Journal of Agricultural Chemistry and Environment, 2020, 9, 59-72
https://www.scirp.org/journal/jacen
ISSN Online: 2325-744X
ISSN Print: 2325-7458

Health Risk Assessment of Compost-Amended Soils

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How to cite this paper: Darimani, H.S. and Ito, R. (2020) Health Risk Assessment of Compost-Amended Soils. *Journal of Agricultural Chemistry and Environment*, **9**, 59-72.
https://doi.org/10.4236/jacen.2020.92006

Received: March 13, 2020
Accepted: May 4, 2020
Published: May 7, 2020

Abstract

Application of improperly treated compost from composting toilet is one of the causes of bacterial contamination in the field, crops, food and water. The study characterized the die-off represented by kinetic inactivation rate coefficient, k (h^{-1}) of *Enterococcus* in clay and sandy loam soils, determined the effect of temperature, compost-to-soil ratio and soil type on the inactivation rates of *Enterococcus* and evaluated the health risk associated with the amendment of compost from composting toilet in real conditions using local climatic data. The soils were amended with compost to soil ratios of 1:10, 1:25, 1:50 and 1:100 held at different temperatures (30˚C, 40˚C and 50˚C). Inactivation of *Enterococcus* (pathogenic bacteria) in the soil with high temperature under different compost application rates was tried in the laboratory test and the Quantitative Microbial Health Risk evaluated. The study results indicated the inactivation rates of *Enterococcus* in clay soils as 0.015 - 0.027 h^{-1}, 0.246 - 0.322 h^{-1}, 0.397 - 0.571 h^{-1} whilst sandy loam soils recorded 0.056 - 0.130 h^{-1}, 0.348 - 0.447 h^{-1} and 0.475 - 0.630 h^{-1} for 30˚C, 40˚C and 50˚C respectively. Inactivation rates of *Enterococcus* in soils amended with compost from the composting toilet depended on temperature and soil type but not on the compost-to-soil ratios and compost from the composting toilet amended to the soils is safe for use in six (6) days.

Keywords

Risk Assessment, *Enterococcus*, Composting Toilet, Soil System, Inactivation Rate Coefficient

1. Introduction

The rural model of composting toilet has been designed and installed in consideration of local material availability and affordability [1]. Although the com-

posting process reduces enteric pathogens [2] [3] the compost removed from the toilet has a potential to trap pathogens derived from infected persons, which increases the possibility for users and farmers to become infected [4]. Reuse guidelines for human excreta has been published by WHO (2006) [5] and also optional post-treatments such as solar heating, drying [3] [6] and increasing pH with alkaline treatment by lime or ash [7] [8] have been investigated to ensure pathogen inactivation. Increasing temperature of the soil layer amended with compost by solarisation also occurs slight drying of the surface of the layer owing to low moisture content of soil layer in arid area. Therefore, thermal treatment may be the main process of inactivation in this case. In a practical view, however, these guidelines and the practice may be misapplied sometimes due to local situations such as lack of attention by traditional users, labour ineffectiveness and lack of materials. In the case of on-site individual composting toilet, particularly, the improperly treated compost contains high levels of enteric bacterial indicators as previously observed [6]. When the improperly treated compost is amended to the field, the soil, possibly intermediates several exposure pathways; contamination in ground water, attachment on vegetable and farmer's ingestion of the contaminated soil. Assuming the improperly treated compost amendment as a worst case, the fate of pathogens in the soil after amendment of compost should be investigated to consider the overall control of pathogens. Little information exists on inactivation of bacteria in compost-amended soils.

Comprehensive information on die-off periods of several pathogens in the soil-plant-waste system has been previously reported [9]. The die-off periods for these organisms range from 30 mins to several years [10]. This makes it difficult to apply it to a particular case. A number of factors are known to influence the inactivation of pathogens and the indicator organism in a soil-waste system: waste pre-treatment, moisture, temperature, sunlight, pH, competitive organisms, available nutrients, organic matter, method and time of application of waste and soil type [9]. On the other hand, there is little information regarding the characterisation of the die-off in soil such as clay and sandy loam in the hot climates of rural communities, where reuse of the compost is expected. Inactivation rate coefficient, k (h^{-1}), would be useful for researchers developing a management type of model to simulate the behaviour of bacteria in the soil system [10]. *Enterococcus* was used as a model for pathogenic bacteria in this experiment to assess the hygienic quality of the compost because it occurs in high numbers in the intestine and associated with human faeces, and has been known as relatively high tolerant bacteria for the environmental conditions [11]. It is often used to determine water quality and waste product quality [12].

The study by Kagambéga *et al.* [13] indicated that wild animals can share the same *Salmonella* serotypes in humans and potentially transmit some of them to humans. As the humans and animals often live in close vicinity in rural Africa and the hygiene control of the meat retail chain is defective, high *Salmonella* carriage rates of the animals can pose a major public health risk in Africa. This underlines the necessity for a joint and coordinated surveillance and monitoring

programs for *salmonellosis* in Africa. *Salmonella* has the potential of re-growth of bacteria under lower temperature conditions. Considering this increase in concentration under lower temperature and the fact that it is a public health concern in Burkina Faso, the health risk of *Salmonella* in the soil should be studied to determine the health risk for the reuse of compost after the post-treatment.

Considering actual practices, solarisation is one of the main processes for disinfection of enteric pathogens, because sunlight could be obtained in any place of farmland. The solarisation relates to the ambient temperature, while the temperature is not constant as shown in Figure 1. Farmland generally has several types of soil. Farmers sometimes change the composition of fertilizers resulting in change of ratio of compost in soil. These factors might affect the inactivation of pathogens in the soil. Therefore, the objectives of this study were 1) to characterise the die-off represented by the kinetic inactivation rate coefficient of *Enterococcus* in soil system amended with compost from the composting toilet, 2) to determine the effect of temperature, compost-to-soil ratio and soil type on the inactivation rates of *Enterococcus*, 3) to determine the health risk of *Salmonella* in the soil system after the amendment of compost.

2. Materials and Methods

2.1. Compost and Soil Preparation

Compost used for the experiment was collected from a pilot site installed in a family in Kamboinse, Burkina Faso. Nine people use the toilet and the compost has been used for 8 months on the site. Soil samples (Clay and sandy loam) were taken from the Kamboinse pilot experimental site on the campus of the International Institute for Water and Environmental Engineering (2iE). The characteristics of the sandy loam and clay soils used are described in Table 1. Moisture contents, *MC* (%), in dry basis for both compost and soils were determined by drying 5 g of the compost/soil sample at 105˚C for 24 h in an oven (manufactured by Memmert GmbH) and described by the equation below:

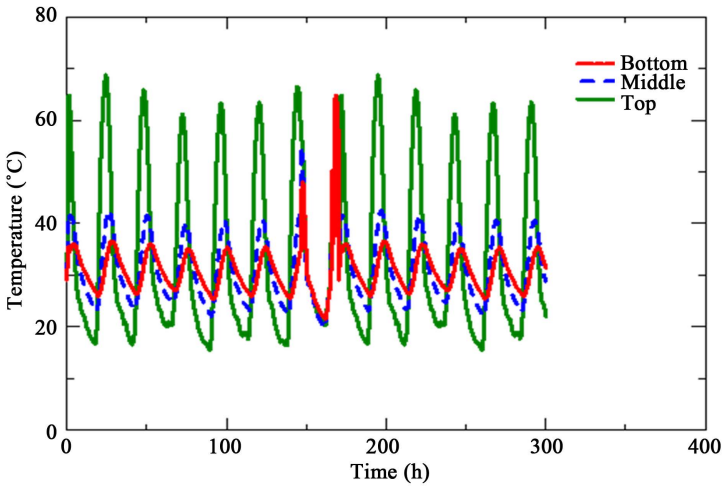


Figure 1. Temperature distribution in the soil system in Ouagadougou, Burkina Faso.

Table 1. Characteristics of sandy loam and clay soils.

Particle size (%)	Sandy loam soil	Clay soil
Silt	23	31
Fine sand	23	9
Coarse Sand	35	2
Total N	0.04 ± 0.01	NM
C	0.54 ± 0.02	NM
SOM	0.93 ± 0.03	NM

**NM, not measured.

$$MC = \frac{W_{wet} - W_{dry}}{W_{wet}} \times 100 \quad (1)$$

where, W_{wet} and W_{dry} are wet and dry weights of compost sample (g). The moisture content was set to 25% with ultra-pure water, because the moisture content at field capacity for sandy loam and clay soils are 14.7% and 22.6% respectively [14].

2.2. Compost Amendment and Inoculations

Enterococcus ATCC 19433 strain was purchased from American Type Culture Collection (ATCC) and was grown in a 10 ml Nutritif Nutrient broth (Difco, France) by incubating at 37˚C over night. Zero-point three millilitre (0.3 ml) of the broth solution was inoculated into 20 g of the compost by a pipette on the surface and then mixed for 5 mins with a sterile spatula to homogeneously distribute the *Enterococcus.* The concentration of *Enterococcus* in the inoculated solution was about 10^6 CFU/ml. After 3 h, each 20 g-dry of the inoculated compost was mixed well with the soil at the specified ratios described in **Table 2**. We checked the pH of the compost-amended soils and the compost with following protocol by adding 5 g of the sample in 250 ml of distilled water. The suspension was allowed to settle for 5 mins. The pH of the liquid was determined by hand held multi-parameter monitor (WTW 330i, WTW GmbH, Germany) with composite pH sensor (Sen Tix 41, WTW GmbH, Germany). The pH of the liquids ranged from 7.06 to 7.18. The amended soils were placed in sterilized bottles with lids (1 litre, 2 litre and 4 litre bottles) then mixed for 5 mins with a sterile spatula to homogeneously distribute the compost over the soil. The bottles were tightly closed and immediately put into an incubator to keep the temperature constant. Ten grams of the sample were taken from each bottle every 2 hours for biological analysis.

2.3. Bacteria Extraction and Measurement

Enterococcus was cultured following the modification of method 9215A in Standard Methods for the Examination of Water and Wastewater [15]. Bacteria were extracted from the soil samples with buffered peptone water. The composition of buffered peptone water in g/litre is Tryptone 10.0, Sodium Chloride 5.0,

Table 2. Description of compost-to-ratio in dry weight.

Samples	Temperatures (˚C)		
	30	40	50
Compost-to-soil ratio (g/g)	Corresponding quantity ratio of compost on soil (g/g)		
1:10	20:200		
1:25	20:500		
1:50	20:1000		
1:100	20:2000		

Disodium Phosphate Anhydrous 3.5, and Potassium Dihydrogen Phosphate 1.5. Ten grams of the compost sample were added to a 90 ml volume of peptone water and agitated for 3 mins with vortex mixer. After adequate dilution (10^1 - 10^7 times) with sterilized Ringer solution, each diluted extract was isolated in Chromocult coliform ES agar (Merck KGaA) by simple layer method. The media were incubated at 37˚C for 24 h, and then, *Enterococcus* colonies were counted. The limit of detection of bacteria was 10 CFU/g.

2.4. Data Analysis

Concentration versus time data obtained from the inactivation experiments were fitted to a first order kinetic model. Nakagawa *et al.* [16] indicated that inactivation of microorganisms follows a first order reaction and it is expressed as:

$$\ln\left(C/C_o\right) = -kt \tag{2}$$

where, C is concentration of microorganism in soil sample in dry basis at time, t (CFU/g-dry solid), C_o is initial concentration of microorganisms in soil samples in dry basis (CFU/g-dry solid), t is reaction time (h). After the estimation of inactivation rate coefficients, k, we tried to evaluate the effect of temperature with the Arrhenius equation described as follows;

$$k = A\exp\left(-\frac{E_a}{RT}\right) \tag{3}$$

where, A is pre-exponential factor (h^{-1}), E_a is activation energy (J/mol), R is the universal gas constant (J/mol/K), T is the temperature (K). A statistical study (nonparametric Kruskal-Wallis test) was carried out to determine significant difference ($p \leq 0.05$) in temperature, the compost-to-soil ratio and the soil type. The analysis was done with IBM SPSS, version 12.0 (IBM Corporation).

2.5. Estimation of Risk of *Salmonella* during Amendment in the Soil

Quantitative Microbial Risk Assessment (QMRA) was used to predict the likelihood of *salmonellosis* transmission during the amendment of compost. The farmer exposure was predicted via a Monte Carlo technique in three scenarios: bottom, middle and top temperature of the soil. The β-Poisson equation was used to assess the dose response of *salmonellosis*. The N_{50} and α used are 17,700

and 0.23475, respectively. The random number is applied for estimation of variables with distributions for simulation with the appropriate equations. The simulation was repeated 10,000 times [17]. Then, 95 percentile of the probability was estimated as the infection risk.

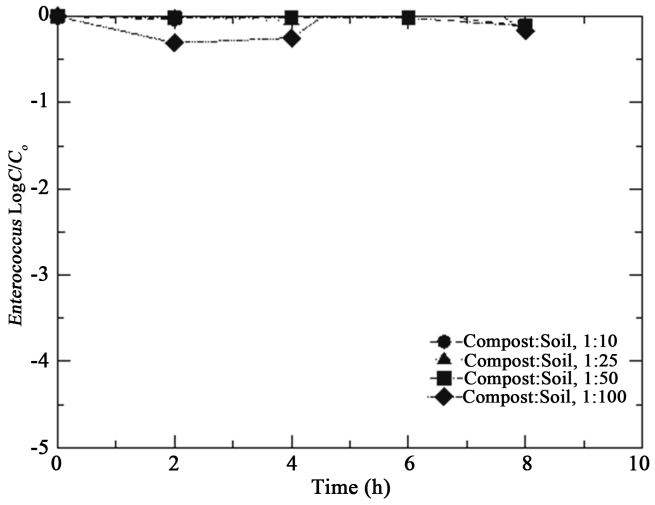
2.6. Temperature Distribution

One-week temperature was measured during February, 2015 in the soil with the aid of ThermoManager sensors. They were placed in the soil at the bottom, middle and top at 10 cm, 5 cm and 1 cm respectively. The temperature distribution is show in **Figure 1** below. The fluctuation of the temperature distribution is observed for the bottom, middle and top respectively. This rise and fall of the temperature is as a result of the day and night.

3. Results and Discussions

Enterococcus concentration in the compost amended clay soils at 30˚C for 8 h did not record any change in concentration for all ratios, as shown in **Figure 2(a)**. **Figure 2(b)** illustrates the inactivation of *Enterococcus* at 40˚C. The concentration decreased in the different compost-to-clay-soil ratio. The ratio 1:10 recorded < 1 log, 1:25 recorded 1.2 log, 1:50 and 1:100 recorded 1 log reduction in 8 hours. The regression coefficient, R^2 for the compost-soil ratios 1:10, 1:25, 1:50 and 1:100 were 0.98, 0.60, 0.81, 0.84 respectively. The declines in the concentrations of *Enterococcus* at 50˚C are represented in **Figure 2(c)**, and the ratio 1:10 and 1:100 recorded 1.5 log reduction, while the ratio 1:50 and 1:100 recorded 1.8 and 2 log in 8 h. The regression coefficient, R^2 for the compost-soil ratios 1:10, 1:25, 1:50 and 1:100 were 0.60, 0.81, 0.85, 0.82 respectively. In the compost amended sandy loam soil, the concentration of *Enterococcus* recorded < 1 log reduction in 8 hours at 30˚C for all ratios, as shown in **Figure 3(a)**. Decline in concentration of *Enterococcus* was observed at 40˚C by 1 log, 1.2 log, 1.5 log and 1.6 log reductions in 8 hours for compost amended sandy loam soil formulations 1:10, 1:25, 1:50, and 1:100, respectively (**Figure 3(b)**). The regression coefficient, R^2 for the compost-soil ratios 1:10, 1:25, 1:50 and 1:100 were 0.99, 0.83, 0.93, 0.87 respectively. **Figure 3(c)** indicates *Enterococcus* decreased at 50˚C by 1.4 log, 1.8 log, 2.4 log and 2.5 log in 8 hours for the 1:10, 1:25, 1:50, and 1:100, respectively. The regression coefficient, R^2 for the compost-soil ratios 1:10, 1:25, 1:50 and 1:100 were 0.96, 0.98, 0.88, 0.90 respectively. **Table 3** summarises the inactivation rate coefficients. Arrhenius plots for *Enterococcus* inactivation for both clay soil and sandy loam soil are respectively shown in **Figure 4** and **Figure 5**. Other factors influencing the die-off of pathogenic bacteria in the soil are soil composition and pH [18]. Under field conditions, other variables, such as solar radiation and dryness, may also affect the survival of pathogens [14].

Increase in temperature showed to increase the inactivation rate of all soil types. The first order kinetics inactivation rate coefficient, k, of *Enterococcus*



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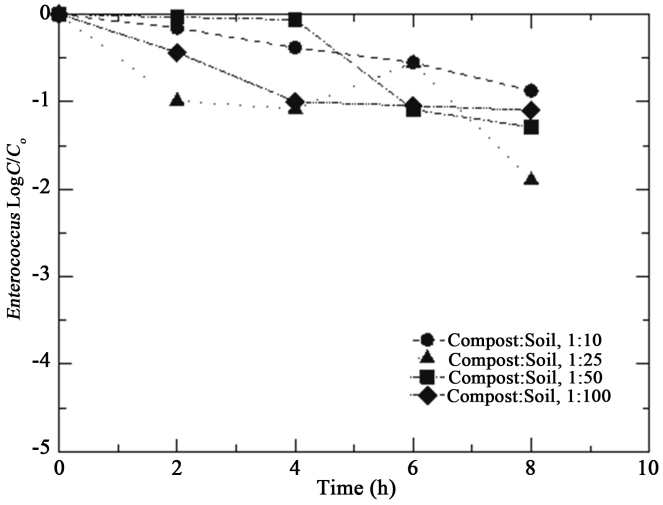

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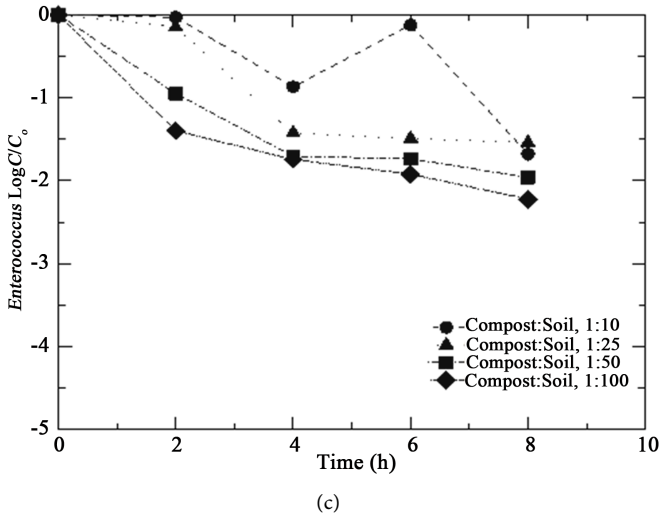
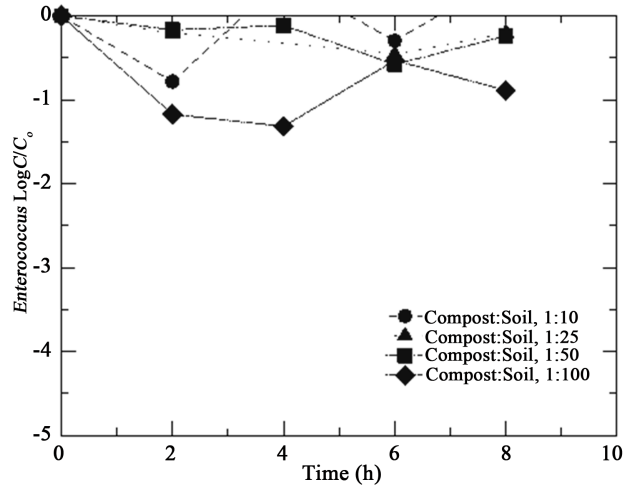


(c)

Figure 2. (a) Inactivation of *Enterococcus* in compost amended clay soils at 30˚C; (b) Inactivation of *Enterococcus* in compost amended clay soils at 40˚C; (c) Inactivation of *Enterococcus* in compost amended clay soils 50˚C.



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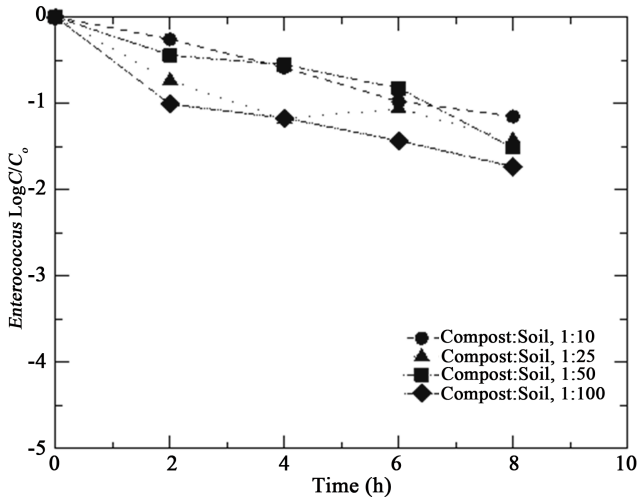

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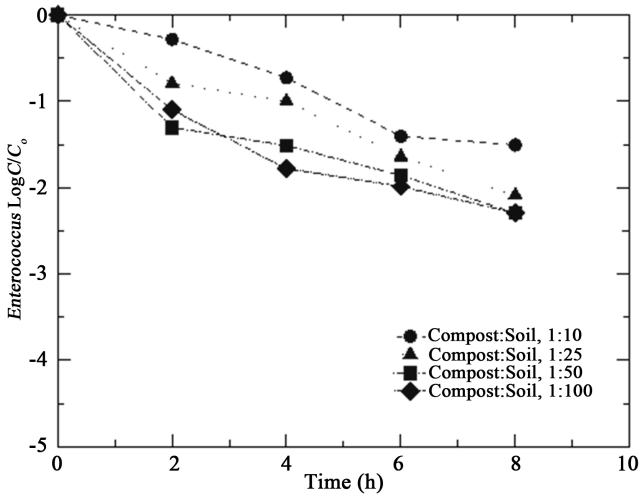

(c)

Figure 3. (a) Inactivation of *Enterococcus* in compost amended sandy loam soils at 30˚C; (b) Inactivation of *Enterococcus* in compost amended sandy loam soils at 40˚C; (c) Inactivation of *Enterococcus* in compost amended clay soils at 50˚C.

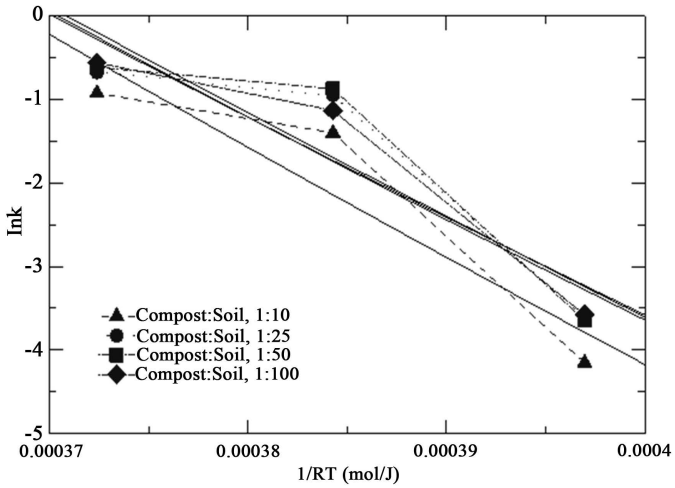

Figure 4. Effect of temperature on *Enterococcus* for compost amended clay soil.

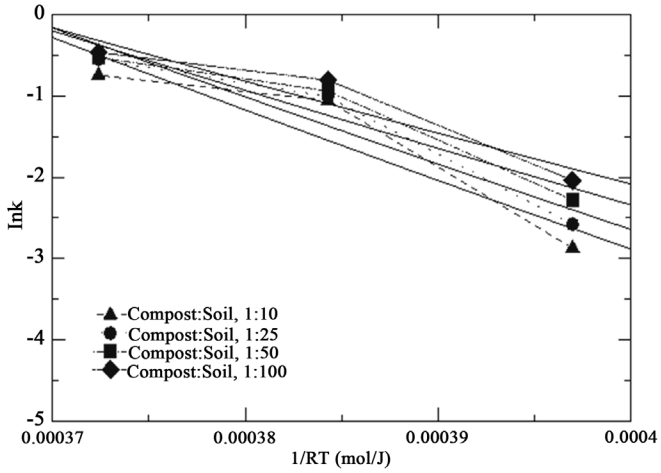

Figure 5. Effect of temperature on *Enterococcus* for compost amended sandy loam soils.

Table 3. Summary of first order kinetics inactivation rate coefficient k (h^{-1}) for *Enterococcus*.

Sample	Temperature (˚C)		
	30	40	50
	First order kinetic inactivation rate coefficient k (h^{-1}) *Enterococcus*		
Clay soil ratio of compost to soils (g/g)			
1:10	0.015	0.246	0.397
1:25	0.027	0.386	0.509
1:50	0.026	0.418	0.541
1:100	0.027	0.322	0.571
Sandy loam soil ratio of compost to soils (g/g)			
1:10	0.056	0.348	0.475
1:25	0.075	0.365	0.578
1:50	0.102	0.392	0.591
1:100	0.130	0.447	0.630

increased with temperature as summarised in (Table 3). Several studies reported the effect of temperature on bacterial inactivation rate [6] [12] [19], indicating that, higher temperature decreasing the survival time of faecal bacteria. Our results showed that inactivation rate coefficient k, values increased as temperature increased from 30˚C, 40˚C to 50˚C. The Kruskal-Wallis test (Table 4) results showed that there was an effect on the performance of different temperatures on *Enterococcus* inactivation. The effect of temperature on the die-off of *Enterococcus* was statistically significant (Kruskal-Wallis test, $p < 0.05$) for sandy loam and clay soils (Kruskal-Wallis test, $p < 0.05$). Arrhenius plots for *Enterococcus* inactivation is shown in Figure 4 and Figure 5. The solid lines are trend lines expressing the effect of temperature on *Enterococcus* at different compost-to-soil ratio. There were strong correlations with temperatures, thus the effect of temperature on the inactivation of *Enterococcus* is described with the trend lines.

Enteric bacteria have a faster die-off in soils possessing a high pH with pH of 6 to 7 being optimum for bacteria survival, and dying quickly under acid soil conditions [10]. In our study pH of compost-amended soils ranged from 7.06 to 7.18 and hence could not cause the die-off of *Enterococcus* because pH levels are good for bacterial survival in soils.

Results showed that, the type of soil influenced the inactivation rate of *Enterococcus*. The inactivation rate coefficient in all compost-amended sandy loam soil formulations was higher than the clay soil formulations. This indicated that the soil type had an influence on the survival of bacteria. Soil type affected the die-off rate because finer soils, especially, clay minerals and humic substances increase the survival of bacteria [20] [21]. Survival of *E. coli* O157:H7 in finer-textured soils (such as the ones rich in clay) resulted in a greater survival rate of coarse-textured soils (Sandy soils) [22]. The clay particles used in the experiments were smaller than the sandy loam soil. Thus, the larger particle size distribution of the sandy loam soil probably increased the inactivation rates. Clay content increased the survival of *Enterococcus* comparatively. The statistical results indicated that inactivation rates of the clay soil (Kruskal-Wallis test, $p < 0.05$; $p = 0.01$) were lesser than sandy loam (Kruskal-Wallis test, $p < 0.05$; $p = 0.007$) in all temperatures. Jamieson *et al.* [23] reported that the single soil property that appears to have the greatest impact on bacterial survival is moisture retention, which is linked to particle size distribution and organic matter content. The capacity to remove organism increases with the decrease in soil-water content. Laboratory and field experiments have shown that many soils have high

Table 4. Kruskal-Wallis test for *Enterococcus*.

	Temperature			Compost to soil ratio			
	Temp_30	Temp_40	Temp_50	1:10	1:25	1:50	1:100
	Mean	Mean	Mean	Mean	Mean	Mean	Mean
Clay soils	0.024_a	0.343_b	0.504_c	0.219_a	0.306_a	0.328_a	0.307_a
Sandy loam soils	0.091_a	0.388_b	0.569_c	0.293_a	0.339_a	0.362_a	0.402_a

retention capacity for bacteria [19]. Retention of bacteria increased with an increase in clay content, cation exchanged capacity of the soil and specific surface area [23]. Another study by Mubiru *et al.* [24] compared the mortality of *E. coli* O157:H7 in two different soil types. They indicated that reduced mortality was primarily influenced by soil type, with soils exhibiting a higher metric potential, showing lower mortality rates. They also stated that as well as enhancing moisture retention, fine soil particles could increase bacterial survival because of an increased ability to retain nutrients. Reddy *et al.* [10] reported that retention of organism is enhanced when the clay is present.

Compost-to-soil ratio showed a variation on the inactivation rate coefficient in our study. In the compost-amended clay soil formulations, the inactivation rate constant values were higher when the soil volume was increased (**Table 3**). This showed that high compost applications recorded lesser inactivation rate coefficient than low compost applications. We found that the clay soil formulation 1:50 at 30˚C and 1:100 at 40˚C values looks deviated a little from the trend observed in 50˚C probably due to the mixing of *Enterococcus* strain with the clay soil.

The statistical results, however, showed that there was no effect among the performance of the different application rate of the compost. Results showed that the differences of compost-to-soil ratio on *Enterococcus* inactivation was not statistically significant Kruskal-Wallis test, $p > 0.05$ for both sandy loam and clay soils. Crane *et al.* [25] followed the die-off of indicator organisms in surface applied poultry manure and indicated that the rate of manure application had no influence on bacterial survival and this conformed to our study.

The average diurnal ambient temperature in Ouagadougou varied from 36˚C to 47.2˚C, these temperatures could reduce the concentration of pathogens in an improperly treated compost amended to the soil as fertilizer to minimise the serious health risk as previously observed [26]. Temperature and humidity change over time due to strong sunlight under field conditions. Therefore, care should be taken in interpreting our results with field studies.

The study simulated the conditions in real situations with the measured temperature in the soil. The result of the change in concentration and annual risk of *Salmonella* in the soil was estimated. **Figure 6** and **Figure 7** shows the change in *Salmonella* concentration and annual risk of infection. The change in concentration and annual risk after 24 h of post-treatment were estimated. The change in concentration after the 24 h for the bottom, middle and top were 2.8×10^{5}, 6.37×10^{6} and 4.03×10^{-15} respectively. Amending of the compost will be done at this point. An assumption of the dilution of compost to the soil ratio was 0.01. The change in concentration at this dilution for the bottom, middle and top soil were 2.8×10^{3}, 6.37×10^{4} and 4.03×10^{-17} respectively. The annual risk of infection at this point was 1 for the top, and bottom and safe level at the top. The bottom and middle temperatures achieved a safe level at 147.5 h with risk values of 1.2×10^{-4} and 1.05×10^{-4} pppy respectively. The top temperature distribution achieved a safe level after the 24 h.

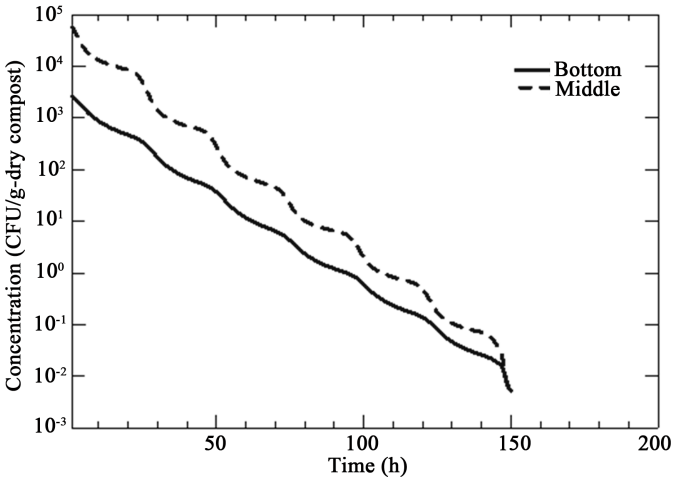

Figure 6. Change in concentration of *Salmonella* at different depth in the soil.

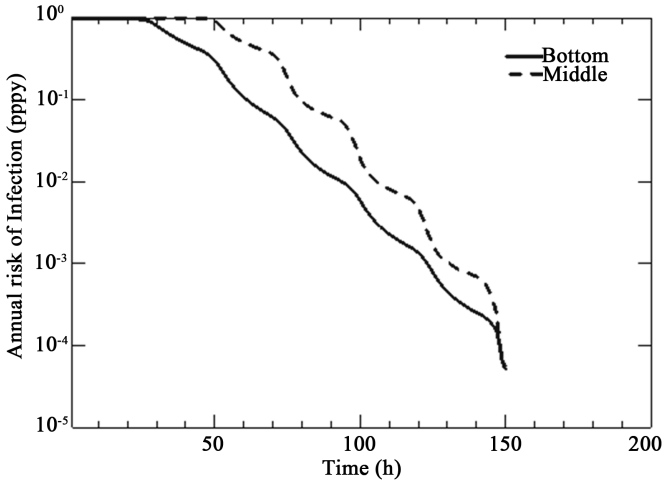

Figure 7. Annual risk of *Salmonella* at different depth in the soil.

4. Summary

Inactivation of *Enterococcus* (pathogenic bacteria) in the soil with high temperature under different compost application rates was tried in the laboratory test. As a result, 1) the inactivation rates of *Enterococcus* in clay soils were 0.015 - 0.027 h^{-1}, 0.246 - 0.322 h^{-1}, 0.397 - 0.571 h^{-1} for 30˚C, 40˚C and 50˚C, respectively. Sandy loam soils were 0.056 - 0.130 h^{-1}, 0.348 - 0.447 h^{-1} and 0.475 - 0.630 h^{-1} for 30˚C, 40˚C and 50˚C, respectively. 2) Inactivation rates of *Enterococcus* in soils amended with compost from the composting toilet depended on temperature and soil type, but not on the compost to soil ratios. This study would be a useful information for researchers and farmers to understand the behaviour of pathogenic bacteria in the sandy loam and clay soils. The experimental conditions in this study are different from the real field situation because temperature and humidity change over time due to strong sunlight under field conditions. Therefore, care must be taken when interpreting the results of this study to estimate the die-off rates in real field conditions. 3) Compost after 24 h of

post-treatment period amended to the soil would be safe in 6 days. Further research is required to understand the behaviour of pathogenic bacteria in field conditions of a hot semi-arid climate. This study succeeded to evaluate the risk of pathogens with initial biological parameters and operational conditions.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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