Toxicity assessment of the extract of compost as a final product from Bio-Toilet

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Abstract Bio-Toilet is the name of a dry closet or composting toilet using sawdust as an artificial soil matrix for bioconversion of human excrement into compost. Since feces and urine contain several chemicals such as pharmaceutical residues and endocrine disruptors and they may still remain in compost after biological reaction in the Bio-Toilet, it is required to examine the possibility of soil and/or groundwater pollution by applying compost to a soil system in farmland. In this study, toxicity of Bio-Toilet compost was evaluated by measuring the viability of human neuroblast (NB-1). The bio-assay was applied to the water extract of compost from the Bio-Toilets which are in practical use in Japan. The assay results showed that (1) the extract of feces showed no toxicity, and the extracts of unused sawdust had no or low level toxicity and (2) the extracts of composts had heavier toxicity than unused sawdust. These results implied that some chemicals that have toxicity were generated by biological reactions or accumulated in toilet system. The bio-assay results with fractionated organic matter by its molecular weight showed that the small molecular weight fraction had stronger toxicity than other fractions. The effect of inorganic matter on toxicity was examined by comparing the dose-response relationship of the extracts of compost with positive control with 1M of sodium chloride solution. The comparison showed that sodium concentration in the extract was too low to develop the toxicity and the effect of inorganic matter could be neglected in this study.

Keywords Compost; dry toilet; feces; fractionation of organic matter; human cell NB-1; toxicity

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Introduction

The wastewater effluent from a household or group of households is made up of contributions from various appliances, such as WC, kitchen sink, wash basin, bath, shower, and washing machine. Elimination of toilet waste (black-water) from the residential wastewater stream by using non-water carriage toilet will reduce the mass of organic matters; pathogenic microorganisms; nitrogen and phosphorus in the remaining waste stream (gray water). We have proposed the Onsite Wastewater Differentiable Treatment System (OWDTS) (Lopez Zavala *et al.*, 2002) based on the concept of a differentiable management and treatment of household wastewater effluents. Figure 1 shows a hypothetical model for onsite wastewater differentiable treatment system. In this system, the separation of household wastewater into three types is essential. Reduced-volume black water, higher-load and lower-load gray water are new concepts that are introduced in this model. Here, treatment of black water conceives a change in the traditional way of using the WC; in other words, the use of water in the WC is just to clean the toilet, not to transport the toilet wastes; this is a very important change.

Bio-Toilet is the name of a dry closet or composting toilet using sawdust as an artificial soil matrix for bioconversion of human excrement into compost which can be used either as organic fertilizer rich in N, P, and K, or as a soil conditioner (Del Porto and

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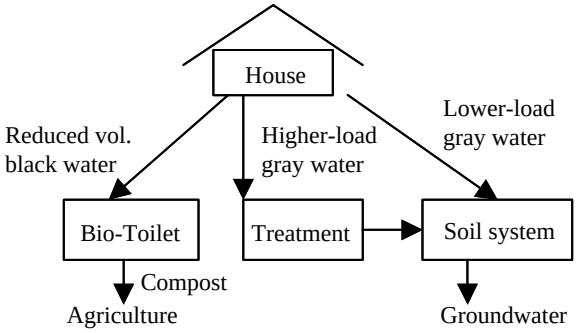

Figure 1 Hypothetical model for Onsite Wastewater Differentiable Treatment System

Steinfeld, 1998; Kitsui and Terazawa, 1999). The bioconversion process has been studied by several researchers, on the other hand, it should be noted that the character of residuals in compost has not been described well yet. The black water contains several chemicals such as pharmaceutical residues and endocrine disruptors, and some of them might be non-biodegradable or partially biodegradable, and non-biodegradable organic matter might be produced during bioconversion process. Since the compost as a final product of Bio-Toilet contains residual chemicals, it may be possible that those chemicals accumulate in soil systems and leads to soil and/or groundwater pollution when the compost is applied to farm land.

In order to characterize residual chemicals in compost, toxicity assessment was applied to the water extract of compost in this study. This work is a part of a study on fate and transport processes in soil systems. Toxicity assessment by bio-assay is one of the effective techniques to characterize mixture of unknown chemicals (Kunimoto *et al.*, 1992; Suzuki and Utsumi, 1998). The bio-assay method of using cultured human cell and mammalian cell lines is an effective method to assess the effect for human system (Kunimoto *et al.*, 1996; Nagashima *et al.*, 2001; Fukushima *et al.*, 2002). In this paper, the results from toxicity assessment on the extract of compost from Bio-Toilet are discussed.

Material and methods

Samples for toxicity assay

Compost. We obtained compost from three kinds of Bio-Toilet (Bio-Toilet A, K and T) operating in Japan. Their operational conditions are summarized in Table 1. Bio-Toilet A uses sawdust of Japanese red pine as a matrix. This Bio-Toilet is installed in office in Asahikawa city and is operating as an unisex toilet (A-M&F). Chips of Japan cedar are used for Toilet K. This Bio-Toilet is placed at the park in Fujishiro city; two units are prepared for male (K-M) and female (K-F). Bio-Toilet T is also placed at the riverside park in Fujishiro and two units T-M and T-F are used, and sawdust of Japan cedar is used in these toilets.

Table 1 Operational conditions of toilets

Mark	Matrix material (majority)	Cumulative user before sampling (%)	Moisture content (%)	Operation temp.
A-M&F	Japanese red pine	–	–	55 °C
K-M	Japan cedar chip	(about)160	55.4	Heating
K-F	Japan cedar chip	(about)50	53.5	Heating
T-M	Japan cedar sawdust	48	76.1	Not heating
T-F	Japan cedar sawdust	25	76.0	Not heating

Feces and fresh sawdust. In order to assess the original toxicity of fresh sawdust and feces, we performed bio-assay for their extract from the fresh sawdust used for Bio-Toilet. Feces are provided by a healthy man who did not take any medicines.

Extraction procedure

The extraction procedure is illustrated in Figure 2. One litre of distilled water was used as solvent and mixed with 100 g of sawdust. The mixture was left at rest for 30 minutes, and the supernatant was filtered by 1 μm paper filter and then by 0.45 μm membrane filter. This filtrate was used for bio-assay and analytical test. Some samples are concentrated by a rotary evaporator to obtain enough response data in a bio-assay.

Analytical methods

Dissolved Total Organic Carbon (DOC) of filtrates was determined according to *Standard Methods* (APHA *et al.*, 1989). Sodium ion (Na^+), ammonium ion (NH_4^+), nitrate, and nitrite concentration were measured by ion chromatography. Electric conductivity (EC) and pH values were also monitored.

Fractionating organic matter by ultra-filtration

The extracts from compost were filtered by ultra membranes (Millipore). We used four membranes with their cut off levels 30,000Da, 10,000Da, 3,000Da, and 1,000Da. These filters were pre-washed with distilled water for more than 1 hour so that we could eliminate contamination by organic matter coated on surface of ultra-filter. With this pre-washing, we reduced contamination of organic matter to less than 1 mg-DOC/L level. In this study, the filtrate of ultra-filter with 30,000Da is designated by "30,000 pass". In the same manner we prepared "10,000 pass", "3,000 pass" and "1, 000 pass".

Bio-assay

Bio-assay using human cell lines was performed. Cultured human neuroblastoma cell line NB-1 was used. This cell was inoculated in 96-well plates and incubated for 48 h in the CO_2 incubator before and after the sample exposure (Kunimoto *et al.*, 1992). Crystal

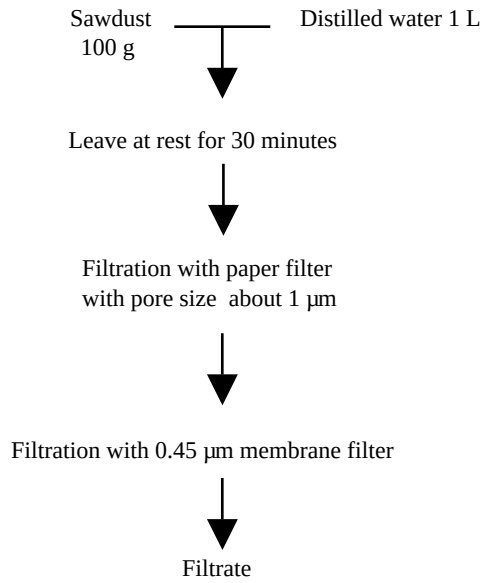

Figure 2 Extraction procedure

violet dyeing method was used for viability measurement. EC50 value was calculated by curve-fitting with sigmoid curve by using data analysis software (GraphPad Prism, GraphPad software Inc).

Results and discussion

Water quality of extracts

DOC, pH, EC, and sodium ion (Na^+) concentration of extracts are summarized in Table 2. The values of pH of extracts of unused sawdust are ranged from 5.5 to 7.6, but the composts from Bio-Toilet have rather high pH. This high pH is due to ammonium nitrogen in compost. Since residual organic matter in compost came out from compost to liquid phase, the extracts of compost have higher DOC that unused sawdust and the amount of organic matter in the extracts depend on how many persons have used the Bio-Toilet.

Electric conductivity and sodium ion (Na^+) concentration represents the extent of accumulation inorganic matter supplied by urine and feces. These concentrations also depend on the number of usage.

Bio-assay results

Feces and fresh sawdust. Figure 3 is the bio-assay results from experiment using the extract of feces given by a healthy man. This figure shows the dose-response relationship, the vertical axis is the viability percentage of NB-1 cell and the horizontal axis is DOC concentration of sample in log-scale. In the bio-assay, the sample was diluted by RPMI-1640 and FCS medium several times and exposed to NB-1 cell (Kunimoto *et al.*, 1992). It is seen from Figure 3 that there is no evidence to show the toxicity since the viability remains nearly 100% at any DOC concentration. Figure 4(a) is the dose-response relationship obtained from the extract of unused sawdust used in Bio-Toilet A, showing that the unused sawdust in Bio-Toilet A does not have any toxicity. Figure 4(b) shows the results of bio-assay from experiments with unused sawdust used in Bio-Toilets K and T. Unlike the results from Bio-Toilet A shown in Figure 4(a), the unused sawdust shows toxicity. This difference in the dose-response relationship may be due to material of sawdust. The sawdust used in Bio-Toilet A is Japanese red pine, and in the other hand, Japanese cedar is used in Bio-Toilets K and T.

Extracts from compost. Figure 5 is the dose-response relationship obtained from bio-assay with the extract of compost from Bio-Toilet A, showing that the extract of compost has toxicity. We performed bio-assay twice on the extract. In order to compare the strength of toxicity, the dose-response relationships with 100 μM $CdCl_2$ solution and 1M NaCl solution are also plotted in Figure 5. It is seen from Figure 5 that (1) the toxicity of the extract may be comparable to 100 μM $CdCl_2$ solution; and (2) high concentration of

Table 2 Quality of extract from compost and unused sawdust

	pH	DOC (mg/L)	EC (mS/cm)	Na (mg/L)
A-M&F	–	277.7	0.72	–
K-M	8.42	251.0	4.39	20.9
K-F	6.32	130.1	3.14	16.2
T-M	8.43	77.9	0.75	2.24
T-F	7.36	51.7	0.15	0.97
A-unused	5.52	174	0.10	–
K-unused	7.58	26.6	0.043	0.90
T-unused	6.57	22.7	0.023	1.85

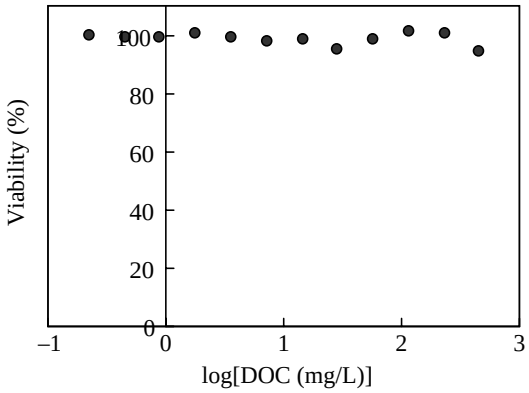

Figure 3 Bio-assay result from experiment with feces

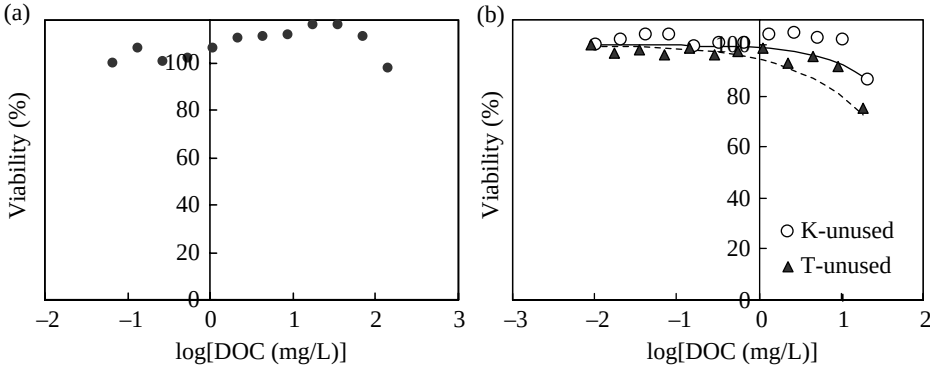

Figure 4 (a) Bio-assay result from experiment with fresh sawdust from Bio-Toilet A; (b) Bio-assay result from experiment with fresh sawdust from Bio-Toilet K and T

NaCl gives low viability of cell, and this effect must be examined for the extract of compost.

Based on the results obtained from the above figures (Figures 3, 4(a) and 5), that (1) feces did not show the toxicity (Figure 3), (2) unused sawdust of Bio-Toilet A did not have toxicity, either (Figure 4(a)), (3) the extract of compost from Bio-Toilet A showed

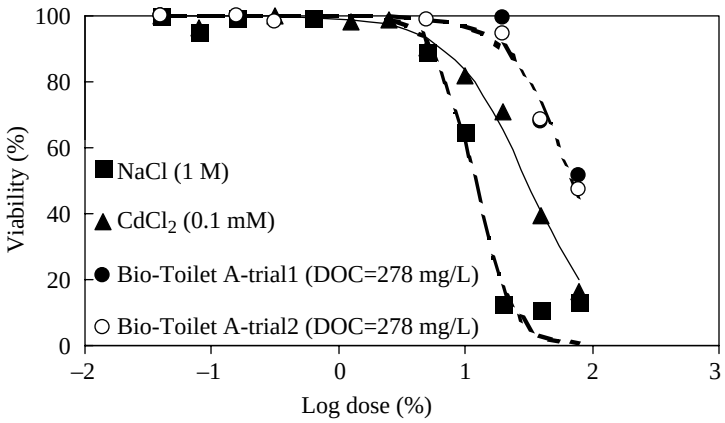

Figure 5 Bio-assay results from experiment with compost from Bio-Toilet A

toxicity, we can say that some chemicals that have toxicity were generated by biological reactions or accumulated in toilet system.

Figure 6 summarizes the bio-assay results for the extracts of compost from Bio-Toilet K and T. In this bio-assay, samples were concentrated by evaporator, and their DOC concentrations range from 520 mg/L (T-F) to 2500 mg/L (K-M). They show that the extract of Bio-Toilet K and T also has the toxicity and it can be seen that there is no significant difference between male and female toilets.

Fractionating of organic matter

In order to obtain the information about which kind of organic matter contributes to toxicity development, we applied bio-assay to filtrate of ultra-filter having different cut-off level of organic matter. Figure 7 is molecular weight distribution of the extract of compost from Bio-Toilet A. It can be seen in Figure 7 that there are two groups of organic matter, that is, organic matter having molecular weight greater than 10,000Da and less than 1,000Da. Figure 8 summarizes the bio-assay results, showing that the "1,000 pass" fraction has larger toxicity than the other filtrates and original sample. This result implies that organic matter with less than 1000Da contributes mainly for developing toxicity of the extract.

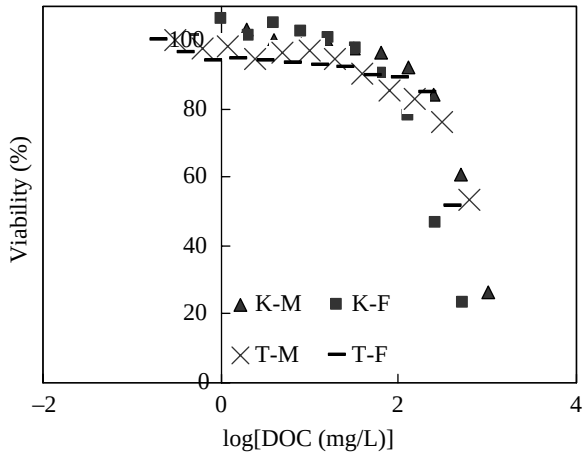

Figure 6 Bio-assay results from experiment with compost from Bio-Toilet K and T

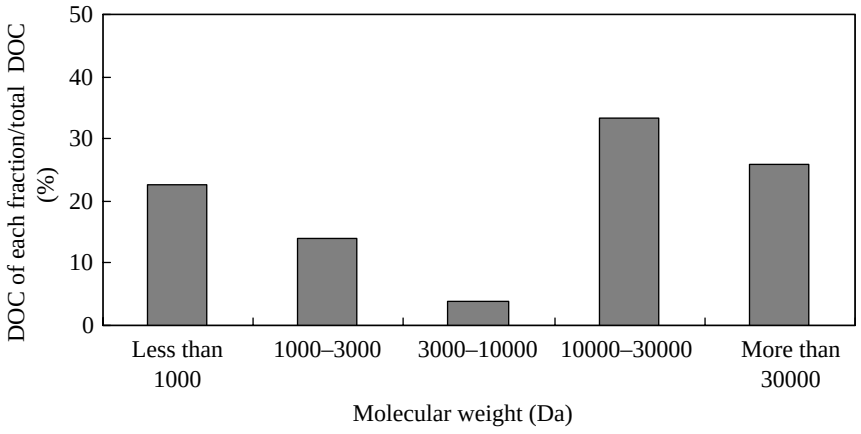

Figure 7 Molecular weight distribution of the extract from Bio-Toilet A

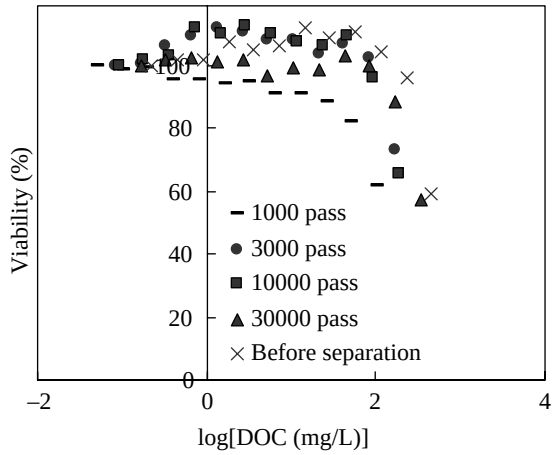

Figure 8 Bio-assay results with filtrate of ultra-filter (Bio-Toilet A)

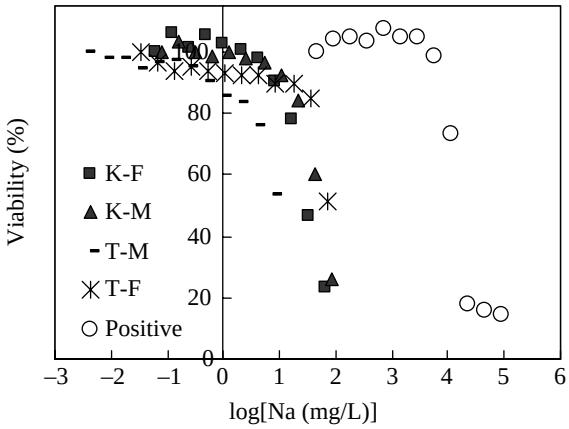

Figure 9 The relation between the sodium concentration and viability of human cell

Effect of sodium ion on bio-assay results

As shown in Figure 5, high NaCl solution has adverse effect on NB-1 cell. Since Bio-Toilet receives urine and inorganic matter is accumulated in the system, it is required to the effect of Na^+ concentration on the dose-response relationship. In Figure 9, we examined the viability of NB-1 cell in terms of the sodium concentration of samples and positive control (1M NaCl in Figure 5). It is seen from Figure 9 that dose-response relationships of the extracts are completely different from that of the positive control. The EC50 of positive control is 3,100 mg-Na^+/L, this value is corresponding to 13.51% dose of positive control in Figure 5. The Na^+ concentration in the extract used in bio-assay ranged from 9.4 to 40 mg/L. From above observation, we may say that sodium concentration in the extract is in the range that there is no adverse effect on the NB-1 cell. But, because the salinity effect is evaluated in terms of osmolarity, further study is needed in future.

Conclusions

In this study, toxicity of water extract of compost from Bio-Toilet operating in Japan was evaluated by measuring the viability of human neuroblast (NB-1). This research led to the following conclusions.

(1) The assay results showed that (i) the extract of feces showed no toxicity, and the extracts of unused sawdust had no or low-level toxicity, and (ii) the extracts of composts had heavier toxicity than unused sawdust. These results implied that some chemicals that have toxicity were generated by biological reactions or accumulated in toilet system.
(2) According to the bio-assay results with organic matter fractionated by its molecular weight, it was shown that the small molecular weight fraction had stronger toxicity than other fractions.
(3) The effect of inorganic matter on toxicity was examined by comparing the dose-response relationship of the extracts of compost with positive control with 1M of sodium chloride solution. The comparison showed that sodium concentration in the extract was too low to develop the toxicity and the effect of inorganic matter could be neglected in this study.

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