Research article

Management of Manure Taking into Account of *E.coli* Loss from Farmland

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Abstract Manure fermented insufficiently includes pathogen such as *E.coli* threatens the environment and human health. Thus, a proper management of the excrement is an important issue. In addition, *E.coli* can be easily transported by surface runoff or percolation from farmland where manure had been applied. So, the loss of *E.coli* from farmlands and its survival limit in manure are big concern from the viewpoints of environmental conservation. Model experiments were conducted under artificial rainfall simulator to investigate *E.coli* loss. Slope plots were filled with soil and then applied 3 types of manure; fresh cow dung, manures fermented for 14 days and 60 days. It was proven that *E.coli* losses occurred even from the manure which was fermented for 60 days. In addition, to acquire a proper treatment of manures, the experiments were conducted to investigate the survival limit of *E.coli* in the manure. It was observed that *E.coli* vanished at the temperature higher than 60 degrees Celsius. Furthermore, the reduction of water content was not enough to eliminate *E.coli*. So, it became clear that controlling temperature of manure is important for vanishing *E.coli* existing in manures.

Keywords *E.coli*, surface runoff, manure, temperature, water content

INTRODUCTION

Large amounts of livestock excrements are being produced in Japan. The Ministry of Agriculture, Forestry and Fisheries of Japan estimated 9,000,000 tons of livestock excrements are being produced per year, and 60% of total livestock excrements are cow dung. At present, these large amounts of excrements are being utilized as manure for improving soil condition. However, large amounts of manure production lead to insufficient fermentation of cow dung. In developing countries, it is difficult to ferment cow dung sufficiently, because of the lack of tools or equipments. There were reports that insufficient fermented manure includes pathogens such as *E.coli*, a microorganism which is normally present in intestinal tract of animals (Chun-Ming Gong et al., 2005). This bacterium will easily propagate in the surrounding environment and can be a threat to sanitation once being discharged with the excrements (Saito et al., 2006). Also, *E.coli* can be easily transported by surface runoff along with soil sediments during heavy rainfall or by percolation into groundwater (Yagura et al., 2006). Thus, a proper treatment of cow dung before applying to farmlands became big concern. Therefore, research interests have been paid to the survival limits of *E.coli* in manure for preventing the loss of *E.coli* from farmlands. So, the objective of this study was to investigate the proper treatment of manure from the viewpoints of environmental conservation.

EXPERIMENTAL METHODS

Experiment on *E.coli* loss

Model experiments were conducted to investigate the *E.coli* loss from farmlands employing slope plots under artificial rainfall simulator as shown in Fig. 1. Cow dung was utilized as the material source of *E.coli* in this study, as it includes more *E.coli* than other animal excrements. The properties of each material are shown in Table 1. The cell numbers of *E.coli* in fresh cow dung, 14 days fermented manure and 60 days fermented manure were 4.1×10^7, 1.6×10^5 and 8.0×10^4 cfu/g dry matters, respectively.

Three stainless slope model plots were prepared at 8 degrees as it is the maximum slope limit on handling agricultural machineries in sloping lands. Soil was compacted into the slope plots at 1.00 ± 0.06 g/cm^3 in dry density (Kawai et al., 2007) and then cow dung or 2 kinds of manure were applied. Fresh cow dung was spread at Plot 1, while Plot 2 and Plot 3 were spread with fermented manures for 14 days and 60 days, respectively. Both cow dung and fermented manures were broadcasted into each plot uniformly. In order to observe and compare the losses of *E.coli* and organic matter from 3 types of plot, artificial rainfall at 60mm/h intensity was simulated for 2 hours.

Discharge of surface runoff was measured at certain interval and sampled from each plots. The *E.coli* in each sample was cultured by the spread plate method on the XM-G agar at 37 degrees Celsius. After 20 hours, the number of blue spots showing *E.coli* colonies appeared on the agar was counted. On the other hand, organic matter was analyzed by means of the ignition loss method.

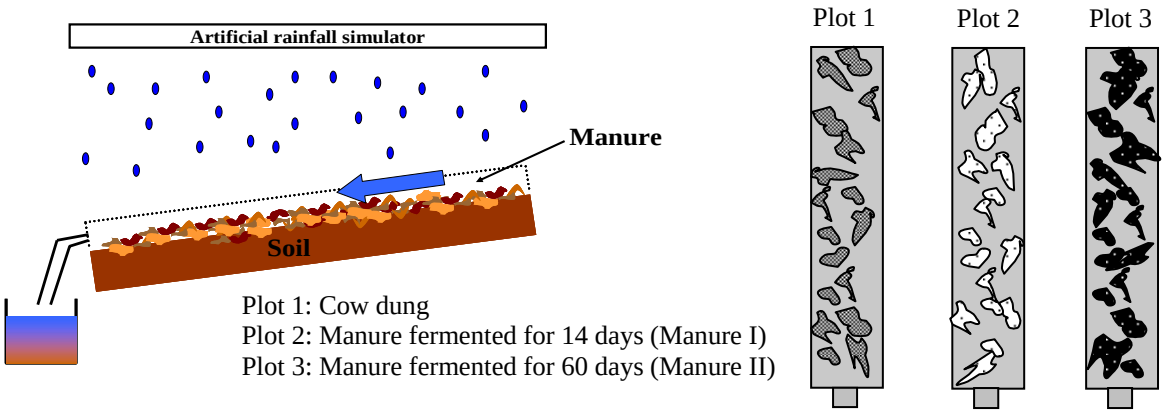


Fig. 1 Slope model experiment

Table 1 Properties of materials

Sample	Sub-material	Fermentation term (day)	*E.coli* (cfu/g dry matter)	Water content (%)
Cow dung	Sawdust	0	4.1×10^7	82
Manure I	Sawdust	14	1.6×10^5	73
Manure II	Sawdust	60	8.0×10^4	72

Experiment on survival limit

Another experiment was conducted to investigate the survival condition of *E.coli* in the manure. As shown in Tables 2 and 3, cow dung and manure fermented for 14 days were used in this experiment. The change in *E.coli* cell number was observed under different temperature or water content.

Cow dung or manure fermented for 14 days was enclosed with plastic wrap to avoid the reduction of water content and kept in the incubator under the different temperature at 30, 40, 50 and 60 degrees Celsius. Additionally, the changes in *E.coli* cell number with water content were observed. Cow dung or manure fermented for 14 days was kept in the greenhouse for enhancing evaporation, and then certain amounts of samples were collected occasionally for analyzing *E.coli* cell number as well as water content.

Table 2 Properties of cow dung or manure used in experiment at different temperature

Sample	Sub-material	Fermentation term (day)	*E.coli* (cfu/g dry)	Water content (%)
Cow dung	Sawdust	0	1.8×10^7	82
Manure I	Sawdust	14	1.4×10^3	72

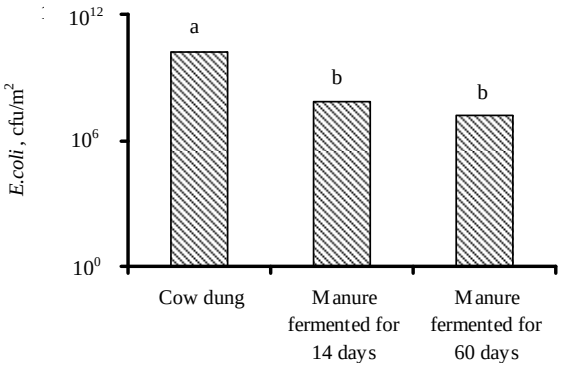
Table 3 Properties of cow dung or manure used in experiment at different water content

Sample	Sub-material	Fermentation term (day)	*E.coli* (cfu/g dry)	Water content (%)
Cow dung	Sawdust	0	1.4×10^8	79
Manure I	Sawdust	14	8.6×10^5	70

RESULTS AND DISCUSSION

E.coli loss from plots applied cow dung or manure

As shown in Fig. 2, *E.coli* loss from the plot applied fresh cow dung was the highest among all plots at 99% confidence interval. The loss of *E.coli* was observed not only from the plot applied fresh cow dung but also from the plot applied manure fermented for 14 days or 60 days. Figs. 3, 4 and 5 show the correlation between the amounts of *E.coli* and organic matter. It showed that *E.coli* loss increased with organic matter in sediments.



**There was a significant difference in total surface E.coli loss at 99% between a and b.*

Fig. 2 *E.coli* loss by surface runoff during rainfall simulation for 2 hours

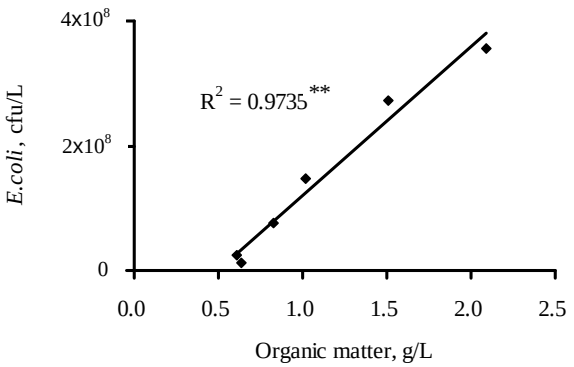


Fig. 3 Relationship between *E.coli* and organic matter in plot applied cow dung

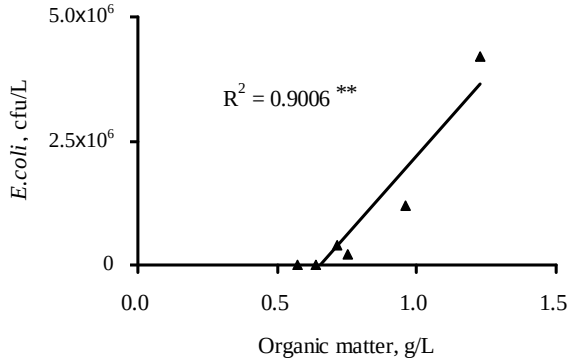


Fig. 4 Relationship between *E.coli* and organic matter in plot applied manure fermented for 14 days

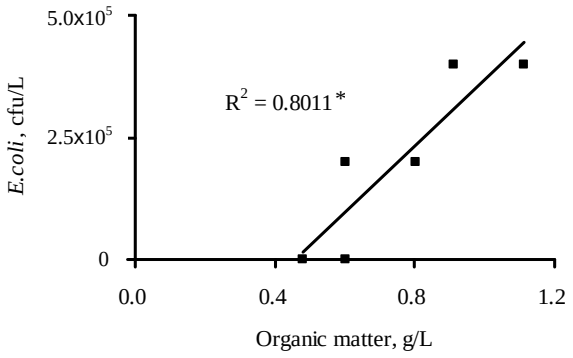


Fig. 5 Relationship between *E.coli* and organic matter in plot applied manure fermented for 60 days

Change in *E.coli* cell number

Based on the results of the experiment employing the incubator, the changes in *E.coli* cell number at different temperature were summarized in Figs. 6 and 7. The cell number of *E.coli* started to decrease at 50 degrees Celsius in either cow dung or manure; however it did not vanish completely. It was observed that *E.coli* vanished completely at 60 degrees Celsius in either cow dung or manure. In opposition, there was no significant difference in *E.coli* cell number among various temperatures under 50 degrees Celsius in either cow dung or manure.

The results of the experiment on the changes in *E.coli* cell number with water content were summarized in Figs. 8 and 9. Water content decreased to 10% in either cow dung or manure for 9 days. There was a tendency for *E.coli* cell number in either cow dung or manure to decrease with the decrease in water content. Additionally, the fluctuation of *E.coli* cell number in cow dung was comparatively smaller than that in manure. Although *E.coli* cell number at 28 days passed was significantly smaller than that at initial stage at 99% confidence interval, the reduction of water content was not enough for vanishing *E.coli.*

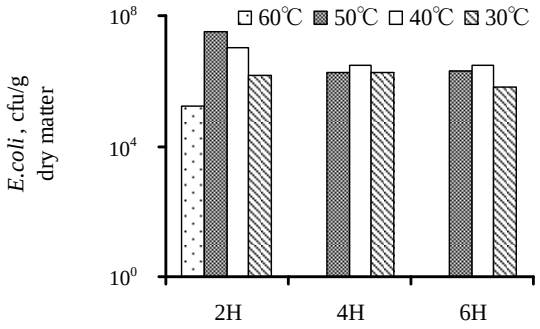


Fig. 6 Changes in *E.coli* with temperature in cow dung

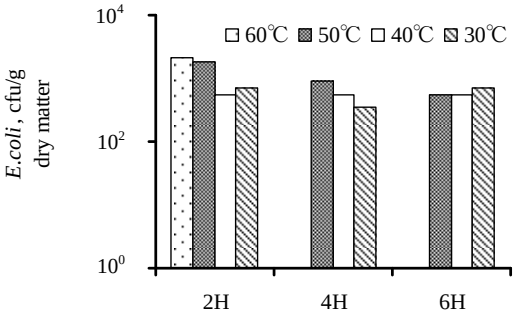
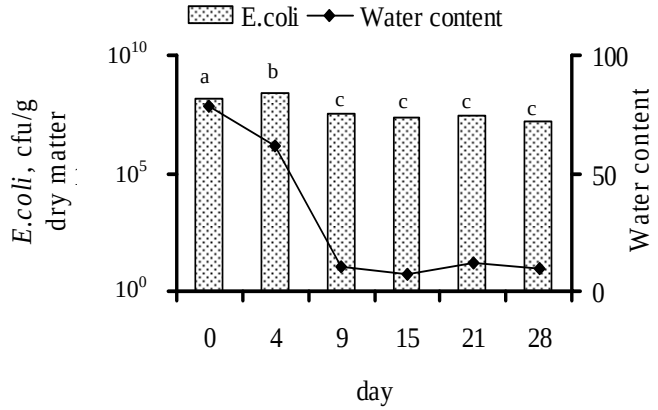
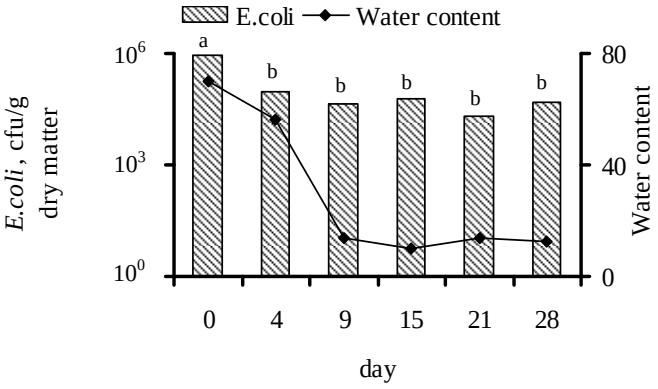


Fig. 7 Changes in *E.coli* with temperature in manure fermented for 14 days



**The data followed by different letters differs significantly at 99% confidence interval.*

Fig. 8 *E.coli* cell number with reduction of water content in cow dung



**The data followed by different letters differs significantly at 99% confidence interval.*

Fig. 9 *E.coli* cell number with reduction of water content in manure fermented for 14 days

CONCLUSION

As the loss of *E.coli* from farmlands is big concern from the viewpoints of environmental conservation, this study dealt with 2 kinds of experiments, one is for observing *E.coli* loss using slope model plots under artificial rainfall simulator and the other is for observing survival limit of *E.coli* in the manure.

It was proven that the loss of *E.coli* was observed not only from the plot applied fresh cow dung but also from the plot applied manure fermented for 14 days or 60 days. Also, *E.coli* loss increased in proportion to the loss of organic matter in sediments. So, it was considered that attentions should be paid more to soil erosion process, as it accelerates *E.coli* loss from upland fields.

Also, it was concluded that *E.coli* vanished at the temperature higher than 60 degrees Celsius.The reduction of water content was not enough to eliminate *E.coli*. So, it became clear that controlling temperature of manure is important for vanishing *E.coli* existing in manures.

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