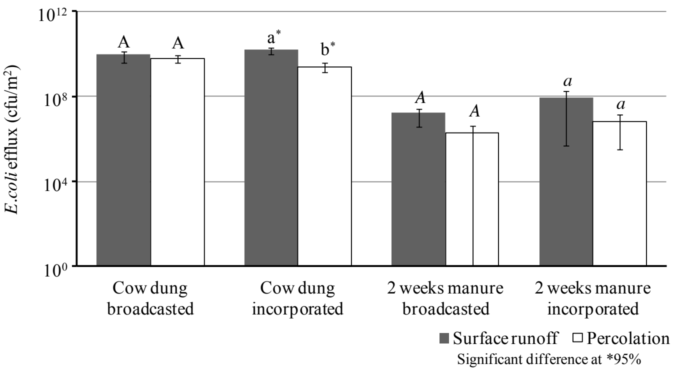

Fig. 8 Total efflux of *E. coli* from plots applied fresh cow dung or 2 weeks fermented manure by broadcasting or incorporating

The total efflux of *E. coli* through surface runoff and percolation are shown in Fig. 8. It was indicated that the efflux of *E. coli* decreased with the fermentation. The tendency of *E. coli* efflux through surface runoff to be larger than that through percolation was also observed. It was clearly indicated that *E. coli* efflux occurred not only through surface runoff but also through percolation. A significant difference at 95% was observed in only the case fresh cow dung applied by incorporating method.

CONCLUSION

Based on the experimental results, it was clearly observed that *E. coli* efflux significantly increased with the increase in total suspended solids in surface runoff from the plots applied fresh cow dung and from the plots applied 2 weeks fermented manure. Also, the same tendency that *E. coli* efflux increased with the increase in total suspended solids by broadcasting and incorporating methods.

The efflux of *E. coli* through percolation was significantly lower than that of *E. coli* efflux through surface runoff. It was also observed that the number of *E. coli* efflux was higher than that of *E. coli* input from both plots applied fresh cow dung and 2 weeks fermented manure.

The existence of *E. coli* were found even in the 2 weeks fermented manure indicating a potential of *E. coli* efflux after applying into farmlands particularly by incorporating application method. Therefore, fermentation of cow dung for more than two weeks before applying into farmlands is highly suggested.

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