Microbial Risk Assessment for Agricultural Production Cycle of On-site Resource Oriented Sanitation Systems: A Case of Burkina Faso

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Abstract

On-site resource oriented sanitation system is one of expectable concepts to address a protection of water resources in developing countries due to low installation cost and resource recovery from human excretes. The present study investigated fates of indicators and pathogens originated from greywater and compost in soil to assess microbial risks when the greywater and compost are reused. Compost and greywater obtained from pilots in Burkina Faso were amended in experimental field with lettuce cultivation and the fate of pathogens in the soil was measured. The results suggested that (i) bacterial fates in compost reuse were fitted to log normal linier and those in greywater were maintained in field soil (ii) the bacterial end-off kinetics in Soudano-Sahelian climate were more rapid than that of reference values. (iii) the reduction of *E. coli* and *Salmonella* in the present condition was significantly different but that of *Salmonella* and *Enterococci* had no difference. (iv) effect of contaminated soil on annual risk probability was lower than direct handling of greywater and compost but not negligible. The present assessment also suggested that presented current model were required further technical improvement from the view of the biological risks.

Keywords: Composting toilet; Greywater reuse; Inactivation rate constant; Salmonella; Ascaris eggs

Introduction

Wastewater reuse is emerging as an integral part of water resource management, promoting the preservation of adequate quality fresh water and reducing both environmental water pollution and overall supply costs. Source separation of feces, urine and greywater (all domestic sewage, with the exception of wastewater generated by toilets and bidets) simplifies each treatment for reuse purposes. In particular, the reuse of greywater for garden irrigation has an estimated potential to reduce domestic water consumption by up to 50% (Friedler 2004). Urine-diverting dry toilets, which can separately treat human feces and urine, have advantages for saving flushing water and sewer pipe networks (Winblad and Simpson-Hebert 2004). Combination reuse of the fecal compost and stored urine can supply nutrients to vegetables as well as chemical fertilizer (Hijikata et al. 2014). Therefore the overall wastewater reuse for the garden can alleviate stress on depleted water resources while both reducing burdens for water and wastewater costs and enhancing garden activities. The wastewater reuses would be an attractive option for rural area in developing countries where often faces poverty problem, poor infrastructure, low efficiency and instability of government, and severe environmental condition (Ushijima et al. 2014). To address the overall reuse concept, one rural model with a low-cost mixing type dry toilet (composting toilet) (Yabui et al. 2012) and a slanted soil greywater treatment system (Ynoussa et al. 2014) has been tested in

houses of small scale farmers, as a case of Burkina Faso where is located in sub-Sahel Africa.

From a hygienic perspective, these resources potentially contain microbial pathogens that mainly cause gastrointestinal infections (WHO 2006). To manage the microbial risk, adequate treatments and handlings for the compost and greywater have been successfully studied (Shuval et al. 1997; Ottoson and Stenström 2003; Nakagawa et al. 2006; Schönning et al. 2007; Maimon et al. 2010). However, little information is available in a scenario of combination reuse of compost and greywater in terms of the microbial risk. In particular, effect of contaminated soil by both compost and greywater on the risk for farmers and consumers is still unclear.

Hygienic safety is generally discussed in terms of quantitative microbial risk assessment (QMRA), which is comprised of four steps: hazard identification, dose-response assessment, exposure assessment and risk characterization (Haas et al. 1999). The QMRA requires researchers to assume exposure scenarios and to use end-off kinetics of target microorganisms for each situation. The exposure scenarios were assumed by agricultural styles and tools (Seidu et al. 2008) and the end-off kinetics in soil are influenced by climate and soil properties (Zaleski et al. 2005). Assuming the combination reuse in a situation of Burkina Faso as a model case, the exposure scenarios and the die-off kinetics in contaminated soil were investigated to assess the risks for gardeners and consumers in the current rural model, in the present study.

1. Material and Methods

Compost and greywater

The composting toilet and slanted soil greywater treatment system was installed in two rural villages where located 40 km far from capital city Ouagadougou, in Burkina Faso. Compost samples were obtained from the composting toilet of 4 pilot families in October 2013 and April 2014. Totally 8 samples were measured. Most contaminated sample was applied for the present field test. Greywater samples were obtained from same 4 pilot families every week during April and March in 2014. The measuring samples were separately collected in the pilot-site but samples for field test was mixed in 200 L plastic tanks and storage in experimental field.

Field experiment

Obtained samples were applied in field cultivation test with lettuce on April to March, 2014 in an experimental site where is localized in a campus of International Institute for Water and Environmental Engineering (2iE) whose geographic details are 12°27’39.74”N and 1°32’54.78”W. Compost amendment with tap water irrigated plots (C+T), Compost amendment with greywater irrigated plots (C+G), chemical fertilizer with greywater plots (NPK+G) and chemical fertilizer with tap water plots (NPK+T) were prepared with three replications. Compost of 2 kg-wet and chemical fertilizer of 100 g was applied for one plot (1.56 m^2). Ten liter of greywater and tap water was applied in the plot every day. Soil samples were collected from 0-5 cm depth every week. Microorganism measurements and dry weight were analyzed.

Biological measurement

Compost and soil samples of 25 g (w/v) were homogenized in 225 mL of buffer phosphate water and a 10-fold dilution series with ringer solution was prepared as extract liquid. *Escherichia coli*, fecal coliforms and fecal *Enterococci* were cultured following a method 9215A in standard methods (APHA 1998). Relevant dilutions were spread on plates in duplicate on the following selective media; chromo cult coliform agar ES (Difco, France) incubated for 24 h at 44.5°C for Fecal Coliforms; same agar incubated at 37°C for *E. coli*; Slanetz Bartley agar incubated for 48 h at 37°C for *Enterococci*. For *Salmonella*, the extract liquid was diluted with

9 mL of Rappaport Vassiliadis media and different dilutions (100 to 10^{-6}) of three to five repetitions are made for each sample. The diluent were incubated for 24h at 37°C as testing process and then it was sown in Chrom Agar media on petri dish. The media was incubated at 37°C for 24h as confirmation process. The results were presented as Most Numbers Probable per gram (MNP/g) with Mac Grady table. For *Ascaris* eggs, compost and soil samples of 25 g were homogenized with 225 mL of 0.1% Tween 80 for 1 min using a blender and screened through 4 layers of wet gauze folded. The filtrate was collected in round bottom flasks and allowed to settle for 3 hours. *Ascaris* eggs were determined by the US EPA protocol (1999) modified by Schwartzbrod (2003) with a modified density of zinc sulfate (Z_nSO_4) saline solution. In the case of greywater measurement, the same method was applied except extraction step.

The results for *E. coli*, fecal coliform, fecal *Enterococci* and *Salmonella* were expressed to log normal No./g-dry. end-off kinetics were presented as inactivation rate constant (Kazama and Otaki 2011) using equation (1):

$$\text{In}\,(N / N_0) = - kt \qquad (1)$$

where, N is concentration of microorganisms at t; N_0 is concentration of microorganisms at time 0; k is inactivation rate constant; t is cultivation time after compost amendment.

One-way ANOVA, two-way ANOVA, Tukey HSD as a post-hoc test, significant correlation and model fitting were conducted with IBM SPSS Statistics 21 software.

Quantitative microbial risk assessment

Compost and greywater potentially contain hazardous of pathogens such as bacteria, virus, protozoa and helminthes that mainly cause gastrointestinal infections (WHO 2006). The present assessment selected risks of *Salmonella,* rotavirus and *Ascaris* infections.

For Dose-response assessment, Beta-Poisson model was used and the annual infection risk was calculated with equation (2) and (3):

$$P_{annual} = 1 - [1 - Pi\,(d)]^{\,n} \qquad (2)$$

$$Pi\,(d) = 1 - [1 + (d / N_{50})\,(2^{1/\alpha} - 1)]^{-\alpha} \qquad (3)$$

where P_{annual} is the annual risk probability, n is the number of the event, Pi (d) is the risk probability per one series of events, d is the ingested number of pathogens, N_{50} is a median infection dose and is a model parameter. Assuming 4 times lettuce cultivation, 4 is plugged in the n. Parameters of N_{50} and α for each pathogens were summarized in Table 2.

Exposure scenario for farmers and consumers in the present model was summarized in Table 1. Expectable exposure events for famers were determined with field observations (Hijikata et al. 2014). The exposure quantities were referred from previous reports (Table 1). Regarding the scenario for consumers, it was assumed that 12 g of lettuce was consumed every week and the lettuce was not washed (Ackerson and Awuah 2012). A quantity of contaminated soil on the lettuce was obtained from the present study: washed the cultivated lettuce in the field test with 0.1% tween 80 of 100 mL, 3 times; the washed liquid was collected and filtered with 0.45 µm pore filter; weight the filter after one day in an oven at 105°C. Dose amounts per 1 series of cultivation/consumption presented a sum of ingestion dose, which calculated with frequency, quantities, pathogen concentration of each matrix and end-off kinetics. The parameters for pathogen concentrations were summarized in Table 2. Values of a worst case and a typical case were obtained from maximum and mean values in the field

observation.

In terms of risk characteristic, 7.7×10^{-4} for *Salmonella* as general diarrheal infection (WHO 2006), 1.4×10^{-3} for rotavirus (Maimon et al. 2010) and 1.0×10^{-2} for *Ascaris* (Mara et al. 2007) was applied as tolerable risk corresponded to WHO guide of DALY 10^{-6} reduction in developing countries.

Table 1. Exposure scenarios of farmers an d consumers for the present risk assessment.

Target	Event	Ingestion matrix	Ingestion scenario	Event no./year	Quantities/event
Farmers	Compost amending	Compost	Compost handling with naked hand	4	300 mg[a]
	Plowing	Soil contaminated by compost	Soil touching at 0 day after plowing	4	100 mg[b]
	Seeding	Soil contaminated by compost and greywater	Soil touching of soil at 1 day after plowing	4	100 mg[b]
	Irrigation	Greywater	Handling of watering cans or bucket	300	2 mL[c]
	Weeding	Soil contaminated by compost and greywater	Soil touching at 20, 40 and 60 days after plowing	4 for each day	100 mg[b]
		Greywater on leaf	Touching of plants leaf	12	1 ml[d]
	Harvesting	Soil contaminated by compost and greywater	Soil touching at 75 days after plowing	4	100 mg[b]
		Greywater on leaf	Plants touching	4	1 ml[d]
Consumers	Eating	Greywater on leaf	Eating lettuce without wash	52	10.8 mL /100g[e] (eat lettuce of 12 g/day[f])
		Contaminated soil on leaf			0.6-2.3 (1.4 g) /100g* (eat lettuce of 12 g/day[f])

a: Nakagawa et al 2007; b: Mara et al. 2007; c: Ackerson and Awuah 2012; d: Maimon et al. 2010; e: Shuval et al. 1997; f: Seidu et al. 2008.
*obtained in the present study

Table 2. Parameters for the present risk assessment.

Constituent	Initial concentration		Concentration in soil		End-off kinetics in soil		Dose-response (Beta-Poisson)	
	compost [No./g-DW]	greywater [No./100mL]	compost [No./g-dry]	greywater [No./g-dry]	inactivation rate constant	T_{90}	α	N_{50}
Salmonella								
typical	4.9×10^{2}*	3.5×10*	2.2×10*	2.1*	4.1×10^{-2} for compost & no decay for greywater	(24±2)	0.3126[b]	23600[b]
worst	2.0×10^{3}	4.1×10^{2}	8.7×10^{2}	4.6×10				
Ascaris								
typical	3.4*	4.6×10^{-1}*	5×10^{-1}*	1.6×10^{-2}*	625[a] for compost & no decay for greywater		0.104[c]	859[c]
worst	27	9.8×10*	4	1.9×10^{-1}*				

a: WHO 2006; b: Haas et al. 1999; c: Navarro et al. 2009.
*obtained from the present mean of Enterococci with existed ratio in analyzed greywater and compost (pathogens/ Enterococci).

2. Result and Discussion

Microbial existence in pilot materials

Microbial existences and the concentration in compost and greywater, which collected from pilot sites, were summarized in Table 3. *Salmonella* and *Ascaris* eggs were rarely observed in pilot samples but these pathogens were existed in only one compost sample. The pathogens contaminated compost sample was used for further experiment in the present study. In the case of greywater, *Ascaris* eggs were not observed and *Salmonella* was sometimes. It has been reported that *Ascaris* eggs were frequently observed in fecal sludge collected from unsewered latrine in urban, Ghana, and the concentration in co-composting sample was initially 81 eggs/ g-dry sludge (Kone et al. 2007). In previous study by Sossou et al. (2014), we also observed existences of helminthes in collected feces in urban, Burkina Faso, and *Ascaris* concentration in compos with the fecal sample

Table 3. Initial concentration of indicators and pathogens in collected materials.

	Compost [No./g-dry]			Greywater [No./100mL]	
	Range	Amended sample	Mean	Range	Mean
E. coli [log_{10}CFU/unit]	1.9-7.7	5.1	4.2	2.8-6.1	4.7
Fecal coliform [log_{10} CFU/unit]	2.9-5.6	4.1	4.1	3.6-7.5	5.3
Enterococci [log_{10} CFU/unit]	4.1-7.8	6.2	5.6	2.7-6.8	4.4
Salmonella [log_{10} MPN/unit]	*n.d.* -3.3	3.3	-	*n.d.* -2.6	-

was initially 106 eggs/g-dry compost. Comparing to these reports, the concentration and existent frequency of *Ascaris* was lower in the present. This may be influenced by food culture in rural area, Burkina Faso, since population in the rural rarely eat fresh vegetables and most of meal was boiled at once. Concerning the geographic context, therefore, the present data was used for the QMRA as a current rural model.

Microbial reduction in compost and greywater reused fields

Concentrations of *E. coli*, fecal *Enterococci* and *Salmonella* in C+T plots were significantly decreased (Figure 1). For C+G plots, the significant decrease was observed in *E. coli* and *Salmonella* but not in *Enterococci*. For NPK+G plots, the concentrations of *E. coli, Enterococci* and *Salmonella* were not changed significantly and maintained at the level of 1-3 log and 1-2 log, respectively. For control plots of NPK+T, *E. coli*, *Enterococci* and *Salmonella* were rarely detected. Regarding on fecal coliform, the clear tendency was not observed in all treatments and the concentration of log 2-3 was detected even in NPK+T plots. From these results, it

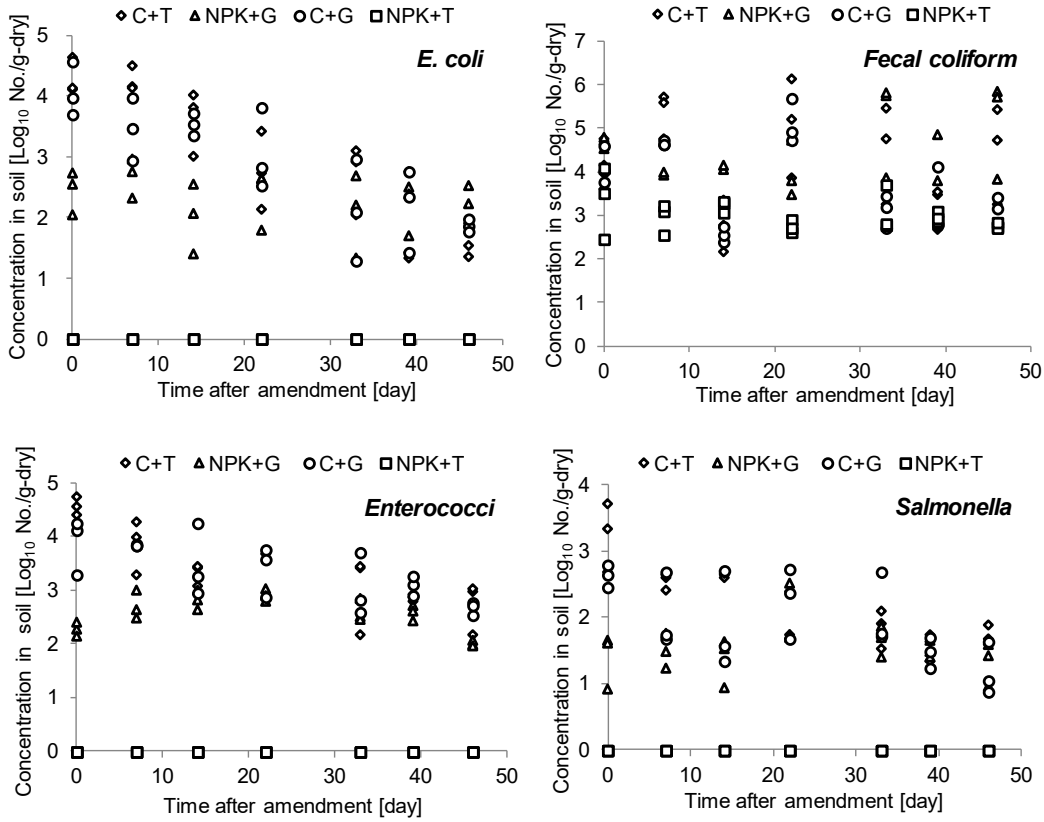

Figure 1. Fate of bacteria in field soil.
C+T, compost amended plots; NPK+G, greywater irrigated plots; C+G, compost and greywater applied plots; NPK+T, chemical fertilizer and tap water applied plots.

was suggested that (i) bacterial concentrations in C+T supposed to be modeled by die-off kinetics (ii) the concentrations in NPK+G was mainly maintained due to continuous greywater irrigation and the concentration would be affected by greywater concentration (iii) the concentrations in C+G might be difficult to fit the simple die-off kinetics but it would be decreased at first and maintained at the same level of NPK+G at the end. In addition, it would be difficult to apply fecal coliform value as an indicator of enteric pathogens for the risk assessment related to soil pathway.

Ratios of *E. coli*, *Enterococci* and *Salmonella* in C+T plots were significantly correlated and fitted to a linier model (Figure 2). Inactivation rate constants for the three bacteria were $5.8\pm0.4\times10^{-2}$, $4.7\pm0.4\times10^{-2}$ and $4.1\pm0.4\times10^{-2}$, respectively. Using the obtained kinetics, times for 90% inactivation (T_{90} value) for the three bacteria were calculated to 17±1, 21±2 and 24±2 days, respectively. The T_{90} values were relatively rapid than reference values of 25±5 for *E. coli* and 35±5 for *Salmonella* in soil (WHO 2006). This difference might be caused by dried and high temperature in Soudano-Sahelian climate. Furthermore, two-way ANOVA and post-hoc test by Tukey HSD showed that the time dependent change of *E. coli* and *Salmonella* ratio was significantly different but not in *E. coli* v.s. *Enterococci* and *Salmonella* v.s. *Enterococci*. This statistic result suggested that it would be better to apply original end-off kinetics of *Salmonella* or that of more tolerable bacterial indicator than *E. coli* for *Salmonella* infection risk related to soil. The possible indicator may be *Enterococci* or *Enterococcus* feacalis as its represent. Although it has been reported that the bacterial group regrows in composting process and was recommended to be eliminated as an indicator for *Salmonella* (Tønner-Klank et al. 2007), the

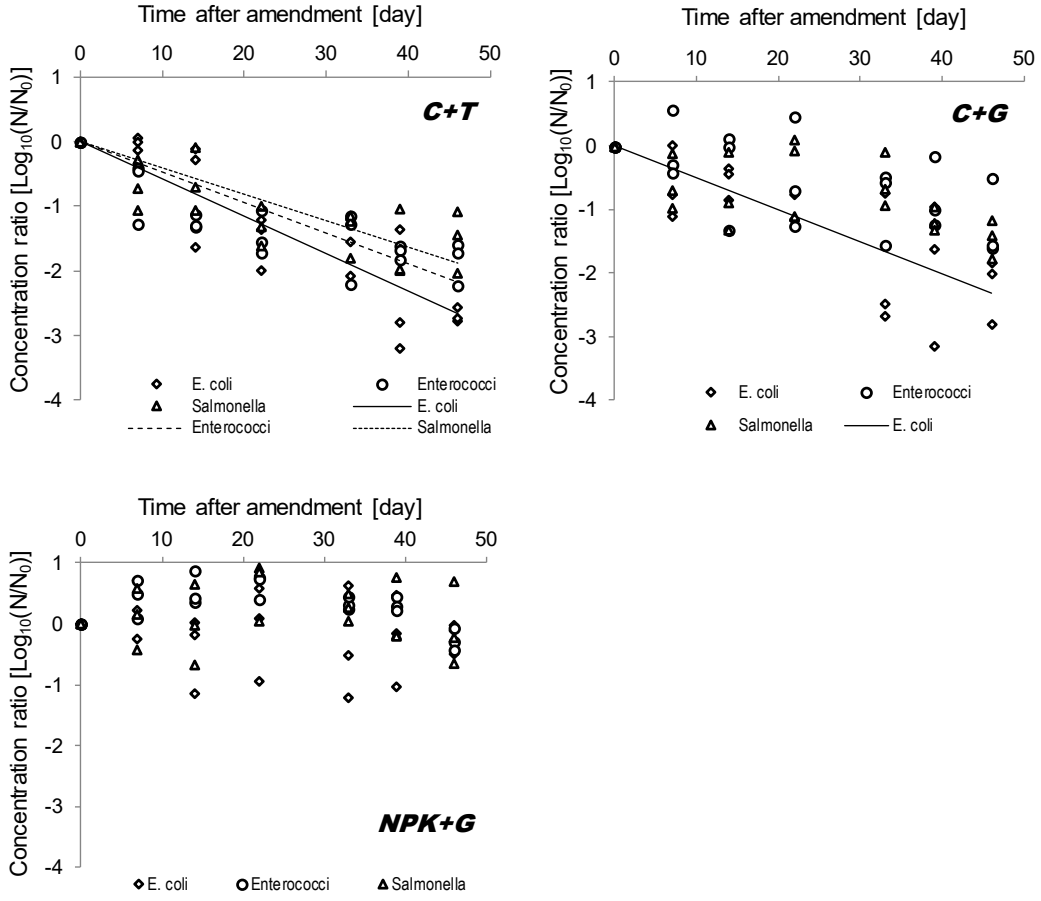

Figure 2. Time dependent changes of bacterial concentration ratio in compost amended plots (C+T), compost and greywater applied plots (C+G) and greywater irrigated plots (NPK+G).
Lines in the figures represents significant model fitting on approximate linier ($p < 0.05$).

tolerability is higher than *E. coli* (Christensen et al. 2002) and the fate is similar with *Salmonella* in greywater (Ottoson and Stenström 2003). In the present study, additionally, regrowth and unclear fate of the group was not observed in soil system and the end-off kinetics was not significant difference with *Salmonella*.

Concentration of *Ascaris* eggs in compost amended soil was initially 4.0±0.4 eggs/g dry soil (Figure 3). The count was not observed in NPK+G and NPK+T plots. Although the count was missed in sometimes probably due to ununiformity of amended compost and low diffusivity of the eggs in the soil, an obvious outlier was not found in the present data set except not detected. Because of the fluctuated data set, the correlation and model fitting was not significant. Therefore, only the initial concentration was used for the present QMRA but die-off kinetics was extrapolated from reference data (WHO 2006).

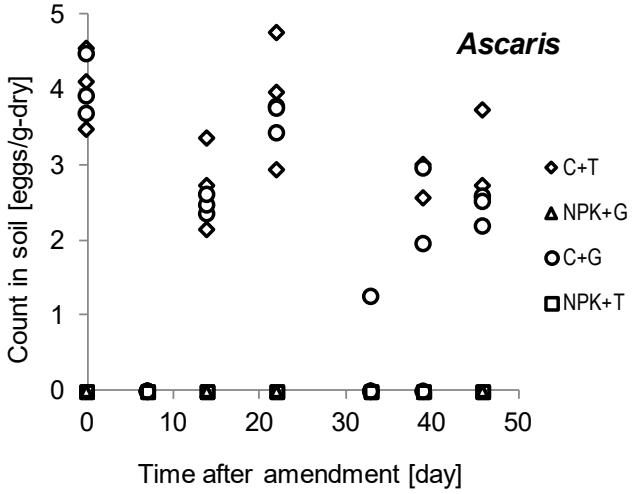

Figure 3. Concentration of Ascaris eggs in field soils.

Quantitative microbial risk assessment

Parameters used in the present QMRA was summarized in Table 2. The obtained data in the field was filled in parameters for *Salmonella* and *Ascaris* risk as a worst case since these microorganisms were rarely observed in the present samples. Parameters for a typical case of these two microorganisms were calculated with means of fecal *Enterococci* concentration in the present samples and existence ratio (pathogens/ *Enterococci*). Parameters for rotavirus risk were calculated with mean (as typical case) and maximum (as worst case) of *E. coli* concentration, using a ratio of rotavirus (1/100,000 *E. coli*) (Mara et al. 2007).

All annual risks by the three pathogens for farmers were exceeded to the target tolerable risks in both typical and worst case (Figure 4). Furthermore, the annual risks for consumers were also exceeded to the target tolerable risks excepting *Salmonella* risk in compost reuse (Figure 5). These results suggested that further investigation for greywater treatment system and compost handling was recommended to improve the safety management from both technical and practical views. Regarding the risks in compost reuse, Schönning et al. (2007) has recommended human manure storage for 6-12 months for adequate handling of urine diverting dry toilet. For mixing type composting toilets, the adequate maturation period would be shorter, since *Ascaris* eggs in co-composting with agricultural bulk agents in the toilet decreased from 106 eggs/g-dry to 1 eggs/g-dry for 2 months (Sossou et al. 2014). To shorten the maturation period, addition of alkaline agents such as lime and ash (Newabaga et al. 2009; Kazama and Otaki 2011), co-composting with urea (Nordin et al. 2009) and solar heating (Redlinger et al. 2001) would be further solution as post inactivation treatments. The present result also indicated that risk probabilities for greywater reuse were almost similar magnitude level with compost

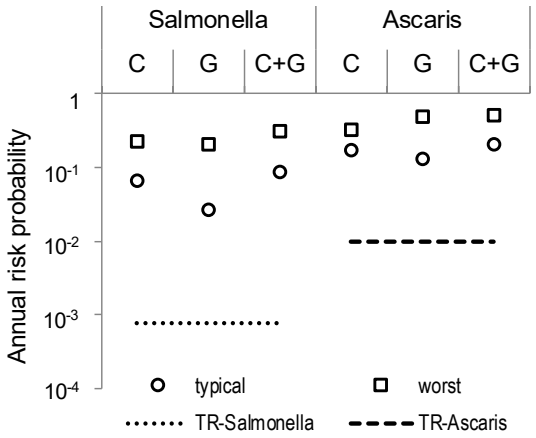

Figure 4. Annual risk probability for famers in a typical and worst case.
TR, tolerable risks corresponded to DALY 10^{-6} reduction.

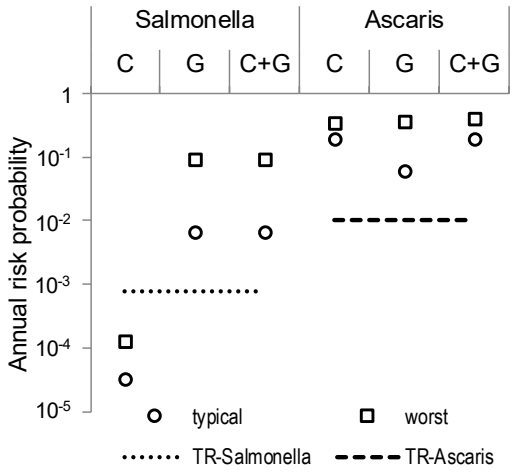

Figure 5. Annual risk probability for consumers in a typical and worst case.
TR, tolerable risks corresponded to DALY 10^{-6} reduction.

reuse. One possible explanation may be higher exposure frequency of greywater even though the contaminant concentration was lower. In Soudano-Sahelian climate, more frequent irrigation is necessary due to high evapotranspiration, particularly in dry season. The geographical characteristics might elevate the microbial risk of greywater reuse. It is well known that drip and subsurface irrigation system is effective both water saving and hygienic risk reducing. More cost effectively, using watering cans with caps also reduce on their spouts reduces water intensity of splashes (Seidu et al. 2008).

Annual risk probabilities by different matrix in compost and greywater reuse were presented in Figure 6. The result showed that effect of contaminated soil on the risks was lower than direct handling of greywater and compost but not negligible. Similar finding has been reported by Seidu et al. (2008) which pointed that on farm soil contamination is the significant health hazard in wastewater reuse for lettuce gardens. Some reports for QMRA have assumed that feces amended soil or greywater irrigated soil are same contaminant concentration with the original materials (Schönning et al. 2007; Mara et al. 2007; Maimon et al. 2010). From the field observation in the present, however, the concentration of indicator was lower than that of original materials. For instance, *Enterococci* ratio of compost and its amended soil was approximately 0.184 (soil/compost) and the ratio of greywater and irrigated soil was approximately 0.014 (soil/greywater). This is probably due to

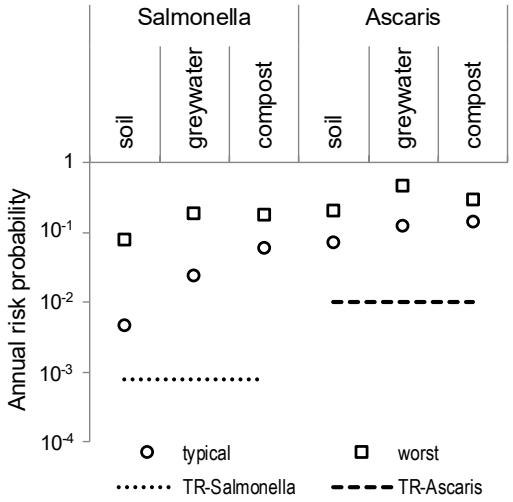

Figure 6. Annual risk probability for famers mediated from contaminated soil, greywater and compost.
TR, tolerable risks corresponded to DALY 10^{-6} reduction.

dilution of compost and permeability of greywater. These ratios would be changed by soil properties and input concentrations. Therefore, fate of indicators in soil and effect of the soil concentration on that on leafs should be further investigated.

Conclusion

The present study evaluated fates of indicator and pathogens originated from greywater and compost in soil to assess microbial risks for production cycle of combined resource oriented sanitation system. The results suggested that (i) bacterial fates in compost reuse were fitted to log normal linier and those in greywater were maintained in field soil, (ii) the bacterial end-off kinetics in Soudano-Sahelian climate were more rapid than that of reference values, (iii) the reduction of *E. coli* and *Salmonella* in the present condition was significantly different but that of *Salmonella* and fecal *Enterococci* had no difference, (iv) fecal coliform did not play a role of indicator in soil and (v) effect of contaminated soil on annual risk probability was lower than direct handling of greywater and compost but not negligible. The present QMRA also suggested that annual risks of current on-site resource oriented sanitation system were exceeded to tolerable risks. Therefore, on farm handling of compost and greywater should be further investigated from practical and technical views.

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