Risk evaluation for pathogenic bacteria and viruses in sewage sludge compost

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Abstract Composting can be regarded as the most available option for recycling of sewage sludge. However, the existence of pathogenic bacteria and viruses in the compost has been scarcely investigated until now. So there is little information on the infectious risk through agricultural activities or gardening in using the compost. In this study, several kinds of composts were investigated for detection of pathogenic bacteria (*Salmonella* spp. and *Escherichia coli* O157) and enteric viruses. It was concluded from the result that these bacteria and viruses could not be detected in 1.0 g-wet of any kinds of composts. Infectious risks through agricultural activities or gardening were evaluated by Monte Carlo simulation in the case that the compost was polluted by *Salmonella* spp., *E. coli* O157:117 and Poliovirus 1. Criteria satisfying the acceptable risk (less than 10^{-4} per year) for these pathogenic bacteria and virus in the compost were determined from the result of simulations. 1.0 [CFU or PFU/g-wet] was available as the criteria for *E. coli* O157 and Poliovirus 1 in the compost. On the other hand, the criterion for *Salmonella* spp. in the compost should be established on a lower concentration than 0.001 CFU/g-wet.

Keywords Compost; infectious risk; Monte Carlo simulation; pathogenic microorganisms; sewage sludge; vegetables

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Introduction

As sewer systems have been widely provided, the volume of sewage sludge has been increasing in recent years. The treatment and disposal of sewage sludge will become more a serious problem in the 21st century. Composting sewage sludge and utilizing the compost as a fertilizer is considered as one of most effective counterplans for the resolution of this problem since it contains rich nutrients and various minerals. However, since sewage sludge also contains a large amount of pathogenic bacteria and viruses, there is the possibility that the compost is still polluted by pathogenic bacteria and viruses without the appropriate treatment. So, the utilization of compost as a fertilizer must be discussed carefully from the viewpoint of public health. Though the rule on the utilization of compost as a fertilizer was established this October in Japan, no criteria for pathogenic bacteria and viruses are included in the rule. On the other hand, in the United States, the similar rule had been already established eight years before and it has obligated to monitor pathogenic bacteria and viruses in the sewage sludge when the sewage sludge or the compost is utilized for various objectives (USEPA, 1992a). However, valuable monitoring data have not been reported enough (especially on enteric viruses) because of inefficiency of conventional detection methods. In order to discuss the safety of utilizing a compost as a fertilizer, it is necessary to develop available detection methods of pathogenic bacteria and viruses in the compost and the reliable model for the risk evaluation by pathogenic bacteria and viruses in utilizing the compost as a fertilizer. This paper focused on the infectious risk through vegetables cultivated using the polluted compost. This paper aims to detect pathogenic bacteria (*Salmonella* spp. and *Escherichia coli* O157) and enteric viruses in some kinds of composts and to evaluate an infectious risk through vegetables cultivated using a compost polluted by pathogenic bacteria and viruses (*Salmonella* spp., *Escherichia coli* O157:117 and Poliovirus 1).

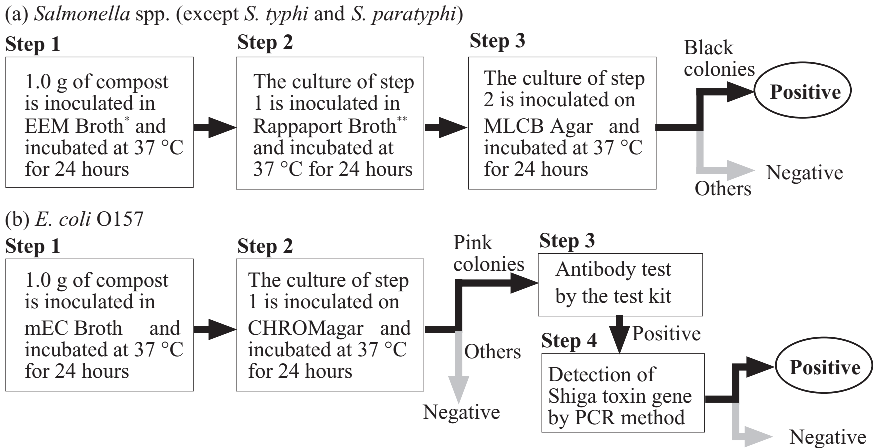
Materials and methods

Detection of pathogenic bacteria in composts

Salmonella spp. (except *S. typhi* and *S. paratyphi*) and *Escherichia coli* O157 were employed as pathogenic bacteria in composts. Procedures for detection of these bacteria are illustrated in Figure 1. These procedures consists of three and four steps for detections of *Salmonella* spp. and *Escherichia coli* O157, respectively. Especially, the antibody test (step 3) and the detection of Shiga toxin gene by PCR method (step 4) are needed since *E. coli* O157 has a lot of close species. According to these procedures, seven kinds of composts were investigated in this study. Table 1 shows materials in each sample compost. The main material for all of these composts was the municipal sewage sludge.

Detection of enteric viruses in composts

Figure 2 shows the procedure for detection of enteric viruses in the compost. In this procedure, enteric viruses were eluted from the compost by 10% beef extract solution. This was the standard method adapted by USEPA (1992b) for the virus elution from sewage sludge. After the centrifugation, the liquid phage was analyzed for the virus detection by the plaque assay using Buffalo Green Monkey kidney cells (BGM cells) according to Japan Water Works Association (1993). BGM cells has the sensitivity against some species of enteroviruses such as poliovirus and coxsackie B virus.



* EEM Broth (Eiken Chemical Co. Ltd., E-MA23)
** Rappaport Broth (Eiken Chemical Co. Ltd., E-MB25)
MLCB Agar (Nissui Medicine Co. Ltd, 05041)
Pearlcore® mEC Broth with novobiocin (Eiken Chemical Co. Ltd., E-MB11)
CHROMagar™ O157 (CHROMagar Microbiology)
E. coli O157 Test Kit (Oxoid, DR620M)

Figure 1 Procedures for detection of pathogenic bacteria in the compost

Table 1 Materials of sample composts

Compost	Materials
A	Sewage sludge (100%)
B	Sewage sludge (100%)
C	Similar to compost B*
D	Sewage sludge (30%), charcoal (20), compost D (30) and others** (10)
E	Sewage sludge (30%), wood chip (20), compost E (30) and others** (10)
F	Sewage sludge (23%), compost F (67) and others** (10)
G	Compost F (50%) and rice bran (50)

* Compost B and C were made from the same materials. However, compost A was made one day before the analysis and compost B was made one month before the analysis
** This item contains coffee beans (80%), rice hulls (10%) and rice bran (10%)

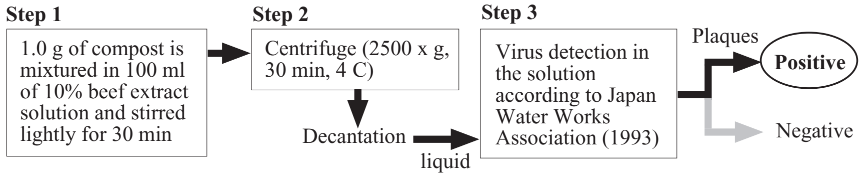


Figure 2 The procedure for detection of enteric viruses in the compost

Risk evaluation by pathogenic bacteria and viruses in the composts

Pathway of exposure by pathogenic bacteria and viruses in the compost. Though various pathways of exposure by pathogenic bacteria and viruses in the compost through agricultural activities or gardening could be listed, eating polluted vegetables was regarded as one of the main pathways in this paper. Lankin *et al*. (1977) mentioned that the concentration of *Salmonella* spp. on the leaf of lettuce reflected the concentration in the soil. Moreover, they concluded that the safety of eating the vegetable could not be assured while *Salmonella* spp. could be detected in the soil. So the worst case that vegetables are polluted by pathogenic bacteria and viruses on the same level as that in the compost should be considered for the risk evaluation.

Dose-response models for infection by pathogenic bacteria and viruses. For evaluation of infection risks through vegetables, *Salmonella* spp., *Escherichia coli* O157:H7 and Poliovirus 1 were employed as pathogenic bacteria and viruses in the compost, respectively. These bacteria and virus were selected corresponding to *S*. spp., *E. coli* O157 and enteric viruses which would be observed in this study, respectively. The reason why Poliovirus 1 was employed is that it has been well investigated on the dose-response relationship. Though several kinds of mathematical models describing dose-response relationships have been already developed, beta models expressed by the following formula were said to be most available for infections by *S*. spp and *E. coli* O157, respectively (Rose and Gerba, 1991; Haas *et al*., 2000):

$$\textit{Salmonella}\text{ spp.: } P(D) = 1-\left(1+\frac{D}{139.9}\right)^{-0.33}$$

$$\textit{Escherichia coli}\text{ O157:H7: } P(D) = 1-\left[1+\frac{D}{596000}\left(2^{1/0.49}-1\right)\right]^{0.49} = 1-\left(1+\frac{D}{191000}\right)^{-0.49}$$

where, D is the dosage of these bacteria and P(D) is the individual infectious probability. On the other hand, Fukushi *et al*. (1998) mentioned that the logistic model given in the following formula was most suitable for the infection data for Poliovirus 1:

$$\text{Poliovirus 1:} P(D) \frac{1}{1+\exp\left(20.0-10.9\log_{10}D\right)}$$

where, D is the dosage of Poliovirus 1 and P(D) is the individual infectious probability.

Relative sensitivity against intestinal infectious diseases. The individual sensitivity against pathogenic bacteria and viruses depends on its immunity. In order to consider this effect by host immunity, Watanabe *et al*. (1999) proposed the relative sensitivity against intestinal infectious diseases as the function of age. Infectious probabilities are weighted by the relative sensitivity, RS(A), as the following equation:

$$P^*(D,A) = P(D)\times RS(A) \tag{4}$$

where, P*(D,A) is the infectious probability with host immunity.

Other assumptions for risk evaluation. In this study, lettuce was employed as the polluted vegetable for the risk evaluation. Ashbolt (1999) adopted 11.5g-wet as the average daily dosage of lettuce for an individual consumers for risk evaluation. This dosage was referenced in this study. Distribution of the concentration of pathogenic bacteria and viruses on the leaf of lettuce was assumed to be Poisson distributed. The assumption that 90 percent of pathogenic bacteria and viruses on lettuce was removed by the wash before being eaten was employed for the risk evaluation.

Procedure for risk evaluation through the polluted vegetable. Figure 3 shows the procedure for risk evaluation through the polluted vegetable (lettuce in this study). At first, the average concentration of the pathogenic bacterium or virus in the compost is considered. This is equivalent to the average concentration of the bacterium or virus in the vegetable. The dosage of the bacterium or virus through the vegetable for one person is determined by the Monte Carlo method so that the frequency distribution of the dosage can be expressed by Poisson distribution. The infectious probability for the person is calculated with the dose-response model modified by the relative sensitivity (Eq. (4)). According to the infectious probability, whether the person will be infected or not in a day is decided by the Monte Carlo method. If the person is decided as not infected, the person will be evaluated for the infection on the next day. On the other hand, the infected person is removed from the evaluation of infection on the next day. The evaluation of infection in one day is repeated for 365 times, that is one year. Moreover, this evaluation is applied to all of the people. Finally, the annual number of infected persons is obtained through this procedure.

Results and discussion

Results of detection for pathogenic bacteria and viruses in the composts

Table 2 shows results of detection of *Salmonella* spp., *Escherichia coli* O157 and enteric viruses in the compost. Pathogenic bacteria and viruses were not detected from any sample composts. Most of the pathogenic bacteria and viruses in the sewage sludge were inactivated by the heat treatment and the dry condition in composting the sewage sludge. However, since 1.0g-wet of the compost was used for the detection of these pathogenic bacteria and viruses in this study, it cannot be denied that any pathogenic bacteria and viruses may be detected from 10g-wet or more of these composts. So, the detection of pathogenic bacteria and viruses from larger volume of compost should be tried in the further study. The infection risk by pathogenic bacteria and viruses at such a low concentration observed in this study was evaluated in the following section. The infection risk was also evaluated in case the compost was accidentally polluted at higher concentrations.

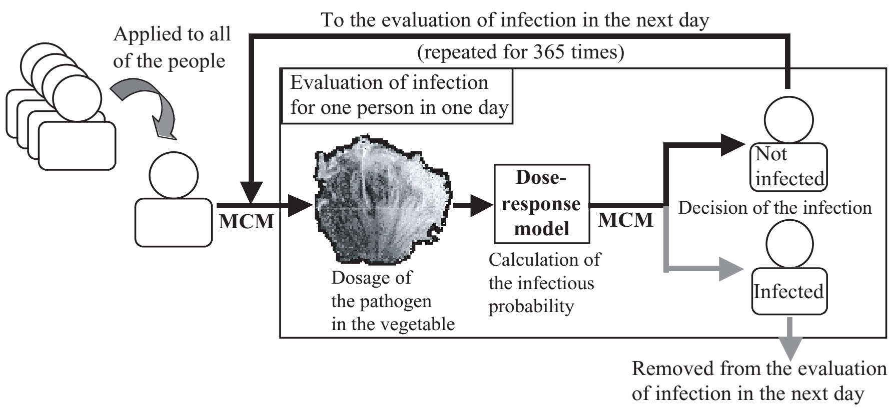


Figure 3 Procedure for risk evaluation through the vegetable polluted by pathogenic bacteria and viruses

Infection risk through the polluted vegetable

Infectious risks were evaluated by Monte Carlo simulation as the number of infected people per 10,000 people per year given that the age distribution was similar to that in Tokyo, 1992. Table 3 shows the number of infected people by *S.* spp., *E. coli* O157:H7 and Poliovirus 1 through eating the lettuce if the concentration of these bacteria and virus in the compost was 0.001, 0.01, 0.1, 1, 10 and 100 CFU or PFU/g-wet, respectively. 95% confidence intervals of average number of infected people were calculated using results of 100 times trials at each conditions. Generally speaking, the acceptable risk for infection by pathogenic bacteria and viruses is desired to be below 10^{-4} per year. According to the result of risk evaluation, the concentration of 1.0 CFU or PFU/g-wet in the compost satisfied this acceptable risk for *E. coli* O157:H7 and Poliovirus 1. So, 1.0 CFU or PFU/g-wet should be employed as the criteria for *E. coli* O157:H7 and Poliovirus 1 in the compost. On the other hand, the infectious risk by *S.* spp in the compost exceeded 10^{-4} even if the concentration was 0.001 CFU/g-wet. So the criterion for *S.* spp in the compost should be established on the lower concentration level than 0.001 CFU/g-wet. However, it may be difficult to employ this criterion since it requires the detection of only one bacterium in the compost of 1kg-wet.

The fate of pathogenic bacteria and viruses in the compost

The regrowth of *Salmonella* spp. in the compost has been reported in several studies (Russ and Yanko, 1981). The possibility of regrowth of pathogenic bacteria in the compost were also indicated regarding total and fecal coliforms, *Salmonella* spp. and fecal streptococci by Haug (1993). The result of detection in our study indicated that most of the pathogenic bacteria in the sewage sludge was sufficiently inactivated by composting. However, it cannot be neglected that pathogenic bacteria surviving in the compost at the undetectable levels may regrow or that the compost may be recontaminated by bacteria from an outside source. The regrowth of pathogenic bacteria in the compost should be investigated in detail for more reliable evaluation of infectious risks through the compost. The fate of pathogenic viruses in the compost is significantly different from that of bacteria since viruses cannot grow outside of the human body. In general, it is said that viruses have slightly higher tolerance against heat treatment and dry condition than bacteria except those in spores. So, pathogenic viruses have higher possibility to survive in composting than pathogenic bacteria. For example, it has been reported that viruses could survive 25 days of composting since the composting mass did not achieve adequately high temperature (Gerba, 1996). On the other hand, all other enteric bacteria were sufficiently inactivated in this case. From these discussions, pathogenic bacteria and viruses in the compost should be monitored before utilizing the compost as a fertilizer.

Table 2 Results of detection of pathogenic bacteria and viruses in composts

Compost	*Salmonella* spp.	*Escherichia coli* O157	Enteric viruses
A	0* (2)**	0 (2)	0 (l)
B	0 (5)	0 (5)	0 (l)
C	0 (5)	0 (5)	0 (l)
D	0 (5)	0 (5)	N.R.†
E	0 (5)	0 (5)	N.R.
F	0 (5)	0 (5)	N.R.
G	0 (5)	0 (5)	N.R.

* This is the number of positive samples
** The number in the parentheses indicates total number of samples
† There was no reliable data

Table 3 Number of infected people by pathogenic bacteria and virus in the compost through the polluted lettuce in case that the individual daily dosage of lettuce is 11.5 [g-wet]

Ave. concentration of pathogenic bacteria or virus in the compost [CFU or PFU/g-wet]	Number of infected people [per 10,000 people per year]		
	Salmonella spp.	*E. coli* O157:H7	Poliovirus 1
10^{-3}	9–10*		
10^{-2}	89–94		
10^{-1}	873–885		
10^{0}	5,984–5,903	Nobody	Nobody
10^{1}	Everybody	37–40	1,383–1,395
10^{2}		918–929	Everybody

* These values indicate lower and upper limits of 95% confidence interval for the average number of infected people

Conclusions

Conclusions obtained in this study are as follows.

1. Pathogenic bacteria (*Salmonella* spp. and *Escherichia coli* O157) and enteric viruses could not be detected in 1.0g-wet of any kinds of seven composts.
2. The concentration of 1.0 CFU or PFU/g-wet was available as the criteria for *Escherichia coli* O157:H7 and Poliovirus 1 in the compost.
3. The criterion for *Salmonella* spp. in the compost should be established on the lower concentration than 0.001 CFU/g-wet.

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