INTERNATIONAL INSTITUTE FOR WATER AND ENVIRONMENTAL ENGINEERING

THESIS

For the grade of:

DOCTOR OF SCIENCE AND TECHNOLOGY IN WATER, ENERGY AND ENVIRONMENT

Speciality: Water

Presented by

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25th June, 2015 **Ref.: 2iE/2015-08**

Title

Design of Thermal Post -Treatment Unit for the Inactivation of Pathogens in Compost after the Composting Process for Food Production

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DEDICATION

First and foremost, I thank the Almighty God for the gift of life, protection and guidance; and I attribute the completion of this work to his will. He is indeed the most merciful and most gracious.

I dedicate this work to my dearest Husband, Alhaji Issah Nurah Danwanaa - it is always stressful being away from you and the children. I am most grateful for your unconditional love, support, patience, understanding and prayers. Thank you for being such a wonderful Husband!

To my little children Ikram Nurah Danwanaa and Ikhlass Nurah Danwanaa, I missed you so much as you missed me during my frequent travel and long absence. Thank you for your love and endurance. I love you so much.

To my parents, Naa Darimani Abudu Saaka and Hajia Habibata S. Darimani for all the love and wonderful upbringing and continuous support of taking care of my children each time I am out of the country.

To my siblings, family members and well-wishers especially Ibrahim Darimani and Nurideen Rashida for their support and prayers during my studies.

Abstract

Composting toilets have been installed in rural areas of Burkina Faso to improve both sanitation and food production. The toilets produce compost from faeces which are separately collected from urine and composting matrix. The compost still contains pathogens due to short reaction time after the final defecation and low temperatures. A post-treatment unit was proposed to ensure inactivation of pathogens based on a multi-barrier concept recommended by WHO. The unit uses solar energy to heat up the compost resulting in no operational cost, which makes it affordable for developing countries in the Sahel. This study aimed to design the post-treatment unit to sanitise the compost withdrawn from the composting toilet to allow a safe reuse of this compost in agriculture. Safety was evaluated with a microbial risk assessment. To perform the risk assessment, the inactivation rate coefficients of indicator pathogens, such as *Enterococcus*, *Escherichia coli*, *Ascaris suum* eggs and MS2 bacteriophage, in the compost and the soil amended compost were estimated. The kinetics of inactivating pathogens were determined at different temperatures with varying moisture content of the compost. To evaluate bacteria behaviour in different environmental conditions when compost is amended to cultivated soils, kinetic inactivation rate coefficients of *Enterococcus* in clay and sandy loam soils were determined with both varying temperature and compost-to-soil ratios. The moisture content was set to 25 % in all conditions. A quantitative microbial risk assessment done by the Monte Carlo model was conducted to evaluate the risk of infectious diseases to farmers during amendment of compost to the soil and the required time to reach a safe level for 4 scenarios evaluated. The health risk of infectious diseases to the farmers working on the soils after amendment was also assessed. The main results of these experiments are as follows: *E.coli* requires post-treatment conditions of 50 °C and 50 % MC and a contact time of over 4.5 h or post-treatment conditions of 70 °C, 50% MC for 20 min. For *Enterococcus*, post-treatment conditions of 50 °C and 50 % MC during 15 h or 70 °C, 50 % during 2.5 h are required. Inactivation of *A. suum* was fast with greater than 2 log reductions achieved within 2 h at 50 °C and 50 % and greater than 3 log reductions at 60 °C and 50 % during 3 h. Statistical analysis showed that the inactivation rates of *A. suum* eggs significantly depends on higher temperature (i.e. 60 °C) and lower MC (i.e. 50 %) of the compost. The inactivation rate coefficients of MS2 statistically depended on a higher temperature, but not moisture content. The inactivation rates of *Enterococcus* in soils amended with compost from the composting toilet depends on temperature and soil type, but not on the compost-to-soil ratios. The risk value assessed in a worse case was 10^{0} pppy for all scenarios, indicating farmers were at a higher risk from pathogens. For *Ascaris*, Norovirus and *Salmonella*, post-treatment with solar thermal heat requires approximately 295 h, 845 h and 969.5 h to achieve the safe level of 10^{-4} pppy respectively for all scenarios. Protocols to design post-treatment unit from the perspective of microbial risk assessment were developed.

***Keywords**:* Pathogens*;* composting toilet; temperature; post-treatment; health risk assessment

Résumé

Des toilettes à compost ont été installées dans des zones rurales du Burkina Faso pour améliorer à la fois l'assainissement et la production d'aliments. Les toilettes produisent du compost à partir des matières fécales qui sont collectées séparément de l'urine et de la matrice de compostage. Le compost contient toujours des agents pathogènes en raison du peu de temps de réaction après la défécation finale et les basses températures. Une unité de post-traitement a été proposée pour assurer l'inactivation des agents pathogènes basée sur le concept multi-barrière recommandé par l'OMS. Cette unité utilise l'énergie solaire pour chauffer le compost obtenu sans coût opérationnel suplémentaire, ce qui la rend abordable pour les pays en développement dans le Sahel. Cette étude visait à concevoir l'unité de post-traitement pour la désinfection du compost retiré de la toilette à compostage pour permettre une réutilisation sûre de ce compost dans l'agriculture. L'innocuité du composte a été évaluée par une évaluation des risques microbiens. Pour effectuer l'évaluation des risques, les taux d'inactivation des indicateurs d'agents pathogènes, tels que les *Enterococcus*, *Escherichia coli*, *Ascaris suum* et du bactériophage MS2, dans le compost et le sol amendé ont été estimés. Le coéfficient d'inactivation des agents pathogènes a été déterminée à différentes températures et à différentes teneurs d'humidité du compost. Pour évaluer le comportement des bactéries dans différentes conditions environnementales lorsque que le compost est apporté aux sols cultivés, la cinétique coefficients du taux d'inactivation de *Enterococcus* dans les sols argileux et sableux riches en terreau ont été déterminées avec des températures et des ratios du compost au sol. La teneur en humidité a été réglée à 25 % dans toutes les conditions. Une évaluation du risque microbien quantitative a été effectuée par le modèle de Monte Carlo pour évaluer le risque de maladies pouvant infecter les agriculteurs lors de l'épandage du compost au sol. Le temps nécessaire pour atteindre un niveau de sécurité pour les 4 scénarios a été évalué. Le risque sanitaire des agriculteurs travaillant sur les sols après épandage du compost a été également évalué. Les principaux résultats de ces expériences sont les suivants: *E. coli* nécessite des conditions de post-traitement de 50 °C de température et 50 % de taux d'humidité (TH) pour un temps de contact de plus de 4,5 h ou 70 °C, 50 % de TH pendant 20 min. Pour les *Enterococcus*, les conditions de 50 °C, et 50 % de TH pendant 15 h ou 70 °C, 50 % de TH pendant 2,5 h sont nécessaires. L'inactivation de *A. suum* a été rapide avec plus de 2 log de réductions en 2 h à 50 °C et 50 % de TH, et, de plus de 3 log de réductions à 60 °C et à 50 % de TH pendant 3 h. L'analyse statistique a montré que les taux d'inactivation des œufs d'*A. suum* dépendent de façon significative d'une température élevée (par exemple 60 °C) et d'un bas TH (soit 50 %) du compost. Les taux d'inactivation de MS2 dépendent statistiquement d'une température élevée, mais pas de la teneur en humidité. Les taux d'inactivation de *Enterococcus* dans les sols amendés avec du compost obtenu à partir des toilettes à compost dépendent de la température et du type de sol, mais pas du rapport compost - sol. La valeur du risque évaluée dans le pire des cas était de 10^0 pppa pour tous les scénarios. Pour *Ascaris*, *Norovirus* et *Salmonella*, le post-traitement avec la chaleur solaire nécessite environ respectivement 295 h, 845 h et 969,5 h pour atteindre le niveau de sécurité de 10^{-4} pppa pour tous les scénarios. Les protocoles de conception de l'unité de post-traitement du point de vu de l'évaluation des risques microbiens ont été développés.

Mots-clés: agents pathogènes; toilette à compost; température; après traitement; évaluation des risques sanitaires

List of Publications

Dissertation submitted for the degree

I. Title

Design of Thermal Post-Treatment Unit for the Inactivation of Pathogens in Compost after the Composting Process for Food Production

II. Published Papers:

- Darimani, H.S., Ito, R., Sossou, S.K., Funamizu N., and Maiga A.H., 2015. Effect of Post-treatment Conditions on the Inactivation rate of pathogenic bacteria after the composting process. *Compost Science & Utilization*, (23) 3, 164-173. Available at: *http://dx.doi.org/10.1080/1065657X.2015.1015082*
- Darimani, H.S., Ito, R., Sou, M., Funamizu, N., Yacouba H., and Maiga A.H., 2015. Design of Post-treatment Unit for Compost from a Composting Toilet with Microbial Risk Assessment. *Journal of Residual Science & Technology*, 12 (2) 43-51. http://www.dpi-journals.com/index.php/JRST/article/view/1213
- Darimani, H. S., Sou/Dakoure, M., Hijikata, N., Sangare, Sawadogo F., Ito, R. and Maiga A H., 2014. Inactivation of *Enterococcus* in Compost-Amended Soils. *Journal of Japan Society of Civil Engineers*, 70 (7), 323-330. Available at: http://dx.doi.org/10.2208/jscejer.70.iii_323.

III. Submitted Paper and Manuscript:

- Darimani, H.S., Ito, R., Maiga, Y., Sou M., Funamizu, N., and Maiga A.H., 2015. Effect of Post-treatment Conditions on the Inactivation of Helminth eggs (*A. suum*) after the Composting Process (Minor Revision; *Environmental Technology*).
- Darimani, H.S., Ito, R., Funamizu, N., and Maiga, A.H., 2015. Effect of Post-treatment Conditions on the Inactivation of MS2 Bacteriophage as indicator for pathogenic viruses (Manuscript).

IV. Conference Papers:

Oral Presentations

- Darimani, H. S., Ito, R., Sou/Dakoure, M., Funamizu N., Yacouba H. and Maiga A.H. 2014. Microbial Risk Assessment Associated with Post-treatment of compost from the composting toilet. *In proceedings of 9th IWA International Symposium on Waste Management Problems in Agro-Industries*, Kochi, Japan (***Won an award for this presentation***).
- Darimani, H.S., Sou/Dakoure, M., Hijikata, N., Sangare, D., Sawadogo F., Ito, R. and. Maiga, A. H., 2014. Inactivation of *Enterococcus* in Compost - Amended Soils. *Committee on Environmental Engineering of Japan Society of Civil Engineers*, Yamanashi, Japan.
- Darimani, H.S., Ito, R., Sossou, S.K., and Maiga, A.H., 2013. Effect of Post-treatment Conditions on the Inactivation of *Escherichia coli* after the composting process. *In proceedings of 36th WEDC International Conference on Delivering, Water, Sanitation and Hygiene Services in an Uncertain Environment*, Loughborough University, UK / Egerton University, Kenya.

Posters

- Darimani, H. S., Ito, R., Sou/Dakoure, M., Funamizu N., Yacouba H. and Maiga A.H., 2014. Microbial Risk Assessment Associated with Post-treatment of compost from the composting toilet. *Africa Water conference*, Ouagadougou.
- Darimani, H.S., Ito R., Sossou, S.K., and Maiga A.H., 2013. Effect of the post-treatment conditions after the composting process on inactivation of *E.coli ATCC 11775*. *7th Edition of 2iE's Science Week and Eco-Innovation for Sustainable Development of Natural Resources*, April 1-5, 2013, Ouagadougou, Burkina Faso.

Acknowledgements

The work performed in this Thesis was financed by JST-JICA and SATREPS through Ameli-EAUR project in a research collaboration programme between the International Institute for Water and Environmental Engineering (2iE) and Hokkaido University. We acknowledge and thank them for the financial support.

We sincerely, acknowledge the financial support of USAID through the HED (Higher Education for Development) program in support of 2iE-Tuskegee University and 2iE-University of Mississippi (Ole Miss) partnership.

Prof. Amadou Hama Maiga, 2iE, Burkina Faso, Director of the Thesis, to whom we owe much profound gratitude, receives our special appreciation for guiding us through a successful completion of this research work. Thank you, Sir. God bless you.

We profoundly thank Dr. Ryusei Ito, Supervisor of this work, assistant Professor of Hokkaido University, Japan, for the inspiring enthusiasm, expertise, scientific skill, devotion, assistance and guidance in the overall planning and execution of the studies. You were also a member of the steering committee for this Thesis. We are very grateful for the constructive comments. May God bless you and increase you in wisdom.

We extend our heartfelt appreciation to Prof. Naoyuki Funamizu, Hokkaido University, Japan and Prof. Hamma Yacouba, 2iE, Burkina Faso for accepting me in their Laboratories in Japan and Burkina Faso respectively. We are sincerely grateful for all the knowledge and guidance during the study period in Japan and Burkina Faso.

We give a special thank you to Améli-EAUR project members of Burkina Faso, especially Dr. Mariam Sou/Dakoure, 2iE for all her contribution to this work. Dr. Mariam Sou/Dakoure you were a steering committee member of this Thesis. We express our heartfelt gratitude for all the constructive comments and guidance throughout this work. May God bless you.

We express our heartfelt appreciation to the Améli-EAUR project Hokkaido University research team in Japan led by Prof. Naoyuki Funamizu, Director of the Department of Environmental Engineering, Dr. Ryusei Ito, Dr. Ken Ushijima and Dr. Nowaki Hijikata for their scientific contributions in this research work.

Successful journeys are first initiated by kind and wonderful people. Our special appreciation goes to Prof. Amadou Hama Maiga, CEO of 2iE, Prof. Hamma Yacouba, Director of research Prof. Harouna Karambiri, Director of doctoral School, Dr Yacouba Konaté, head of LEDES, who kindly accepted me within the institution and for doctoral training at 2iE Burkina Faso.

We also want to give a special thank you to the Jury members who have done us the honour to review this work. Our heartfelt gratitude to Prof. Sampson Kwaku Agodzo of Kwame Nkrumah University of Science and Technology, Ghana for accepting to be a rapporteur of this work. We heartily thank Prof. Stewart M. Oakley, California State University, U.S.A for accepting to be a rapporteur of this study. We extend our sincere appreciation to Prof. Naoyuki Funamizu, Hokkaido University for accepting to be the president of the jury.

We express our deepest gratitude to Dr. Ynoussa Maiga for his relevant remarks in reviewing some of the manuscripts and also for giving very useful pieces of advice and suggestions.

We thank all lectures of 2iE, especially, Dr Yacouba Konaté, Dr. Lambert Ettien Anne, Dr. Hela Karoui, Dr. Mahamadou Koita, Dr. Awa-Diaye Koita, Dr. Maimouna Bologo-Traore, Dr. H. Anderson Andrianisa, Dr. Ahmed Bagre for their advice and encouragement.

Prof. G.K.S Aflakpui, Rector of Wa Polytechnic, management and staff of Wa Polytechnic, Ghana, thank you for your support and concern.

We are grateful to research engineers, technicians and support staff both in the laboratory and at the experimental site that contributed to the data collection: Seyram Sossou, Boukary Sawadogo, Moustapha Ouedraogo, Noel Tindoure, Hema Somai Bernard Zongo, Innocent D. Zerbo and Koale Ido the driver.

To all PhD students of 2iE, especially Maxime G. Ahoule, Abdouramane D. Gado, Djafarou A.

Moumouni, Noellie Kpoda, Sangare Drissa, Katherine Lownsbery, Nka Nnomo Bernadette, Dimitri D. Soro, Aida Zare, Vivien C. Doto, Beteo Zongo, David B. Tsuanyo, Tadjouwa Kouawa, Amare Adugna, Cheick O. Zoure, Christine L. Razanamahandry, Yanaba Roland Ousmane, Tazen Fowe and Quenum Arnord Xavier François, we are grateful for the friendship and support during this Thesis. Also, our appreciation to Geneviève Yameogo, coordination officer of the doctoral School of 2iE.

Certainly we would be remiss if we fail to acknowledge friends at the Laboratory on Engineering for Sustainable Sanitation, Hokkaido University, Japan, especially Reem Abukmeil, Wakana Oishi, Shuto Kenako, Midori Yoshimoto, Sebastian Charchala, Masaki Ishiuguro, Steve John Kabore, Benedicte Nikiem, Daisuke Honoki, Rei Sato. Thank you all for your support.

Finally, Sawadogo Fatoumata an MSc student under this Thesis receives our special appreciation.

Résumé Substantial en Françias

Conception d'une unité de traitement solaire pour l'inactivation des agents pathogènes dans le compost après le processus de compostage en vue d'une valorisation agricole du compost

Introduction

Chaque année, des millions de personnes dans le monde souffrent de crises régulières de diarrhée ou d'infections de vers parasitaires causées par des services d'assainissement inadéquats, tandis que le nombre des décès dus uniquement aux maladies diarrhéiques est de l'ordre de 1,4 millions par an (Prüss-Üstün, 2008). Les maladies diarrhéiques constituent l'une des principales causes de morbidité et de mortalité dans les pays en développement, en particulier chez les enfants de moins de cinq ans (Kosak *et al*., 2003; Prüss *et al*., 2002). L'importance de l'amélioration de l'assainissement pour la sauvegarde de la santé et le bien-être des populations est bien documentée (Cairncross, 2003; Moe et Rheingans, 2006). En 1977, la Conférence organisée par l'ONU sur l'Eau à Mar del Plata, Argentine avait recommandé que les années 80 soient proclamée « Décennie Internationale de l'Eau Potable et de l'Assainissement » (DIEPA). L'un des plans d'action prévoyait que tous les pays atteigent une couverture de 100 % dans l'approvisionnement en eau et en assainissement à l'horizon 1990. Bien que la fourniture de services ait augmenté de façon générale, dans de nombreux pays, les installations d'assainissement ne suivent pas le rythme de la croissance démographique, ce qui explique que le pourcentage de personnes n'ayant pas un accès à un assainissement adéquat ait augmenté depuis lors (DFID, 1998). Actuellement, on estime à 2,5 milliards le nombre de personnes qui manquent encore d'assainissement adéquat (OMS/UNICEF, 2014). La cible 7 des l'objectifs dumillénaire pour le développement des Nations Unies est de réduire de moitié la proportion des personnes sans accès à l'eau potable et à l'assainissement de base d'ici à 2015. L'échéance de ces objectifs étant très proche, force est de constater que cette cible ne sera pas atteinte. Les régions du monde qui restent encore loin de cette cible sont principalement en Afrique subsaharienne, une partie de l'Asie et de l'Océanie. L'Afrique subsaharienne reste la région qui a enregistré le moins de progrès soit une augmentation de 5 % entre 1990 (26 % de taux d'accès) et 2012 (30 % de taux d'accès) 2012 (OMS / UNICEF, 2014).

Les économies des pays de la région du Sahel sont largement tributaires de l'agriculture, avec

environ 80 - 90 % de la population active engagée dans l'agriculture (PNUE, 2012). Le Sahel est une zone semi-aride comprise entre le désert dans Sahara au Nord et les savanes tropicales au Sud (Herrmann, 2005). Il constitue une bande qui traverse le Sénégal, la Mauritanie, le Mali, le Burkina Faso, l'Algérie, le Niger, le Tchad, le Soudan du Sud, le Nord-Soudan et l'Érythrée (PNUE, 2012). Les sols sahéliens sont principalement sablonneux (Bationo *et al*., 2014) avec prédominance d'Entisols et d'Alfisols (Kang, 1985). Il y manque particulièrement le phosphore et l'azote (Bationo *et al*, 2014; Breman *et al*., 2001). Dans le Sahel, l'extensification et l'intensification agricoles dues à la croissance démographique et aux pratiques agricoles non durables contribuent à réduire la couverture végétale des terres et à accentuer la dégradation (Doso, 2014). L'érosion est la principale cause de dégradation des terres dans le Sahel. Cette érosion entraîne la perte de nutriments, et réduit par conséquent la productivité agricole (Doso, 2014). L'ajout de matières organiques dans les sols comme le paillage et l'ajout d'engrais organique permettent d'améliorer la production agricole et la lutte contre la dégradation des terres (Doso, 2014). Dans les pays sahéliens comme le Burkina Faso, la sécheresse fréquente et la faible fertilité des sols constituent des facteurs clés à l'origine des pénuries alimentaires. Cependant des technologies innovantes, à faibles intrants qui reconstituent simultanément les nutriments du sol et la matière organique, ainsi que l'amélioration de la disponibilité en eau du sol, peuvent conduire à une augmentation significative de la production agricole et ainsi atténuer la pénurie alimentaire.

Dans la gestion conventionnelle des cultures dans les pays sahéliens, les engrais chimiques sont appliqués aux doses recommandées pour reconstituer rapidement la fertilité du sol et améliorer ainsi le rendement des cultures. Dans ces pays, cependant, le taux d'application recommandé d'engrais est onéreux et, in fine, contribue à l'insécurité alimentaire (Doso, 2014). En 2012, la demande mondiale en azote (N), en phosphore (P) et en potassium (K) respectivement était de 1,6 ; 2,4 et 2,0 % (FAO, 2012). La forte demande de ces engrais les rend très coûteux. Un sac d'engrais au Burkina Faso coûte en moyenne 16.000 FCFA. Ces prix élevés limitent leur utilisation par les populations les plus pauvres, ce qui entraîne de faibles rendements et une baisse des productions (FAO, 2012).

Les matières fécales peuvent être une bonne source de matière organique capable d'améliorer les conditions physiques du sol ; tels que la structure du sol, l'aération du sol et le drainage dans les sols sahéliens pauvres. Les excréments humains sont riches en N, P, K. La teneur en éléments nutritifs des matières fécales provient de la nourriture consommée. Ceci indique que la quantité de N, P, K varie d'une personne à une autre. Cependant, Wolgast (1993) a signalé que chaque année, une personne produit 50 kg de matières fécales et cela contient quelques 10 kg de matière sèche. Une personne produit environ 5,7 kg d'azote, 0,6 kg de phosphore et 1,2 kg de potassium par an (Wolgast, 1993). La population de l'Afrique est de 1,1 milliard (Données mondiales de la population, 2013). En utilisant les statistiques de la population, le potentiel en engrais de l'Afrique peut être estimé à 6,27, 0,66, 1,32 milliard kg/ an respectivement pour l'azote, le phosphore et le potassium. Ce potentiel d'engrais à partir des fèces peut réduire la demande en engrais de l'Afrique.

L'apport d'excréments sur les sols cultivés (sous forme de boue de vidange) permet de fertiliser les sols. C'est une pratique courante dans plusieurs pays comme le Ghana, le Burkina Faso, le Japon, la Chine, le Guatemala, le Zimbabwe, la Suède, le Vietnam, etc. En Chine, l'amendement des sols par les excrétas a été pendant longtemps l'unique option pour évacuer ces déchets dans les zones sans système d'assainissement (Mara *et al*., 1989). L'expérience Chinoise, a contribuer à maintenir la fertilité des sols pendant des milliers d'années (Mara *et al*., 1989). En 1965, par exemple, environ 90 % de tous les excréments humains produits en Chine ont été utilisés comme engrais (Chao, 1970). En plus de fournir des nutriments, les excréments sont très précieux dans l'augmentation de la teneur en humus du sol, ce qui améliore de façon significative la structure du sol et sa capacité de rétention d'eau.

Malgré les avantages agricoles prouvés, de nombreuses sociétés ont une forte aversion socio-culturelle à l'utilisation agricole des excréments, bien que l'utilisation de certains produits dérivés des excréments soit connus et socialement acceptable. Au Royaume-Uni, 47 % de toutes les boues des stations d'épuration municipales sont amendées sur les terres agricoles (Water Autorities Association, 1985). Dans de nombreuses régions comme le Ghana, certains agriculteurs fertilisent leurs exploitations agricoles avec les boues vidange contenues dans des

camions de vidanges de fosses septiques (Cofie et Adamty, 2009). Les auteurs ont signalé les observations suivantes:

- une bonne demande pour l'utilisation des boues de vidange dans l'agriculture en raison de la perception généralement positive
- le compost à base de boues de vidange produit une circonférence d'épis de maïs plus important comparé à l'engrais inorganique ou le sol sans amendement
- les rendements des parcelles traitées avec les boues de vidange sont 10 % supérieurs à ceux obtenus avec des engrais chimiques.
- plus de 50 % des glands de maïs en provenance des parcelles de boues de vidange forment de la soie et mûrissent une semaine plus tôt que les cultures sur les parcelles traitées à l'engrais chimique.
- les grains frais du traitement à base de boues de vidange étaient plus doux que ceux des engrais inorganiques.

En 2001, des expériences ont été menées dans la banlieue de Ouagadougou pour recueillir, traiter et utiliser les urines et les fèces pour la culture et la production de céréales. Deux ans après la mise en œuvre de ce projet, les résultats de l'expérience ont conforté le principe du recyclage des excréments humains pour la production agricole. Les populations ont été impressionnées par le rendement des cultures produites par fertilisation avec des engrais provenant d'excréments humains (Arzouma, 2012).

Toutes ces expériences permettre de soutenir le fait que pour atténuer la demande mondiale en engrais, réduire les maladies infectieuses pathogènes et construire une société durable fondée sur le recyclage des matières fécales, il est nécessaire de lier l'assainissement et l'agriculture soutenue par plusieurs recherches scientifiques. En effet, un système durable pour les pays en développement, robuste, à faible coût et stable a été développé pour lier l'assainissement à l'agriculture par la récupération et la réutilisation des nutriments et des eaux grises comme eau d'irrigation. Ce modèle de projet se base sur la collecte séparée des effluents domestiques en trois phases : les urines, la matière fécale et les eaux grises. Le principe de base est de gérer les déchets solides et liquides séparément sans les mélanger afin de mieux les valoriser. Les trois

effluents sont traités sur place à travers des systèmes d'assainissement autonomes : les urines par inactivation solaire, la matière fécale par compostage et les eaux grises par filtration.

L'OMS (2006) a recommandé une approche multi barrières dans la cadre de la réutilisation des sous-produits de l'assainissement pour protéger la santé publique. Cette approche permet d'avoir une bonne compréhension de la façon dont la réutilisation des excréments peut être faite en toute sécurité. Le concept est également utilisé dans l'approvisionnement en eau et la production d'aliments et est généralement compris comme une série d'étapes de traitement et d'autres mesures de sécurité pour prévenir la propagation des agents pathogènes.

Objectifs de l'étude

L'objectif général de cette thèse est d'assurer l'inactivation des agents pathogènes présents dans le compost produit à partir des excréments humains dans le but de une valorisation saine de ce compost. Le processus de compostage permet une dégradation de la matière organique contenue dans les fèces et mélangé avec une matrice qui peut être de la sciure de bois ou des résidus agricoles. Toutefois, des études préliminaires ont montrées que le compostage ne permettait par une réduction satisfaisante des organismes pathogènes, ce qui implique la mise en œuvre un post-traitement, objet du la présente étude. Le choix du post-traitement s'intègre dans l'approche multi-barrières.

Les objectifs spécifiques de l'étude étaient:

i. Caractériser le taux d'inactivation des bactéries, virus et parasites dans le compost par un post-traitement thermique.

ii. Concevoir un dispositif pilote de traitement thermique et tester ces performances dans le cadre d'une évaluation de risque sanitaire.

iii. Déterminer le risque sanitaire lié aux agriculteurs travaillant sur les sols amendés avec le compost.

Le document de thèse est structuré en 5 parties : les trois premières parties comprennent les résultats du taux d'inactivation sur les bactéries (partie 1) les parasites (partie 2) et les virus (partie 3). La partie 4 présente les résultats de la conception et du dimensionnement du dispositif

pilote de traitement et la partie 5 traite de l'évaluation de risque sanitaire sur les sols amendés par le compost.

Méthodologie

Effet de la température sur le taux d'inactivation des bactéries, virus et parasites

Cette partie de l'étude a été mise en œuvre à partir de quatre indicateurs d'agents pathogènes :

- *Enterococcus et Escherichia coli* pour le suivi des bactéries pathogènes,
- *Ascaris suum* pour le suivi des œufs d'helminthes et
- les bactériophages MS2 pour le suivi des virus pathogènes.

Pour les bactéries pathogènes, la cinétique d'inactivation d'*Enterococcus* et de *d'Escherichia coli* a été déterminée au cours du post-traitement à trois températures (37 °C, 50 °C et 70 °C) et trois taux d'humidité (50 %, 60 % et 70 %). L'effet du pH a également été testé sur l'inactivation des bactéries avec les deux indicateurs. Pour ce faire, trois doses de chaux (1,0 g, 0,5 g et 0,1 g) ont été ajoutées à des échantillons de compost maintenus à des taux d'humidité de 60 %.

En ce qui concerne les œufs d'helminthes, la cinétique d'inactivation des œufs d'*Ascaris suum* a été déterminée au cours du post-traitement à quatre différentes températures (30 °C, 40 °C, 50 °C et 60 °C) avec des taux d'humidité de 50 %, 60 % et 70 %.

Pour les virus, la cinétique d'inactivation des macrophages MS2 a été déterminée au cours du post-traitement à trois températures différentes (30 °C, 40 °C et 50 °C) selon trois taux d'humidité (50 %, 60 % et 70 %).

La conception post-traitement et l'évaluation des risques pour la santé

Pendant l'utilisation du compost, les gens peuvent ingérer accidentellement le compost avec les agents pathogènes par voie orale. Les personnes exposées à des agents pathogènes auraient des maladies avec une probabilité estimée par l'évaluation des risques. Nous avons mis 4 scénarios, la température à 3 positions (1 cm, 5 cm et 10 cm) dans la boîte en acier (S-1) comme un post-traitement pour l'évaluation. Des températures plus basses ont été fixé pour moins cinq, moins dix et moins quinze de distribution de la température de la boîte en acier. Ces températures plus

basses ont été obtenues à partir de l'hypothèse que la température mesurée dans la boîte en acier au Burkina Faso est de moins cinq, moins dix et moins quinze inférieures aux températures réelles mesurées. C' est pour tenir compte de la température plus basse au cours de l'année. Cela correspond à la température à différentes positions (1cm, 5 cm et 10 cm), soit température basse (-5 °C) comme (S-2), température plus basse (-10 °C), S-3 et température basse (-15 °C), à S-4. Les différentes profondeurs sont définies comme bas, milieu et haut. Pour le calcul de la concentration dans le compost, les coefficients de notre précédente mesure des taux d'inactivation ont été utilisés (Darimani *et al*, 2015).

Etude de l'évaluation de risque sanitaire

Une évaluation du risque microbien quantitatif a été effectuée par le modèle de Monte Carlo pour évaluer le risque de maladies infectieuses pour les agriculteurs et la durée nécessaire d'exposition solaire au cours du post-traitement. Le scénario envisagé est l'ingestion accidentelle de compost (à des doses excessives comprises entre 0,5 et 0,8 g) et les risques que cela entrainerait en termes d'infection parasitaire (modèle *Ascaris)*, d'infection a virale (modèle *Norovirus) et d'infection bactérienne* (modèle *Salmonella).*

Dans le cas particulier de l'exposition des agriculteurs, l'inactivation de *Enterococcus* a été suivi dans des sols amendés avec du compost aux ratios compost: sol suivants, 1:10, 1:25, 1:50 et 1: 100. Les mélanges ont été exposés à différentes températures (30 °C, 40 °C et 50 °C) à un taux d'humidité de 25 % pour tous les essais. Les données obtenues ont été utilisées pour évaluer le risque sanitaire des *Salmonella* dans le système du sol.

Analyse des données

Le taux d'inactivation (*k*) des micro-organismes dans le compost, obtenu à partir des expériences d'inactivation thermique, suit un modèle cinétique de premier ordre selon Nakagawa *et al*., (2005), se traduisant par l'équation (1):

$$\ln k = C/C_o = -kt \qquad (1)$$

où « C », exprimé en œufs, en CFU ou en PFU / g de compost sec selon qu'il s'agissent

- *de parasites, de bactéries, ou de virus, est la concentration des micro-organismes dans l'échantillon de compost,*
- *« C_o » est la concentration initiale de micro-organismes dans l'échantillon de compost (exprimé en œufs, en CFU ou en PFU / g de compost sec),*
- *k est le taux d'inactivation (h^{-1}), et*
- *t est le temps de traitement thermique (h).*

A partir l'estimation du taux d'inactivation, nous avons évalué l'effet de la température avec l'équation d'Arrhenius décrite comme suit;

$$k = A\exp\left(-\frac{E_a}{RT}\right) \qquad (2)$$

- *où A est le facteur pré-exponentiel (h^{-1}),*
- *Ea est l'énergie d'activation (J/mol),*
- *R est la constante des gaz parfaits (J/mol/K),*
- *T est la température (K).*

Remarque : l'énergie d'inactivation (E_a) n'a pas de sens dans le processus d'inactivation thermique, il sera considéré comme égal à 1 dans la simulation de l'effet de la température sur le taux d'inactivation.

Une analyse statistique ANOVA a été réalisée pour déterminer les effets significatifs ($p \leq 0,05$) de la température et de l'humidité sur l'inactivation du micro-organisme.

Résultats et discussion

Effet de la température sur l'inactivation des bactéries

Les agents pathogènes indicateurs (*E. coli* et *Enterococcus*) étudiés ont montré des différences dans le coefficient du taux d'inactivation.

Les taux d'inactivation d'*E. coli* ont montré que le traitement à 37 °C était très pauvre dans tous les post-traitements comparé aux températures plus élevées (50 et 70 °C). La plus basse température à 37 °C a occasionné une protection contre l'inactivation bactérienne.

Le traitement à 50 °C a enregistré des réductions bactériennes de 5 log, 3 log et 1 log, respectivement pour les taux d'humidité de 50 %, 60 %, et 70 % durant 8 heures d'exposition.

Le traitement à 70 °C a entraîné une réduction bactérienne de 7 log avec les trois taux d'humidités de 50 %, 60 % et 70 %, pour un temps d'exposition de 80 minutes.
Les résultats d'analyse de variance ont montré que la température d'exposition avait un effet sur l'inactivation des *E. coli* ($p \leq 0{,}05$). En revanche, les taux d'humidité n'ont pas montré d'effet significatif sur l'inactivation de cet indicateur. On retiendra donc que seule la température est un facteur important dans l'inactivation d'*E. coli.*

Les taux d'inactivation d'*Enterococcus* ont montré des tendances similaires *E. coli*, avec des taux quasiment nuls à 37 °C. Les valeurs du taux d'inactivation à 50 °C étaient plus élevées que les taux d'inactivation à 70 °C, car la durée d'exposition à 70 °C a été réduit à 80 minutes, de sorte que les *Enterococcus* soient dénombrées lorsqu'elles sont soumises à des températures élevées.
Les expositions à 50 °C testé à 50 %, 60 % et 70 % de taux d'humidité ont permis des réductions bactériennes de 3,2 log, 2.3, et de 1,8 log pour des temps d'exposition de 8 heures.
Les expositions à 70 °C testés à 50 %, 60 % et 70 % de taux d'humidité ont permis des réductions de 3,4, 2,3 et 1,9 log en 80 minutes d'exposition.

Les résultats d'analyse de variance ont montré que la température d'exposition avait un effet sur l'inactivation d'*Enterococcus* à l'exclusion des données pour 70 °C où le temps d'exposition avait été réduit ($p \leq 0{,}05$). Les taux d'humidités n'ont pas non plus montré un effet significatif sur le taux d'inactivation, ce qui permet de retenir également que seule la température est un facteur important dans l'inactivation *Enterococcus*

Les taux d'inactivation ont enregistré des valeurs plus élevées dans toutes les conditions expérimentales avec la teneur en humidité à 50 %, suivi de 60 % et enfin 70 %. Cette même tendance a été observée dans toutes les conditions pour *E. coli* et *Enterococcus*. Nos résultats sont similaires à plusieurs études (Nakagawa *et al* 2005; Kazama et Otaki 2011), qui ont indiqué que, une température élevée et une faible teneur en humidité peuvent rapidement inactiver *E. coli* et *Enterococcus.* Cependant, ce n'était pas statistiquement significatif. À haute température, la teneur en humidité n'était pas critique sur l'inactivation d' *E. coli* et *Enterococcus*.

Effet de la température sur l'inactivation des œufs d'helminthes (œufs Ascaris suum)

Les valeurs du coefficient du taux d'inactivation à 30 °C étaient presque nulles, indiquant l'absence de réduction de la concentration des œufs à 30 °C pendant la période de traitement de 3 heures. La variation au cours du temps de la concentration des œufs *A. suum* avec des teneurs variables en humidité de 50 %, 60 % et 70 % à 40 °C a été légèrement supérieure à 30 °C. La teneur en humidité inférieure a montré la plus grande valeur de'inactivation. Le traitement à 50 °C avec une teneur en humidité respectivement de 50 %, 60 % et 70 % a abouti à 2.00 ± 0,14 unités log, 1,00 ± 0,18 unités log et 0,8 ± 0,14 unités log de réductions en 2 heures. L'unité de réduction log de 60°C à une teneur en humidité de 50 %, 60 %, et 70 % était de 3,60 ± 0,21 unités log, 3,00 ± 0,14 unités log et 2,00 ± 0,14 unités log respectivement en 3 heures. Ces caractéristiques de *A. suum* pendant l'inactivation thermique ont également été observées dans des études antérieures (Hawksworth *et al*., 2000; Pecson *et al*., 2007). L'effet de la température et de l'humidité sur l'inactivation des œufs d'*Ascaris* étudié ici montre que les taux d'inactivation augmentent lorsque la température augmente. Une température élevée augmente la dessiccation et donc la destruction des cellules d'*Ascaris* (Feachem *et al*, 1983; Pecson *et al*, 2007; Capizzi-Banas *et al*., 2004). L'effet de la température et de l'humidité sur l'inactivation des œufs peut être vu lorsque l'on compare toutes les températures, 30, 40, 50 et 60 °C à 50 %, 60 % et 70 % d'humidité. Le taux d'inactivation a augmenté tandis que l'humidité diminue. A 30 °C, il n'y avait aucun changement dans la concentration des œufs après trois heures de traitement à tous les niveaux d'humidité, ce qui indique que la température inférieure offrait plus de protection en ralentissant le taux de dessiccation des œufs. Les températures supérieures à 40 °C avec 3 heures de temps de contact ont inactivé les œufs.

Effet de la température sur l'inactivation du bactériophage MS2 (virus)

Les taux d'inactivation étaient presque nuls à 30 °C pendant la période de traitement de 8 heures. Les coefficients de taux d'inactivation à 40 °C étaient de 0,252, 0,165, 0,088 pour les taux d'humidité de 50, 60 et 70 %. A 40 °C, la réduction de l'unité log enregistrée pour 50 % était de 2 log, 60 % d'humidité était de 1 log et 70 % enregistrée <1 unité log en 8 heures. Les coefficients du taux d'inactivation à 50 °C à 50 %, 60 %, 70 % d'humidité étaient respectivement

de 0,447, 0,308 et 0,100. Le traitement à 50 °C avec 50 %, 60 %, 70 % d'humidité a enregistré des réductions de 3 log, 1 log et <1 log en 8 heures.

Les résultats ont montré qu'il n'y avait pas de changement dans la concentration de MS2 après la période de traitement de huit (8) heures à 30 °C, ce qui indique que des températures plus basses facilitent la survie de MS2. Une variation de température de 40 à 50 °C et au-dessus sont capables d'inactiver MS2 comme indiqué dans les résultats. Toutefois, les taux varieront évidemment avec la variation de température.

Les conditions d'humidité inférieure ont augmenté les coefficients de taux d'inactivation k h^{-1} pour toutes les températures d'exposition expérimentées. Cependant, la différence dans les valeurs de *k* n'est pas statistiquement significative. Les taux d'humidité inférieurs à 50 % ont enregistré les taux d'inactivation les plus élevés indiquant que le compost avec une teneur en humidité inférieure à 50 % peut améliorer le processus d'inactivation pendant le post-traitement. Les taux d'inactivation sont plus élevés lorsque la température augmente et que le taux d'humidité diminue. Kazama *et al*. (2011) ont montré que les coefficients de taux d'inactivation, à 50 °C et 40% d'humidité était de 0,40 et la présente étude obtient un taux de 0,447 à 50 °C et 50 % d'humidité. La présente étude montre que l'inactivation des coliphages est plus rapide à haute température. La condition de post-traitement le plus performant était de 50 °C 50 % d'humidité, avec un réduction de virus supérieure à 3 log en 8 heures d'exposition.

Conception d'un dispositif de post-traitement et évaluation du risque sanitaire après le post-traitement du compost

Le risque annuel d'infection lié à la manipulation du compost avant le post-traitement (95 % d'intervalle de confiance) est quasiment égal à 1 pour les trois types de risque (*Ascaris, norovirus* et *Salmonella) et* pour tous les scénarios. Cela signifie que les personnes qui utilisent le compost seraient fortement exposées à un risque de contamination par ces agents pathogènes. Elles seraient infectées à moins qu'un post traitement ne parvienne à réduire la concentration d'agents pathogènes. Schönning et al. (2007) ont également signalé un risque de 1 (à 95 % d'intervalle de confiance) de *rotavirus* et *Ascaris* pour 0 mois de stockage dans le pire des cas.

Une boîte en acier a été dimensionnée pour permettre l'exposition solaire du compost après le processus de compostage dans les toilettes. Les résultats d'évaluation de risque après le poste

traitement dans ce boitier montrent les risques pour l'*Ascaris* pour la boîte en acier, la température inférieure -5 °C, -10 °C et -15 °C ont été réduits et atteint un niveau fort respectivement à 97,5 h, 138 h, 190 h et 295 h. Les répartitions de température dans le boîtier en acier ont enregistré un temps plus bref que les températures plus basses. La durée du traitement peut être réduite si l'appareil est amélioré. Il y a une augmentation de température suffisante dans la journée, mais elle diminue brusquement vers le soir et les nuits. Ce phénomène provoque une inactivation suffisante par l'équilibre du taux d'inactivation élevé à haute température et la faible inactivation à basse température. Pour réduire le temps de traitement, nous devons améliorer l'unité de post-traitement en élevant la température maximale et en maintenant la température pendant la nuit.

Le temps nécessaire pour atteindre le niveau de sécurité pour les *norovirus* pour la boîte en acier, les températures plus basses -5 °C, -10 °C et -15 °C étaient respectivement de 264 h, 362,5 h, 554 h et 845 h. Le temps nécessaire pour atteindre le niveau de sécurité pour les *salmonelles* dans la boîte en acier températures inférieure, -5 °C, -10 °C et -15 °C ont été respectivement de 90,5 h, 143 h, 356,5 et 969,5 h. En comparant l'*Ascaris*, les *norovirus* et la *salmonelle* on se rend compte que la *salmonelle* nécessite plus de temps à des températures inférieures que l'*Ascaris* et les *norovirus* pour atteindre le niveau sécuritaire de 10^{-4} pppa (Regli *et al*., 1988). Ceci est probablement dû au fait que des températures plus basses sont des conditions favorables pour les bactéries. Par conséquent, la *Salmonelle* est l'indicateur le plus important pour la conception de l'unité, même si les œufs d'*Ascaris* ont la possibilité de survivre plusieurs mois dans un système de sol (Seidu *et al*., 2008).

Inactivation de l'*Enterococcus* dans les sols amendés au compost

La température a augmenté le taux d'inactivation pour tous les types de sol. Le coefficient cinétique de premier ordre du taux d'inactivation, d'*Enterococcus* augmente avec la température. Plusieurs études ont rapporté l'effet de la température sur le taux d'inactivation bactérienne (Reddy *et al.*, 1981; Jiang *et al.*, 2002; Gerba *et al.*, 1975), ce qui indique qu'une température plus élevée réduit la durée de survie des bactéries fécales. Nos résultats ont montré que les taux d'inactivation augmentent quand la température augmente de 30 °C, 40 °C et à 50 °C. Les résultats du test de Kruskal-Wallis ont montré qu'il y avait un effet de la température sur les

performance d'inactivation d'*Enterococcus*. L'effet de la température sur la mortalité de masse de l'*Enterococcus* était statistiquement significative (test de Kruskal-Wallis, $p < 0,05$) pour les sols riches en terreaux et argileux (test de Kruskal-Wallis, $p < 0,05$). Les résultats ont montré que le type de sol influençait le taux d'inactivation d'*Enterococcus*. Le taux d'inactivation dans tous les sols sableux et riches en terreaux avec du compost amendé était plus élevé que les sols argileux. Ceci indique que le type de sol a une influence sur la survie des bactéries. Le type de sol a affecté le taux de mortalité massive parce que les sols plus fins, en particulier, des minéraux argileux et substances humiques augmentent la survie des bactéries (Crane *et al.*, 1983; Gerba et Bitton, 1984). La survie de *E. coli* O157: H7 dans les sols à texture fine (tels que ceux riches en argile) a abouti à un taux de survie plus élevé que dans les sols à texture grossière (sols sableux) (Chauret, 2011). Les particules d'argile utilisées dans les expériences étaient plus petites que le sol sableux riche en particules. Ainsi, la large distribution de taille de particules du sol sableux riche en terreaux a probablement augmenté les taux d'inactivation. La teneur en argile a augmenté la survie de l'*Enterococcus*. Les résultats statistiques ont indiqué que les taux d'inactivation au niveau des sols argileux (test de Kruskal-Wallis, $p < 0,05$; $p = 0,01$) étaient moindre que les taux au niveau des sols sableux riches en terreaux (test de Kruskal-Wallis, $p < 0,05$; $p = 0,007$) et cela pour toutes les températures testées. Jamieson *et al.* (2002) ont rapporté que l'unique propriété du sol qui semble avoir le plus grand impact sur la survie bactérienne est l'humidité, qui est liée à la distribution de la taille des particules et à la teneur en matière organique. La capacité d'inactivation augmenterait donc avec la diminution de la teneur en eau du sol. Des expériences en laboratoire et sur le terrain ont montré que de nombreux sols ont une haute capacité de rétention de bactéries (Gerba *et al*., 1975). La rétention des bactéries augmente avec une augmentation de la teneur en argile, une capacité d'échanges de cations du sol et d'une surface spécifique (Jimieson *et al*., 2002).

Le rapport compost/sol a montré une variation sur le taux d'inactivation dans notre étude. Dans les formulations de sols argileux amendés avec du compost, les taux d'inactivation augmente avec l'augmentation de la fraction de sol. Cela a montré que les applications élevées de compost ont enregistré de moindre coefficient d'inactivation que les applications faibles de compost. Les résultats statistiques, cependant, ont montré qu'il n'y avait pas d'effet entre le taux d'inactivation et les ratios sol/compost. Les résultats ont montré que les différences de ratio du compost et du

sol sur l'inactivation de l'*Enterococcus* n'étaient pas statistiquement significatives ; test de Kruskal-Wallis, $p > 0,05$ à la fois pour les sols sableux riches en terreau que les sols argileux. Crane *et al*. (1981) ont suivi la mortalité des organismes indicateurs à la surface du sol avec une application de fumier de volaille et a indiqué que le taux d'application de fumier n'a eu aucune influence sur la survie bactérienne, ce qui corrobore avec les résultats de notre étude.

Les taux d'inactivation d' *Enterococcus* dans les sols amendés avec du compost à partir des toilettes à compost dépendent de la température et du type de sol, et non pas des ratio compost sol. Cette information a été utilisée pour évaluer le risque sanitaire lié au *Salmonella* dans le système du sol. En effet entre 2012 et 2013 au Burkina Faso, Berger (2015) a rapporté 32,8 % de cas de bactériémies dus au non-tyhpoid *Salmonella* chez des enfants fébriles admis à l'hôpital. Il a aussi signalé que la *Salmonella typhi* reste persistant pendant plus de 12 mois dans les selles d'un porteur sain. Cela souligne la nécessité de programmes conjoints et coordonnés de surveillance et de suivi de la *salmonellose* en Afrique. L'étude a simulé les conditions dans des situations réelles avec la température mesurée dans le sol. Le résultat de la variation de la concentration et le risque annuel de *Salmonella* dans le sol a été estimé.

Le changement de concentration et le risque annuel après 24 h de post-traitement ont été estimés. Le changement de concentration après les 24 h pour le bas, le milieu et le haut du dispositif de traitement était respectivement de $2,8 \times 10^5$, $6,37 \times 10^6$, and $4,03 \times 10^{-15}$ pppa. La modification du compost se fera à ce moment. Une hypothèse de la dilution du compost pour le rapport du sol était de 0,01. Le changement de concentration à cette dilution pour le bas, le milieu et le haut du sol était respectivement de $2,8 \times 10^3$, $6,37 \times 10^4$ et $4,03 \times 10^4$ 10^{-17} pppa. Le risque annuel d'infection à ce stade était de 1 pour le haut, et en bas et 0 au sommet. Les températures au fond et le au milien ont atteint un niveau de sécurité à 147,5 h avec des valeurs de risque de $1,2 \times 10^{-4}$ et $1,05 \times 10^{-4}$ pppa respectivement. La distribution de la température de pointe a atteint un niveau de sécurité après 24 h. Le compost après une période de post-traitement amendé au sol de 24 h atteindrait un niveau de sécurité en 6 jours.

Conclusion

De cette recherche, nous avons étudié le traitement thermique du compost avec une unité de

post-traitement. Pour une réutilisation saine du compost par les agriculteurs en agriculture; les conclusions suivantes ont été faites:

Pour assurer une inactivation thermique suffisante (réduction de log 6) des bactéries pathogènes dans le compost, avec *E. coli* comme indicateur bactérien, les conditions de post-traitement sont une température de 50 °C un taux d'humidité de 50 % et un temps d'exposition d'au moins 4,5 h. Une autre alternative est 70 °C à 50 % de taux d'humidité avec un temps de contact d'au moins 20 minutes.

Avec *Enterococcus* comme indicateur bactérien, les conditions de post-traitement sont 50 °C, 50 % avec un temps de 15 h ou de 70 °C, 50 % de taux d'humidité avec un temps de contact de plus de 2,5 h.

En synthèse, l'inactivation de bactéries à 37, 50 et 70 °C avec des taux d'humidité de 50, 60 et 70 % conduit aux équations ci-dessous. Les taux d'inactivation et d'élimination du rapport de log *E. coli* et *Enterococcus* peuvent être estimés à l'aide des équations suivantes:

- *E.*coli à 50%; $\ln k = -156601/RT + 52{,}426$
- *E.coli* à 60%; $\ln k = -168936/RT + 62{,}386$
- *E.coli* à 70%; $\ln k = -182521/RT + 66{,}594$
- *Enterococcus,* à 50%; $\ln k = -114172/RT + 41{,}135$
- *Enterococcus,* à 60%; $\ln k = -119070/RT + 42{,}483$
- *Enterococcus,* à 70%; $\ln k = -126725/RT + 45{,}110$

• Les taux d'inactivation de *A. suum* (indicateur de la contamination parasitaire) dans le compost dépend de la température et de l'humidité. La meilleure combinaison de conditions de post-traitement pour l'inactivation efficace des œufs d'helminthes sous la chaleur solaire thermique serait de 50 °C à 50 % de taux d'humidité. Le taux de réduction d'œufs d'helminthes dans le compost sous désinfection thermique solaire serait 0,42 à 0,92 h^{-1}. Lorsqu'une chaleur suffisante est appliquée avec une faible humidité d'environ 50 % et en-dessous, une efficacité élevée de 3,60 ± 0,210 unité de log peut être atteinte en 3 heures au cours du post-traitement. Les taux d'inactivation de *A. suum* à 30, 40, 50 et 60 °C avec TH 50, 60 et 70 % ont conduit aux équations

ci-dessous. Les taux d'inactivation et les rapports d'élimination de log de *A. suum* peuvent être estimés en utilisant les équations suivantes:

- at 50%; ln k = -63196/RT + 23,122
- at 60%; ln k = -62564/RT + 22,702
- at 70%; ln k = -125499/RT + 45,501

• Les coefficients de taux d'inactivation du bactériophage MS2 dépendent d'une température très élevée, et non du taux d'humidité. Les températures (>50 °C) avec un temps plus long vont inactiver avec succès les agents pathogènes viraux. Les taux d'inactivation de MS2 (virus indicateur) à 30, 40 et 50 °C avec TH de 50, 60 et 70 % conduit aux équations ci-dessous. Taux d'inactivation et les ratios d'élimination log des virus peuvent être estimées en utilisant les équations suivantes:

- at 50% ; ln k = -63932/RT + 23,066
- at 60% ; ln k = -848665/RT+ 30,556
- at 70% ; ln k = -64780/RT + 22,041

• Pour réduire efficacement les pathogènes pendant le post-traitement et également réduire le temps de traitement, la boîte en acier a besoin d'un isolant pour maintenir la température.

Les lignes directrices pour la conception de l'équipement de post-traitement sont: pour les *Ascaris*, la boîte en acier et la température inférieure de -5 °C, -10 °C et -15 °C, le post-traitement nécessite environ 295 h pour atteindre le niveau de sécurité de 10^{-4} pppa. *Norovirus* nécessite environ 845 h pour atteindre un niveau de sécurité. *Salmonella* nécessite 969,5 h pour atteindre un niveau de sécurité.

• Les taux d'inactivation de *Enterococcus* dans les sols amendés avec du compost à partir des toilettes à compost dépendent de la température et du type du sol, et non des ratios sol/compost. Le compost après 24 h de post-traitement amendé au sol serait sans danger en 969,5 h (40 jours), même à des températures plus basses dans le sol. Les basses températures ne sont pas recommandées pour la mortalité de masse du pathogène. Par conséquent, les agriculteurs devraient éviter la réutilisation du compost dans les périodes de très basses températures. Les

taux d'inactivation de l'*Enterococcus* dans les sols argileux à 30, 40 et 50 °C ont donné lieu à des équations ci-dessous. Taux d'inactivation et les ratios d'*Enterococcus* d'élimination log dans les sols argileux peuvent être estimés en utilisant les équations suivantes:

- au rapport de 10 ; ln k = -132320/RT + 48,725
- Au rapport de 25 ; ln k = -120515/RT + 44,599
- Au rapport de 50 ; ln k = -124457/RT + 46,149
- Au rapport de 100; ln k = -123590/RT + 45,77

• Les taux d'inactivation de *Enterococcus* dans les sols sableux et riches en terreaux à 30, 40 et 50 °C ont conduit aux équations ci-dessous. Taux d'inactivation et les ratios d'Enterococcus d'élimination dans le sol peuvent être estimés avec les équations ci-dessous:

- Au rapport 10 ; ln k = -87256/RT + 31,997
- Au rapport 25 ; ln k = -83088/RT + 30,574
- Au rapport 50 ; ln k = -71780/RT + 26,356

• La quantité d'eau a eu une influence sur les taux de mortalité de masse de certains agents pathogènes, des teneurs en humidité basses sont recommandées pendant le post-traitement. Il y a suffisamment d'énergie solaire et donc le séchage se produit rapidement. Par conséquent, aucune importance ne doit être accordée à la teneur en humidité au cours de la saison sèche. La manipulation du compost doit être évitée pendant la saison des pluies. Pour obtenir une inactivation des agents pathogènes réussie, la teneur en humidité ne doit pas dépasser 60 %.

• Le protocole de modèles mathématiques pour concevoir/système de post-traitement du point de vue de l'évaluation des risques microbiens ont été développés. L'application de ces modèles saunait guider les agriculteurs pour traiter le compost à un niveau sécuritaire avant de les réutiliser pour la production alimentaire.

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Acronyms

ATCC	American Type Culture Collection
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
DFID	Department for International Development
DM	Dry matter
DWD	Directorate of Water Development
E. coli	*Escherichia coli*
Ecosan	Ecological Sanitation
EcoSanRes	Ecological Sanitation Research Program, Sweden
FAO	Food and Agriculture Organisation
IDWSSD	International Drinking Water Supply and Sanitation Decade
K	Potassium
LB	Luria Bertani
MC	Moisture content
MDGs	Millennium Development Goals
N	Nitrogen
NBRC	National Biological Resource Centre
OM	Organic matter
OWDTS	On-site Wastewater Differentiable Treatment
P	Phosphorus
QMRA	Quantitative Microbial Risk Assessment
UDDT	Urine Diversion Dry Toilet
UN	United Nations
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency
WHO	World Health Organisation
DALY	Disability Adjusted Life Year
TFTM	Too Fast to Measure

Chapter 1

Introduction

1. Introduction

1.1 Problems on Sanitation

Every year, millions of people in the world suffer from regular bouts of diarrhoea or parasitic worm infections caused by inadequate sanitation services, while a number of deaths from diarrhoea diseases alone comes up to 1.4 million per year (Prüss-Üstün, 2008). Diarrhoeal disease is one of the leading causes of morbidity and mortality in developing countries especially among children under the age of five (Kosak *et al*., 2003; Prüss *et al*., 2002). The importance of improved sanitation in safe-guarding the health and wellbeing of human kind is well documented (Cairncross, 2003; Moe and Rheingans, 2006). In 1977, the UN Water Conference in Mar del Plata, Argentina recommended that the 1980s should be proclaimed the 'International Drinking Water Supply and Sanitation Decade' (IDWSSD). One of the action plans aimed that all countries should achieve 100 % coverage in water supply and sanitation by 1990. Although generally the provision of services did increase, in many countries the increase in sanitation facilities could not keep pace with the rising population, meaning that the percentage of unserved people continued to rise (DFID, 1998). Unfortunately, today 2.5 billion people still lack adequate sanitation (WHO/UNICEF, 2014). The UN millennium development goal 7 target 7.C aims at halving the proportion without access to safe drinking water and basic sanitation by 2015, yet many are people living without it. Regions that could not keep the target are Sub-Sahara Africa, Asia and Oceania. In fact, Sub-Sahara Africa recorded the least progress of only 5 %: from 26 in 1990 to 30 % in 2012 (WHO/UNICEF, 2014).

The WHO (2014) reported an estimated proportion of the population without access to improved sanitation for the period 1990 - 2012 as 81 % in Burkina Faso. In fact, most countries in Sub-Sahara Africa recorded high percentages of the population without the access. Sub-Saharan Africa has not made progress in reducing open defecation that causes a serious wide-spread of diseases. In fact, it has decreased by only 36 per cent since 1990 (WHO/UNICEF, 2014). Excreta can contain large concentrations of pathogenic viruses, bacteria, protozoa and helminth eggs (Faechem *et al*., 1983; WHO, 2006). They have the possibility to enter the human body by a number of routes due to poor domestic and personal hygiene and these routes involve food, water and hands. Indeed, in our study region, Burkina Faso, mortality distribution of causes

of death for diarrhoea among children of under 5 years only reduced from 13 % in 2000 to 11 % in 2012 (WHO, 2014). Therefore, continued efforts for improving sanitation are needed especially, to manage human excreta in Sub-Sahara Africa to bring sanitation 'on track' (WHO/UNICEF, 2014). Management of human excreta has become increasingly very important to all governments in Sub-Sahara Africa. Centralised sewage systems, leading to wastewater treatment plants seem to be unsuitable in rural areas of arid zones of developing countries because of their enormous investment, operating and maintenance cost, their high water consumption and other drawbacks (Lopez Zavala *et al.*, 2004).

1.2 Problems on Food Security and Land Management

The economies of the countries in the Sahel region are largely dependent on agriculture, with about 80 - 90 % of the population actively engaged in agriculture (UNEP, 2012). The Sahel is a semi-arid grassland and shrub land transition zone stretching across the African continent between the Sahara desert to the north and the tropical savannahs to the south (Herrmann, 2005). It covers parts of Senegal, Mauritania, Mali, Burkina Faso, Algeria, Niger, Chad, South Sudan, North Sudan and Eritrea (UNEP, 2012). Figure 1.1 shows the Sahel region of Africa. The Sahelian soils are mainly sandy (Bationo *et al*., 2014) with the dominant soil types being Entisols and Alfisols (Kang, 1985). Phosphorus and nitrogen are mostly deficient (Bationo *et al*., 2014; Breman *et al*., 2001). Table 1.1 shows some soil properties in the Sahel. In the Sahel, agricultural extensification and intensification due to population growth and unsustainable agricultural practices that reduce vegetation cover on lands have contributed to land degradation (Doso, 2014). Soil degradation through soil erosion is the main form of land degradation in the Sahel, resulting in nutrient loss, soil physical degradation and salinisation and consequently reducing agricultural productivity (Doso, 2014). Practices that promote organic matter additions to soils such as mulching and the addition of organic manure can also have positive benefits for both agricultural production and addressing land degradation (Doso, 2014). In Sahelian countries like Burkina Faso, frequent drought and poor soil fertility are key factors behind food shortages. But innovative, low-input technologies that simultaneously replenish soil nutrients and organic matter, as well as improving soil water availability, can lead to significant increases in crop production and reduce acute food shortages. Under conventional crop management in Sahelian

countries, chemical fertilisers are applied at recommended rates to rapidly replenish soil fertility and thus improve crop yield. Here, the world demand for nitrogen, phosphorus and potassium was forecast to grow by 1.6, 2.4 and 2.0 % over the previous year, respectively (FAO, 2012). The high demand of these fertilisers in the world makes their prices very expensive for the poor people. A bag of fertiliser in Burkina Faso sells averagely at 16,000 FCFA. These high prices of fertiliser causes low yields resulting in serious food insecurity, because farmers can apply less amount of the fertilisers (FAO, 2012).

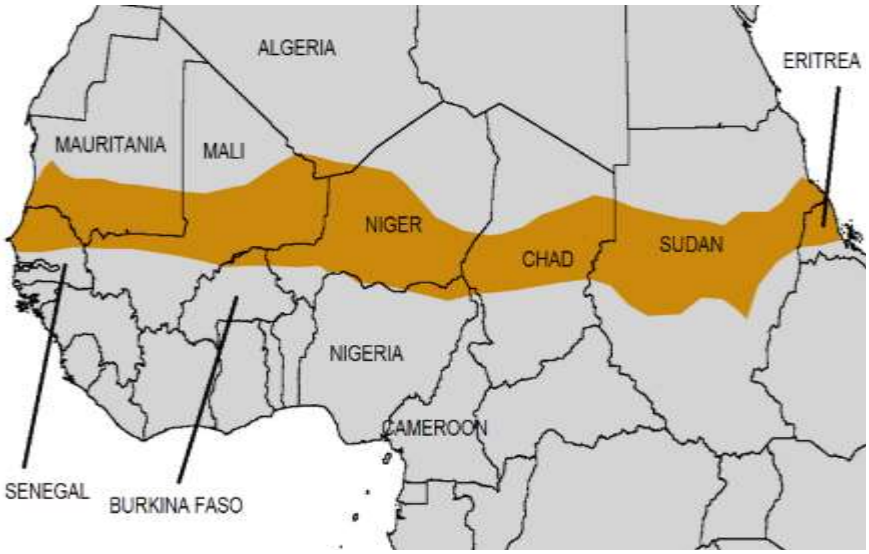


Figure 1.1: *The Sahel region of Africa (UNEP, 2012).*

Table 1.1 General soil properties in the Sahel

Property	**Characteristic**
Structural stability	Poor
Nutrient holding capacity	Low
Water retention capacity	Low
Organic matter content	Low
Effective cation exchange capacity	Low
Drought susceptibility	High
Texture	Coarse

Source: (Kang 1985; Bationo and Mokwunye, 1991)

1.3 Advantages and Disadvantages on the Reuse of Excreta

As with anything in life, there are advantages and disadvantages of reuse of excreta and composted faeces in agriculture. Positive health benefits may arise from the safe use of excreta, which may result in increasing household food security and nutritional variety, and generating household income, as observed in several countries. These can be used to support health-promoting activities such as education or access to better health care (WHO, 2006). Bio-solids, sludge and excreta in particular, apart from the major issues above, it also provides numerous micronutrients such as cobalt, copper, iron, manganese, molybdenum and zinc, which are essential for optimal plant growth (Jiménez *et al*., 2010). Reuse of these fertilisers could reduce the demand for chemical fertilisers. Indeed, considering the global phosphorus crisis, excreta can be critical sources of phosphorus (Rosemarin, 2004).

Among the disadvantages of using untreated excreta, the most obvious are the health risks from pathogens. Some references will be provided here in order to give an idea of the magnitude of the problem. Firstly, it should be stated that diseases are linked to the nature of the pathogen in the excreta thus vary locally following the local public-health pattern. Secondly, the risks are not limited to farmers, but can be observed in four groups: agricultural workers, their families, crop handlers, or consumers of crops (Jiménez *et al*., 2010). Within these groups the most vulnerable sections of the population are the farmers in our observations. Thirdly, observed responses may vary considerably between developing and developed countries (Jiménez *et al*., 2010). This is because different distributions and concentrations of pathogens which these groups are exposed to are different as well as the level of resistance to disease (Jiménez, 2007; Jiménez and Wang, 2006). It is also reported that excessive concentrations of nitrogen in excreta can lead to over-fertilisation and cause excessive vegetative growth, delayed or uneven crop maturity and reduced quality (Jiménez, 2006; Qadir *et al*., 2007). Excessive concentrations of some trace elements may also cause plant toxicity (Jiménez *et al*., 2010). In fact, recommended amount of application are encouraged.

1.3.1 Potential of reuse of faeces and urine as fertiliser

Human faeces can be a good source of organic matter which can improve soil physical conditions such as the soil structure, soil aeration and drainage in poor Sahelian soils. It is also a

rich source of N, P, K and other micro elements, which can fertilise the soil. The nutrient contents of faeces originate from the food consumed. This indicates that amounts of nutrients in excreta vary from person to person and day by day. However, Wolgast (1993) reported that one person annually produces 50 kg of faeces, while this contains 10 kg of dry matter. One person produces approximately 5.7 kg of N, 0.6 kg of P and 1.2 kg of K per year (Wolgast, 1993). The population of Africa is 1.1 billion (World population data, 2013). Using the population figures, estimated fertiliser potential in Africa would be, 6.27, 0.66 and 1.32 billion kg/year of nitrogen, phosphorus and potassium, respectively.

The application of treated excreta or composted excreta to the land to fertilise crops is a common practice in Ghana, Burkina Faso, Japan, China, Guatemala, Zimbabwe, Sweden and Vietnam, etc. In some countries like China, it was the only agricultural use option in areas without a sewerage system. On-site sanitation technologies that readily permit the use of treated and composted excreta: for example, alternating twin-pit and composting toilets as used in such places as Guatemala, Vietnam and Burkina Faso should be encouraged (Mara and Caincross,. 1989). Historically the importance of treated excreta use in agriculture would be judged by the experience in China, where soil fertility has been maintained by this practice and other methods (application of other organic matter, chemical fertilisers etc.) for thousands of years. In 1965, approximately 90 % of all human excreta produced in China was used as fertiliser (Chao, 1970). In addition to supplying nutrients, excreta is very valuable in increasing the humus content of the soil, which significantly improves the structure and water-retaining capacity of the soil. Notwithstanding the clear agricultural advantages, there is in many societies a strong socio-cultural aversion to the agricultural use of excreta, although the use of some excreta-derived products is common and socially acceptable. In the United Kingdom, for example, 47 % of all sludge from municipal wastewater plant is applied to land (Water Authorities Association, 1985). Although large quantities of faecal sludge are dumped into depressions or watercourses, in many areas in Ghana, some farmers like to empty septic trucks onto their farms (Cofie and Adamty, 2009). Investigations were carried out among selected farming communities in two urban agglomerations (Tamale and Bolgatanga) in the North and one district, Manya Krobo in the southern part of Ghana. In all, 150 farmers were interviewed through focus group discussion and questionnaire survey between year 2003 and 2006. The adoption of faecal sludge as an

alternative to inorganic fertiliser in crop production was investigated. The study also identified factors that influence its use, the constraints as well as the agronomic and economic benefits (Cofie and Adamty, 2009). The study by Cofie and Adamty (2009) reported the following observations; 1. Good demand for faecal sludge use in agriculture due to generally positive perception; 2. Faecal sludge based compost produced bigger girth of maize cobs compared to inorganic fertiliser or soil without amendment; 3. The yield of faecal sludge plots was 10% higher than that for chemical fertiliser; 4. More than 50 % of maize crops of faecal sludge plots tassel, from silk and matured one week earlier than crops on fertiliser treated plots; 5. Fresh grains from t faecal sludge plots were sweeter than those from inorganic fertilisers.

Burkina Faso also has experience in excreta reuse. In 2001, experiments were conducted in the suburbs of Ouagadougou to collect, treat and utilise urine and faeces for crop and cereal production. Two years after the implementation of this environmental project, the results of the experiment are reassuring for those in favour of recycling human excrement for agricultural production. People were impressed by the crop yield grown using fertilisers produced from human intestines (Arzouma, 2012). Cabbage, okro and wheat fields fertilised using treated human urine and faeces can be admired in the city of Ouagadougou. Farmers that tested these fertilisers found them good. Although they were somewhat off-putting in the beginning, today many farmers use these "made in Burkina Faso" fertilisers. For them, these fertilisers have a much greater yield than chemical fertilisers. The natural fertilisers manufactured in Burkina Faso are produced using a scientific process (Arzouma, 2012). To mitigate the global fertilizer demand, to reduce infections pathogenic diseases and to build a sustainable society based on recycling faecal matter, there is the need to link sanitation and agriculture supported by several scientific researches.

There are several studies on human excreta and their nutrient values. Faeces produced per person ranges between 30 and 110 kg, wet weight, per person and year. This corresponds to 10 - 15 kg of dry matter (Lentner *et al*., 1981; Vinnerås, 2002; Niwagaba *et al*., 2009). Other studies indicated that the faecal excretion rate in the developing countries is on average 350 g/p per day in rural areas and 250 g/p per day in urban areas (Feachem *et al*., 1983). Gao *et al*. (2002) measured 315 g/p per day while Pieper (1987) measured 520 g/p per day in Kenya. Schouw *et al*. (2002) measured faecal generation of 15 individuals in three different areas in Southern Thailand

and obtained wet faecal generation rates of 120 - 400 g/p per day. The faecal excretion rate is on average one stool per person per day, but it may vary from one stool per week up to five stools per day (Lentner *et al*., 1981; Feachem *et al*., 1983). Estimates show that food nutrient content is distributed to the faecal fraction in the proportions: 10 - 20 % nitrogen (N), 20 - 50 % phosphorus (P) and 10 - 20 % potassium (K) (Berger, 1960; Lentner *et al*., 1981; Guyton, 1992; Vinnerås *et al*., 2006). Wolgast (1993) reported that one person produces approximately 90 g N, 190 g P and 170 g K per year. Faecal nitrogen is mainly found as organic nitrogen and has therefore to be mineralized before it becomes available for plants. Phosphorus is mainly found as small grains of calcium phosphates in the faeces (Frausto da Silva and Williams, 1997) and this phosphorous is available to plants. Potassium is mainly found in its water-soluble ionic form (Berger, 1960) and is therefore readily available. Figure 1.2 shows the nutrients found in household waste fractions and biowaste in Sweden.

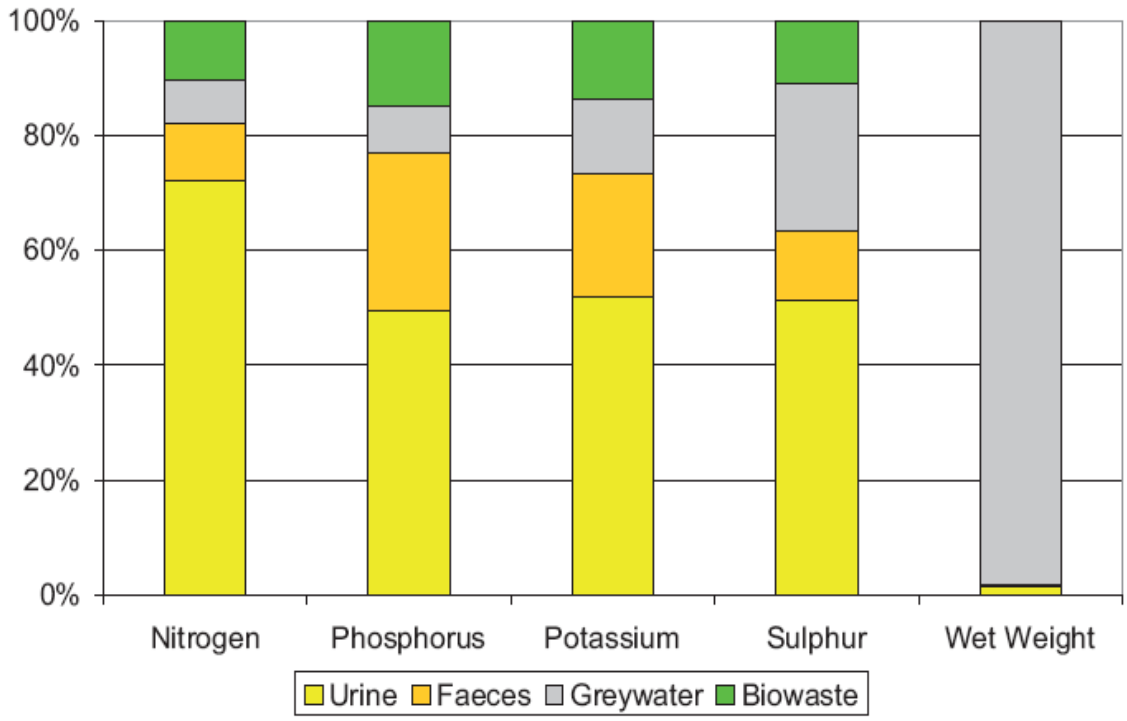


***Figure 1.2**. Proportions of nutrients found in household wastewater fractions and biowaste in Sweden. Source: Jönsson et al., 2005.*

Urine is the excreta fraction that is filtered from the blood by the kidneys (Guyton, 1992). Urine is used by the body as a balancing medium for liquids and salts and the amount of urine excreted

by a person therefore varies (Jönsson *et al*., 2004). Urine largely consists of water, approximately 93-96 % and large amounts of plant nutrients that are mainly in water-soluble form (Jönsson *et al*., 2004). Figure 1.2 shows the nutrient proportions of urine. The urine generation rate for most adults is between 1000 and 1300 g/p per day (Feachem *et al*., 1983). The nutrients in urine are in ionic form (Johansson *et al*., 2001; Kirchmann and Pettersson, 1995; Kvarmo, 1998). Urine contains large amounts of P and K for plant growth, but due to its large content of N, its P/N and K/N ratios are lower than in many mineral fertilisers used for vegetable production (Jönsson *et al*., 2004).

Indeed, humans excrete approximately 7.5 kg of fertiliser each year, mainly nitrogen, phosphorus and potassium, which are sufficient to grow 230 kg of cereal when treated appropriately (Wolgast, 1993). After pathogen destruction through dehydration and/or decomposition (composting), the useful material can be applied to soil to increase the quality of soil as mentioned earlier (Mara *et al*., 1989). Humus from the decomposition process also helps to maintain a healthy population of beneficial soil organisms that actually protect plants from soil-borne diseases (Esrey *et al.*, 1998).

There are recommendations for use of faeces and urine in cultivation. For a starting point when deciding the application rate of urine and faeces, local recommendation for use of conventional N (preferably urea or ammonium fertilisers) and P fertilisers should be considered. If local recommendations for reuse of faeces and urine are not available, a starting point for reuse can be to estimate the amounts of nutrients removed by the crop (Jönsson *et al*., 2004). For a few crops the removal per metric ton of harvested edible fraction is given in Table 1.2 below. These amounts should be multiplied by the estimated harvest to get the amounts of plant nutrients removed (Jönsson *et al*., 2004). It is important to remember that an application rate corresponding to the amount of nutrients removed by the edible fraction of the crop is lower than the application rate needed for highest crop yield, especially on soils of low fertility. The fertiliser supplied has to provide nutrients for the root system, crop and crop residues removed from the field, and there are usually some additional losses of N, K and S in particular through leaching, and of N also through volatilization (Jönsson *et al*., 2004). Some nutrients are also lost if the waste from processing the crop is not recycled to the field as fertiliser. Therefore, the amounts calculated from Table 1.2 give the minimum application level needed for maintained

fertility (USDA, 2004). Higher application rates, often twice as high, are needed to simultaneously increase the fertility of the soil, which is needed to get a high yield off poor soils such as the Sahelian soils. However, if N is supplied to N-fixing crops, e.g. beans and peas, their N-fixing ability is not fully utilised (Jönsson *et al*., 2004).

Table 1.2 Amount of N, P and K (kg/ha) removed per metric ton of harvested edible fraction for different crops

Crops	Crop Amount	Water content	N	P	K
	kg/ha	%	kg/ha	kg/ha	kg/ha
Cereals					
Maize, dry	1000	10	15.1	2.1	2.9
Maize, fresh	1000	69	6.2	1.1	2.9
Millet	1000	14	16.8	2.4	2.2
Rice unpolished	1000	12	12.4	3	2.3
Sorghum	1000	11	17.6	2.9	3.5
Wheat	1000	14	17.5	3.6	3.8
Green beans. fresh	1000	90	2.9	0.4	2.4
Irish potatoes	1000	80	2.9	0.3	4.7
Lentils. Dry	1000	12	38.4	3.8	7.9
Onions	1000	91	1.9	0.4	1.9
Pumpkin	1000	92	1.6	0.4	3.4
Red beans, dry	1000	11	35.2	4.1	9.9
Soybeans, dry	1000	10	59.5	5.5	17
Spinach	1000	94	3	0.3	5.6
Tomatoes	1000	93	1.4	0.3	2.1
Water melon	1000	91	1	0.1	1.2
White cabbage	1000	92	2.2	0.3	2.7

United States Department of Agriculture (USDA), 2004

1.3.2 Transmission of pathogens in faeces and urine

Indeed, faeces contain approximately 10^{10} - 10^{13} microorganisms per gram of dry matter, some of which can be pathogenic (Vinnerås *et al*., 2003; Stenström, 2004). The faeces of a healthy person contain large numbers of bacteria of many non-pathogenic species, referred to as normal intestinal microbiota. Gastrointestinal pathogenic microorganisms do not occur as a natural part of normal intestinal microbiota (Feachem *et al*., 1983). Their presence in faeces is an indication of infection amongst the population contributing to the faeces analysed. However, on occasion, some of the commensal bacteria otherwise referred to as the normal intestinal microbiota may give rise to disease. This situation is likely to happen when the immune system of the human being has been compromised, for example, during sickness or old age, giving rise to opportunistic infections (Madigan and Martinko, 2006). Pathogens are from the gastrointestinal track of an infected person. Infections are always an exception and not general situation for an individual. Infections of individual may in rare case be chronic, bacterial and viral disease. The individuals are then called "carriers". Parasitic worms (helminths) may establish themselves for long periods in the human body and have high prevalence rate in societies with run sanitary conditions (Madigan and Martinko, 2006). An individual will normally excrete large amounts of microorganisms in faecal material. Groups of pathogens found in faeces are:

- Bacteria e.g. *Escherichia coli* (EIEC, EPEC, ETEC, EHEC), *Salmonella spp*. etc.
- Virus e.g. Rotavirus, Adenovirus, Norovirus etc.
- Parasitic protozoa e.g. *Giardia* intestinalis, *Crytosporidum* parvum, *Entamoeba* histolytica etc.
- Helminths e.g. *Ascaris lumbricoides*, *Taenia solium* etc. (Schönning and Stenström, 2004).

In this study all groups of pathogens found in faeces were measured except parasitic protozoa, although they were present in our compost. Preliminary investigations showed that Entamoeba histolytic was present, however, we could not obtain them in higher concentration for the kinetic analysis.

As previously stated several intestinal pathogenic microorganisms enter a new host by ingestion (water, food, dirt on fingers) (Feachem *et al*., 1983). After infecting the host, large numbers of pathogens may be excreted. Depending on the health of the population, several species of pathogenic bacteria, viruses, parasitic protozoa and helminths may be found in the faeces from the population. From a hygiene point of view, any exposure to fresh/untreated faeces constitutes a risk (Feachem *et al*., 1983; Schönning and Stenström, 2004; WHO, 2006).

In a healthy individual, urine in the bladder is sterile. However, different types of bacteria are picked up in the urinary tract. Freshly extracted urine normally contains <10 000 bacteria per ml. From the patient of the urinary tract infections, significantly higher amounts of bacteria are excreted (WHO, 2006). Some pathogens, such as *Leptospira interrogans*, *Salmonella typhi*, *Salmonella paratyphi*, *Schistosoma haematobium* and some viruses can be excreted in urine. A range of others has been detected in urine, but risk of environmental transmission may be considered insignificant (WHO, 2006). Urinary excreted pathogens are of less concern for environmental transmission than faecal pathogens. Experiments in Sweden have established that, should faecal contamination of source-diverted urine occur, six months of storage time is probably sufficient for the destruction of pathogenic organisms. However, this is also dependent on the temperature and dilution of the mixture – lower temperatures and higher dilutions tend to increase the survival time of the pathogens (Olsson, 1996; Höglund *et al.*, 1998).

In developing countries especially, excreta-related diseases are very common, and the excreta thus contains high concentrations of pathogens that cause diseases in man. This study focus on the hazard associated with handling of faeces based compost by farmers. Pathogenic organisms can enter the human body by a number of routes, as illustrated in Figure 1.3. It should be noted that poor domestic and personal hygiene involving food and hands, often diminishes or even negates any positive impact of improved excreta disposal on community health. Technology by itself cannot break the cycle of disease transmission and accompanying ill health if hygiene awareness in a community is at a low level (Aussie, 2002). The faecal matter should preferably be treated on site at the point of collection to avoid handling of the pathogen-containing material, as one of the major transmission routes is direct contact with raw untreated faecal matter (Faechem *et al*., 1983). Indeed, if treatment is not complete, pathogens may find their way into the human body.

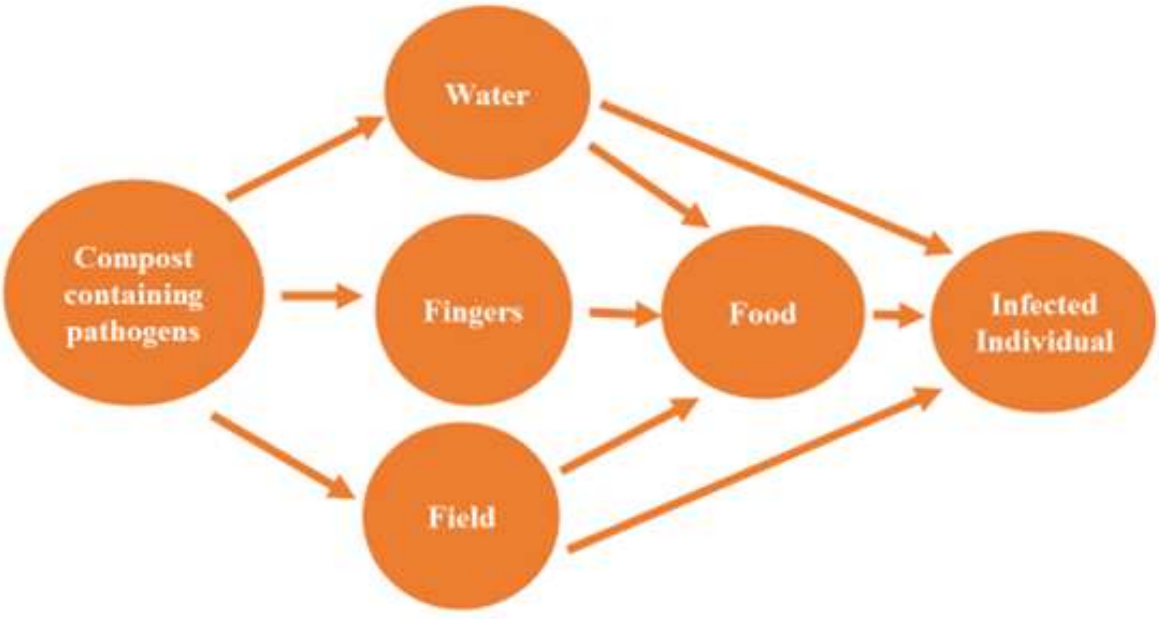


Figure 1.3: *Transmission route of pathogens*

N.B: The diagram shows the different routes that diseases take from faeces-based compost, through the environment to an infected individual. This can take the form of faecal oral route through contamination of food and water (mostly bacterial, protozoa, virus), soil transmitted helminth through the field contamination and fingers e.g. Ascariasis (roundworm), Trichuriasis (whimpworm), Hookworm, sometimes through host like uncooked beef and pork (Taeniasis) (Caincross and Feachem, 1999). See Annex 1 for the life cycles of excreted helminth.

For the agricultural use of excreta to pose an *actual* risk to health requires *all* of the following to occur:

(a) *either* an infective dose of an excreted pathogen reaches the field *or* the pathogen multiplies in the field to form an infective dose;

(b) the infective dose reaches a human host;

(c) the host becomes infected; and

(d) the infection causes disease or further transmission.

The risk remains a *potential* risk if only (a), or (a) and (b), or (a), (b) and (c) occur, but not (d).

Even if there is an actual risk involved, the agricultural use of excreta will be of public health importance only if it causes a measurable excess incidence or prevalence of disease or intensity

of infection (Aussie, 2002). Therefore, the health risk reusing the compost for farmers should be assessed, based on academic evidence.

1.4. Methods of inactivation of pathogens in compost

As discussed above, faeces often contain high concentration of pathogens that has to be reduced to an acceptable level. The reduction of concentration is achieved by the decay of the microorganisms, inhibiting their growth and accelerating their decay rate. Environmental conditions that influence the decay rates are nutrition, water activity, pH, temperature, toxic materials to damage their metabolisms etc. There are several practices to inactivate pathogens in faeces based compost. Among them are long time storage, heat treatment, UV radiation, alkaline treatment, incineration (Schönning and Stenström, 2004) etc. Some studies used a combination of the above methods. Koné *et al*., (2007) showed that *Ascari*s eggs, which is most resistant to the bad environment, could be inactivated with temperature. Low moisture content in the operating process was recommended by Koné *et al*. (2007), Sanguinetti *et al*. (2009), Singh *et al*. (2011) etc. to help facilitate pathogen reduction. Sanguinetti *et al*. (2009) indicated that apart from low moisture content and temperature contributing to successful inactivation of pathogens, high pH (addition of lime) >12 could aid in the inactivation process. Singh *et al*. (2011) added that factors such as carbon/nitrogen ratio (C/N), and types and population of indigenous microflora around the pathogens could affect the inactivation process. Several authors recommended high temperatures to inactivate pathogens, it is really difficult to attain such high thermophilic temperatures during the composting process.

1.4.1 Current available composting toilets

Our project model is the "On-site Wastewater Differentiable Treatment System (OWDTS)" (Figure 1.8), which has "Don't mix and Don't collect" principle where household waste is separately collected with pollution level as faeces, urine, and greywater then treated individually. The merit of this system is to reduce costs for piping system and to allow the use of special treatment units. The toilet systems handle faeces and urine separately to produce fertilisers. A slanted soil treatment system treats greywater to produce water for irrigation. A pilot study is

being performed to investigate the feasibility of the rural model urine diverting composting toilet in three villages in Burkina Faso namely; Kamboinse, Kologondiesse and Barkoumba.

The composting toilet technology is decentralised, requires no water, creates a value product (fertiliser) and can possibly reduce the burden on the current infrastructure as a sustainable sanitation approach (Nakagawa *et al*., 2006) (Annex 3). The toilet has a reactor with mixing, heating and ventilation devices under the toilet bowl. The reactor captures faecal matter, then mixes with organic material such as sawdust in vaults or pits. Several other material such as rice husk, millet husks etc. can be used as composting matrix (Annex 2). The heating device produces heat to control the moisture content of the matrix and to accelerate the biodegradation rate. The ventilation removes emission of moisture, carbon dioxide and other gases, and supply fresh air into the reactor. Under properly managed conditions, aerobic decomposition takes place so that excreta is transformed into humus-like compost suitable for use as a fertiliser. This system has a rapid biodegradation rate of organic matter of faeces which finishes in two (2) days after final input of faeces (Lopez Zavala *et al*., 2004).

In the world, there are many cultures regarding the usage of toilets, e.g. using paper or water after defecation, sitting or squatting styles, availability on flushing water, open defecation, regulations, religious experience to use toilets etc. and also chain of the materials around the toilet. To adopt this situation, many designs of toilet should be available considering the public acceptance. The rural model of composting toilet system has the same functions above but do not use electricity. Figure 1.6 shows the different arrangement type composting toilet.

Figure 1.4: *Example of Japanese type composting toilet (Source: H. Darimani)*

Figure 1. 5*: Rural model composting toilet used in the study showing the inner view of the composting reactor (Source: R. Ito).*

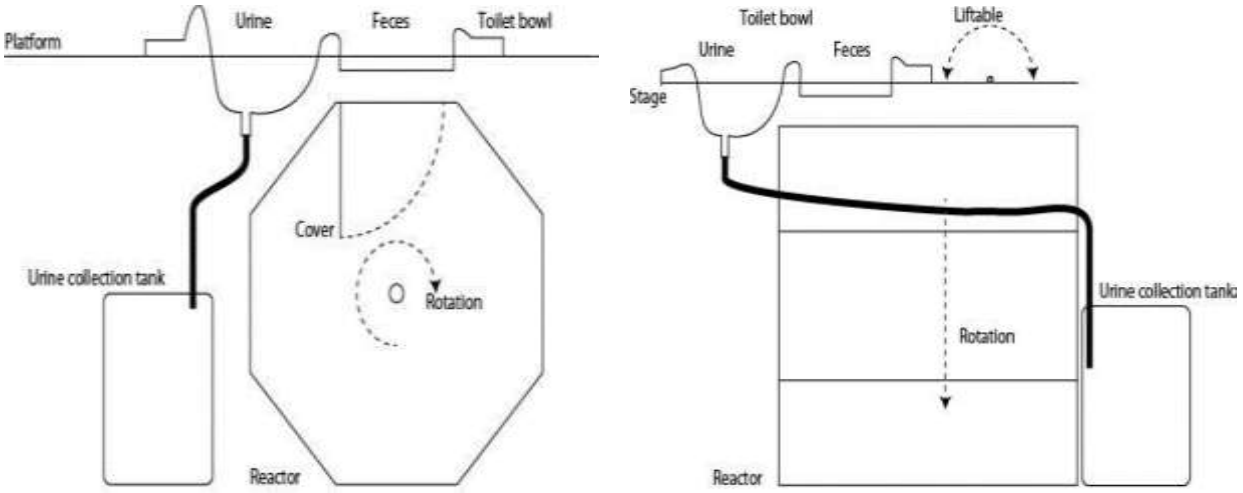
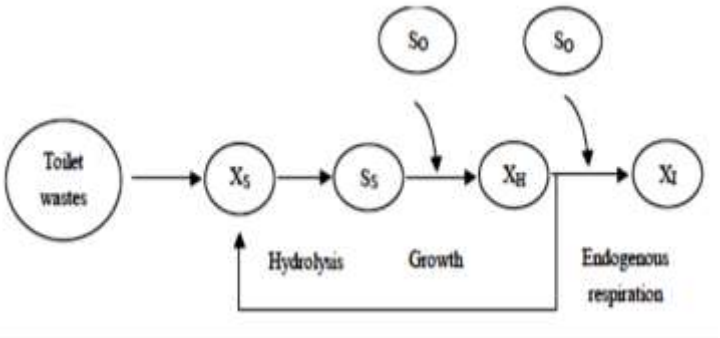


Figure 1.6: *Arrangement of composting toilet showing the squatting type and sitting style (Ito et al., 2012)*

1.4. 2 Reactions in composting process

Biodegradation rates of organic matter in the composting toilet system are very important because of the continuous use of the composting toilet and the aim of accelerating decomposition (Lopez Zavala *et al*. 2004). For bioconversion of faeces into compost, the composting toilet must function properly to achieve pathogen destruction. Inactivation of pathogens is a thermophilic process. Non-uniformity in temperature distribution leads to a reducing overall performance of pathogen destruction (Lopez Zavala *et al*. 2004). Lopez Zavala *et al*. (2004) adopted the concepts and notation of the activated sludge models for biodegradation of faeces as illustrated in Figure 1.7; where, slowly biodegradable substrates (X_S) and particulate substrates, must undergo cell external hydrolysis before they are available for degradation. It is assumed that a product of hydrolysis is readily biodegradable (S_S) material, a soluble fraction directly available for biodegradation by heterotrophic organisms. They reported that biodegradable fraction of faeces is constituted by slowly biodegradable organic matter (X_S). This material is transformed into readily biodegradable organic matter (S_S) by hydrolysis and used by heterotrophic biomass (X_H). One portion of the consumed S_S is oxidised to provide energy and the remainder of the substrate molecules is reorganised into new cell mass. Under the death-regeneration approach, decaying biomass is split into two fractions: inert matter (X_I) and slowly biodegradable matter (X_S); the latter is subsequently hydrolysed into readily biodegradable substrate.



Y_H: Yield coefficient for heterotrophic biomass, g COD (g COD)$^{-1}$.
S_O : oxygen concentration in the bioreactor, g O_2.
S_S: readily biodegradable organic matter, g COD.
X_H: heterotrophic organisms, g COD.
X_S: slowly biodegradable organic matter, g COD.
X_I: inert organic material, g COD.

Figure1. 7: *Modelling approach for the aerobic biodegradation of toilet waste (Lopez et al., 2004).*

1.5 Excreta treatment requirements

The transmission cycle of pathogens (Figure 1.3) can be interrupted by putting barriers in place to block transmission paths and prevent cycle completion. Faecal matter needs to be treated to an adequate hygienic level depending on the purpose of reuse. The World Health Organization (WHO) guidelines for safe agricultural practice published in 1998 specified one or less helminth egg/g total solids (TS) for unrestricted irrigation (WHO, 1998). However, the more recent WHO Guidelines for the Safe Use of Wastewater, Excreta and Greywater in Agriculture and Aquaculture (WHO, 2006) places less emphasis on treatment thresholds but rather highlights on a multi-barrier approach where lower levels of treatment may be acceptable when combined with other treatment along the sanitation chain. This concept of a multi-barrier approach combined with a risk assessment and risk management system is designed to protect public health (WHO, 2006). The first barrier for beneficial users is provided by the treatment of faecal matter. Further treatment mentioned below can be selected; restriction of use on crops like eating them raw, withholding periods between application and harvest to allow pathogen die-off, drip or subsurface irrigation methods, restricting worker and public access during application, use of personal protective equipment, and safe food preparation methods such as thorough cooking, washing or peeling. Considering the risk of infection, all potential exposure groups should be accounted for which can be broadly categorised as farm workers and product consumers. In Volume 2 of WHO Guidelines, it was generally said that wastewater treatment is required to reduce the *E. coli* count by 4 log unit reduction. The corresponding reduction of raw faecal material will thus be 6 log unit, while normally a 2 log unit reduction will suffice for urine and

greywater. In order to achieve the health-based target which is usually set at 10^{-6} DALY per person per year, microbial reduction can be evaluated based on the results of microbiological studies.

The multiple barrier concept of reuse, which is the key cornerstone of this thesis, has led to a clear understanding on how faecal matter reuse can be done safely. The concept is also used in water supply and food production and is generally understood as a series of treatment steps and other safety precautions to prevent the spread of pathogens. In this study, a composting toilet treatment of faeces-based compost was first treated by the composting toilet. A post-treatment unit was proposed for secondary treatment, but firstly, the die-off rate of the indicator pathogens (Bacteria, Helminth eggs and Viruses) with heat in the compost must be known. These parameters would be a guide to design the post-treatment unit for effective heat treatment. Secondly, the temperature distribution with solar thermal heat of the unit at different depth should be characterised. The compost would be amended to the soil by the farmers. Therefore, the temperature of the soil at different depths should also be characterised. Thirdly, utilisation of the compost would pose some health risk to the farmers amending the cultivated soils. Therefore, the health risk for the utilisations of compost should be assessed. Finally, farmers working in the field would also be at risk for compost-amended soils. The health risk associated with the farmers working on compost amended soils should be assessed.

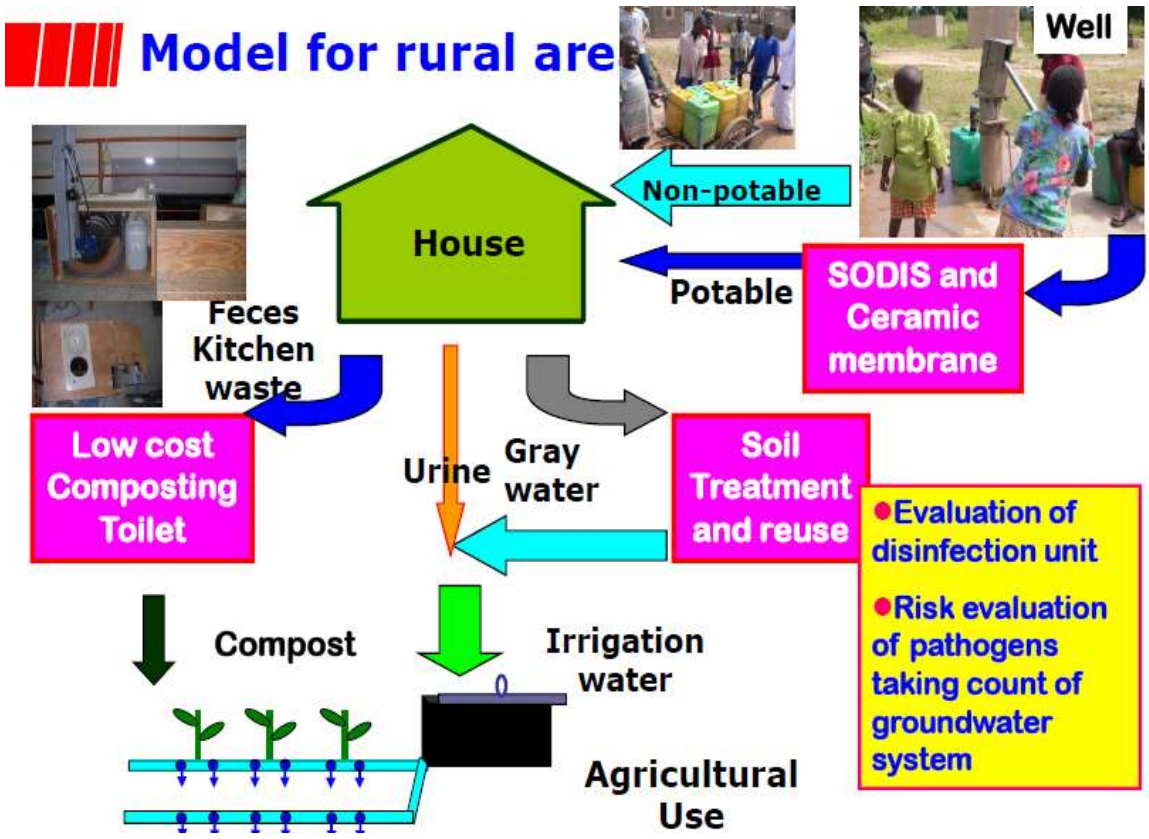


Figure 1.8: *Onsite Water Differentiable Treatment (OWDTS) Concept (Adopted from Améli-EAUR project)*

1.6 Objectives

The compost is produced from human faeces with the aim of promoting safe nutrient recycling. The pathogens in faeces are first treated by the composting toilet. The composting toilet has shown to result in a more degraded final compost, but its effectiveness for pathogen destruction was not effective due to lower temperature in the composting toilet. Based on a multi-barrier approach to protect public health, our research group proposed the composting toilet with post-treatment to further inactivate the pathogens with the solar thermal heat before the amendment of compost into farmland. The main objective of this thesis is to design the post-treatment unit and its operation plan with consideration of the worst case that the first composting process failed to inactivate the pathogens in the compost. To achieve the objective, the following specific objectives were set:

i. to characterise the thermal die-off represented by kinetic inactivation rate coefficient of pathogens in compost and in the soil.

ii. to characterise the temperature of the unit and of the cultivated soil.

iii. to simulate the performance of post-treatment unit by assessing the health risk during the amending of compost by the farmer.

iv. to determine the health risk associated with farmers working on the cultivated soils after amendment of the treated compost.

1.7 Thesis structure

Chapter 1 establishes certain ideas and concept that will reappear throughout this thesis. It provided information on most of the issues raised in this Thesis. The first of these is a necessary discussion of how faecal matter is disposed indiscriminately. It also tried to explain the fact that if faecal matter is properly treated, it can be a source of fertiliser for cultivated soils. In fact, it explained extensively how faecal matter can be treated with the composting toilet for the safe reuse and a multiple treatment recommended. The major issue of concern in the reuse of faeces-based compost is the health risk. The importance of the risk was also delebrated on.

Chapter 2 through to **Chapter 4** discussed one of the central ideas of this thesis. Inactivation of indicator pathogens is the basis for the recycling of faecal matter in agriculture. From the discussion in **Chapter 1**, pathogens are a major cause of disease to man therefore the inactivation rates of indicator pathogens were well characterised with temperature and moisture. **Chapter 2** deals with pathogenic bacteria, **Chapter 3**, Helminth eggs (*A. suum eggs*) and **Chapter 4** viruses (MS2 bacteriophage). From these chapters mathematical models were developed to design the post-treatment unit for safe compost reuse.

After, the characterisation of indicator pathogens, design of a post-treatment unit for compost from the composting toilet with microbial risk assessment as a recommended secondary treatment was done in **Chapter 5**. This chapter characterised the temperature of the unit from the top, middle and bottom of the compost pile in the unit. Using the inactivation rate parameters (**Chapter 2** to **4**) measured, the quantitative microbial risk assessment by the Monte Carlo

simulation was used to assess the health risk associated with reuse of compost by the farmer and the length of time required for the compost to reach a safe level determined. Next, the inactivation of indicator bacteria (*Enterococcus*) in two soil types i.e. clay and sandy loam soils were considered in **Chapter 6**. This Chapter also characterised the temperature of the cultivated soils at different depths, i.e. top, middle and bottom. It was assumed that due to human error compost might not be safe, but farmer may mistakenly apply it to the soil. Therefore, characterisation of the inactivation rate of *Enterococcus* in the soils was done. The health risk associated with farmers working on the soil was also assessed. **Chapter 7**, summarises all the conclusions made by this study. It shows the rate of inactivation of indicator pathogens and showed the established equations to estimate the inactivation rate of the selected pathogens given any temperature conditions. The log removal ratio can also be determined by these equations. Protocols to design post-treatment system from the perspective of microbial risk assessment were developed. The design of the post-treatment unit and operational protocols was successfully achieved.

Annex 1 shows the life cycle of Helminth eggs. **Annex 2** shows the composting matrix available in Burkina Faso. **Annex 3** shows the commercial composting toilet in Japan. **Annex 4** show an example of the calculation of pathogen concentrations for the estimation of risk. **Annex 5 a, b, c** show the QMRA-MC model for the estimation of health risk.

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Chapter 2
Effect of Temperature on the Inactivation Rate of Pathogenic Bacteria after the Composting Process

2.1 Introduction

Each year, an estimated 2.5 billion cases of diarrhoea occur among children under five years of age, and estimates suggest that overall incidence has remained relatively stable over the past two decades (WHO/UNICEF, 2014). Diarrhoea is a common symptom of gastrointestinal infections caused by a wide range of pathogens, including bacteria, viruses and protozoa which are usually found in faecal matter. However, just a handful of these organisms is responsible for the most acute cases of childhood diarrhoea. Major bacterial pathogens responsible for diarrhoea include *E. coli, Shigella, Campylobacter and Salmonella*, along with *V. cholera*. These bacterial pathogens are responsible for about 40 per cent of all hospital admissions due to diarrhoea among children under five worldwide (WHO/UNICEF, 2009). Therefore, proper control of faecal matter is required to cut the linkage from pathogens to children. On the other hand, faecal matter is rich in nutrient (Malisie *et al*., 2007) therefore, can be used as valuable fertiliser to enhance crop productivity and thus the availability of agricultural products, especially in poor countries or areas in which people cannot buy chemical fertilisers.

Our rural model of urine diverting composting toilet could not achieve the 6 log units reduction assumming an initial concentration of 10^7 CFU/g of microorganism as recommended by WHO (2006). Therefore, we recommend an additional treatment for compost from our toilet to meet the 6 log reduction target. There is no information available for the post-treatment of compost in Burkina Faso. In spite of the importance of bacteria as pathogen causing a wide range of infectious diarrhoea, nausea, vomiting throughout the years in developing countries, studies on die-off rates of bacteria are limited. It is a well known fact that temperature (above 50 °C) can inactivate pathogens, however, when considering compost sanitisation, in rural communities the specific conditions required for the inactivation are important.

The preliminary experiments in the pilot sites showed some pathogens still remained in the compost after withdrawal from the composting toilet after three months of operation. This fact motivated us to develop additional treatment in the pilot system to minimise the health risk through recycling faeces into farmland. Thermotolerant coliforms, *E. coli* and *Enterococcus* are enteric bacteria and are not always pathogenic but are often used as indicator bacteria to assess the hygienic quality of treated organic waste (Redlinger *et al*., 2001; Christensen *et al*., 2002). The destruction of pathogens requires high temperatures and high pH values. However, this is

difficult to achieve in the reactor of the urine diverting composting toilet, because the composting process with matrix works under very low organic load resulting in less heat generation and less alkaline sources like ammonia or calcium hydroxide. The Japanese urine diverting composting toilet system for human excreta has a heating device while in the conventional composting processes ash is added as alkaline source. The alkaline source does not contribute to the composting process, but inhibit bacterial activity. The proper conditions for inactivating pathogens are inhibited by less biological activities of the composting reactions. Due to these facts, the composting process and inactivation process should be done separately. Therefore, the compost requires post-treatment to further inactivate the pathogens after the composting process, while it provides options, such as to increase temperatures by solar thermal heat, to dry compost or to add ash as alkaline. The objective of this study was to determine the kinetic parameters of inactivation rates of *Enterococcus* and *E. coli* during a post-treatment of compost.

2.2 Materials and Methods

2.2.1 Compost preparation

Compost used for the experiment was collected from Kamboise pilot site in Burkina Faso. Nine (9) people used the toilet and the compost was used for 8 months on the site. One hundred grammes (100 g) of compost was put into a sterilised bottle. Ultra-pure water was added to adjust the moisture content of the compost. The combinations of temperature and moisture content for the experiment is summarised in Table 2.1. Temperatures were set to 37 °C which is easily achieved in the actual toilet, while 50 °C and 70 °C were considered higher temperatures for the thermal inactivation process. Treatment of compost was done in 8 hours for 37 °C and 50 °C while 70 °C was done in 40 mins for *E.coli* and 80 mins for *Enterococcus*. This was to ensure that moisture is controlled at the end of the experiment. Moisture contents were set to 50%, 60% and 70 %. The composting toilet normally operates around 50 to 60 % (Kazama and Otaki, 2011), while composting matrix can keep water in its micro pore to remove a thick water layer on the surface of the particles which has low transportability of oxygen. Seventy percent (70 %) moisture will promote anaerobic reaction by thick water layer owing to over capacity of water. Here, the moisture content is defined by the following equation:

$$MC = \frac{W_{wet} - W_{dry}}{W_{wet}} \times 100 \qquad (2.1)$$

where, W_{wet} and W_{dry} are wet and dry weights respectively of compost sample (g). Generally microorganism activities are affected by water activity or relative humidity but moisture content was suitable for our study because it is determined gravimetrically and practical in our case. Water activity indicates the amount of water in the total water content which is available to micro-organisms while moisture content or water content is the quantity of water contained in a sample.

Table 2.1 Experimental Conditions for Treatment of bacteria

No. of sample	Temperature (°C)	Moisture content
1		50%
2	37	60%
3		70%
4		50%
5	50	60%
6		70%
7		50%
8	70	60%
9		70%

2.2.2 Bacterial culture preparation

Compost samples contained insufficient concentrations of pathogens for kinetic analysis after the composting process. High concentration of the indicator bacterial pathogens were inoculated for this investigation, so that treatment can be done to observe reduction of the bacteria. *Escherichia coli* ATCC 11775 and *Enterococcus* ATCC 19433 strains were purchased from American Type Culture Collection (ATCC) and was grown in a 10 ml Nutritif Nutrient broth (Difco, France) by

incubating at 44 °C over night. These strains are type strain for *E.coli* and *Enterococcus* are non-pathogenic.

2.2.3 Compost inoculation with *Enterococcus* and *E.coli*

Enterococcus and *E. coli* were used as a model of pathogenic bacteria in this experiment to assess the hygienic quality of the compost because they occur in high numbers in the intestine and are associated with human faeces. They are often used to determine water quality and waste product quality (Bendixen, 1999). The inoculation procedure was similar for both *Enterococcus* and *E. coli*. The 100 g compost in the bottle was inoculated with 0.3 ml of the broth, which contains about 10^7 CFU/ml of *Enterococcus* and *E. coli* strain. The compost was agitated for 1 min to ensure that the bacteria are uniformly mixed. The bottles were tightly closed and immediately put into the incubators (manufactured by Memmert) for microbiology to control temperature. Ten grammes (10 g) of the compost sample was taken from each bottle every 2 hours for analysis.

2.2.4 Bacteria extraction and measurement

The bacteria were extracted from the compost samples with buffered peptone water extraction solution (Sidhu *et al*., 1999; Kazama and Otaki, 2011). The composition of buffered peptone water in g/L is Tryptone 10.0, Sodium Chloride 5.0, Disodium Phosphate Anydrous 3.5, and Potassium Dihydrogen Phosphate 1.5. Ten g of compost sample was added to a 90 ml volume of peptone water and agitated for 3 mins. After adequate dilution (10-10^7 times) with sterilized ringer solution (Sidhu *et al.,* 1999) each diluted extract was isolated respectively in Chromucult coliform ES agar (Merck KGaA 64271 Darmstadt, Germany) and Slanetz bartley agar for *E.coli* and *Enterococcus* by simple layer method. The media was incubated at 37 °C for 24 h, and then, *Enterococcus* and *E. coli* colonies were counted. The limit of detection of bacteria was 10 CFU/g. The recovery rate of *E.coli* using this method was 70 – 100 % (Kazama and Otaki, 2011). This study confirmed this throughout the experiment for *E.coli* and *Enterococcus.*

2.2.5 Alkaline treatment

For the alkaline treatment, the known amount of $Ca(OH)_2$ i.e. 1.0 g, 0.5 g and 0.1 g, was added to 25 ml of distilled water each and mixed properly. This mixture was added to compost with

water to adjust moisture content of 60 %, where the total amount of wet compost was 100 g. After inoculation of *Enterococcus* and *E. coli*, the compost was agitated for 1 min to ensure *Enterococcus* and *E. coli* were uniformly mixed. The bottles were tightly closed and immediately placed at 37 °C in an incubator (manufactured by Memmert) for microbiology. Ten grammes (10 g) of the compost sample was taken from each bottle every 10 mins. Five grammes (5 g) of compost sample was added to 50 ml of distilled water, and then the pH of mixture liquid was measured with a multi-parameter probe (WTW 330i).

2.2.6 Data Analysis

Concentration versus time data obtained from the inactivation experiments were fitted to a first order kinetic model. This was done for thermal and alkaline treatment of *Enterococcus* and *E.coli.* Nakagawa *et al*. (2005), indicated that inactivation of microorganisms follows a first order reaction and it is expressed as:

$$\ln k = C/C_o = -kt \qquad (2.2)$$

where, C is concentration of microorganism in compost sample on dry basis at time, t (CFU/g-dry solid), C_o is initial concentration of microorganisms in compost sample on dry basis (CFU/g-dry solid), k is inactivation rate coefficient (h^{-1}), t is treatment time (h). After the estimation of inactivation rate coefficients, k the effect of temperature was evaluated with Arrhenius equation described as follows;

$$k = A\exp\left(-\frac{E_a}{RT}\right) \qquad (2.3)$$

where, A is pre-exponential factor (-), E_a is activation energy (J/mol), R is universal gas constant (J/mol/K), T is temperature (K).

Taking the natural logarithm of Arrhenius' equation yields:

$$\mathrm{In}\left(k\right) = \frac{-E_a}{R}\frac{1}{T} + In(A) \qquad (2.4)$$

This has the same form as an equation for a straight line:

$$y = mx + c \qquad (2.5)$$

So, when a reaction has a rate constant that obeys Arrhenius' equation, a plot of ln (k) values versus 1/RT gives a straight line, whose gradient and intercept can be used to determine E_a and A. A statistical study (Two-way ANOVA without replication) was carried out to determine significant effect ($p \leq 0.05$) due to increasing temperature and moisture on the inactivation of *E. coli* and *Enterococcus.*

2.3 Results and Discussion

2.3.1 Change in concentration of *E.coli*

The inactivation rate coefficient values for *E.coli* are shown in Table 2.4. The concentrations are plotted on a logarithmic scale to easily fit with Equation (2.2). The decline in *E.coli* concentrations is shown in Figure 2.1a, b, c. The inactivation rate values during the post-treatment were almost 0 at 37 °C, while increasing with time. The urine diverting composting toilet system is operated at 37 °C without a heat source (Kazama and Otaki, 2011). Treatment at 37 °C was very poor in all post-treatments comparing with the higher temperatures. The lowest temperature at 37 °C afforded protection against inactivation. Treatment at 50 °C with varying moisture content levels, 50 %, 60 %, and 70 % recorded 5, 3 and 1 log unit respectively within 8 hours. At 70 °C, the log unit reduction in the three different moisture levels, 50 %, 60 % and 70 % were all 7 log units within 80 mins. The ANOVA results showed that, there was an effect on the performance of different temperatures on *Ecoli* inactivation. The effect of temperature on the die-off of *E.coli* was statistically significant ($p \leq 0.05$). The statistical results showed that, there was no effect between the performance of the three different levels of moisture content. Results showed that, the differences of moisture content on *E.coli* inactivation was not statistically significant ($p \geq 0.05$). The temperature was the significant factor in the inactivation of *E.coli.*

Table 2.2 Analysis of Variance for *E.coli*

Source of Variation	*Sum sq.*	*DOF*	*Mean Sq.*	*F*	*p-values*
Anova Temp.	976.37	2	488.18	29.2994	0.004083
Anova Moisture	41.61	2	20.80	1.2486	0.379023
Residuals	66.65	4	16.66		

2.3.2 Change in concentration for *Enterococcus*

The change in concentration of *Enterococcus* is presented in Figures 2.2 a, b, c. The inactivation rates of *Enterococcus* showed similar trends to *E.coli;* like inactivation rate coefficient values were almost zero at 37 °C. The inactivation rate values at 50 °C were higher than the inactivation rates at 70 °C because the duration of treatment was 80 mins, allowing counting of *Enterococcus*. Treatments with 50 %, 60 % and 70 % of moisture content at 50 °C were respectively 3.2 log, 2.3 log, and 1.8 log reduction within 8 hours. At 70 °C, with varying moisture level, 50 %, 60 % and 70 % recorded 3.4 log, 2.3 log and 1.9 log reduction within 80 mins respectively. The contact time was reduced so that colonies would be counted when subjected to high temperature such as 70 °C. The ANOVA results showed that, there was an effect on the performance of different temperatures on *Enterococcus* inactivation excluding the data for 70 °C because the time was 80 mins. The effect of temperature on the die-off of *Enterococcus* was statistically significant ($p \leq 0.05$). The statistical results showed that, there was no effect between the performance of the three different levels of moisture content. Results showed that, the differences of moisture content on *Enterococcus* inactivation was not statistically significant ($p \geq 0.05$). The temperature was the significant factor in the inactivation of *Enterococcus.*

Table 2.3 Analysis of Variance for *Enterococcus*

Source of Variation	*Sum sq.*	*DOF*	*Mean Sq.*	*F*	*p-values*
Anova Temp.	5.7825	2	2.89124	33.6188	0.003153
Anova Moisture	0.2670	2	0.13349	1.5522	0.317009
Residuals	0.3440	4	0.08600		

2.3.3 Comparison of two species

The performances at 50 °C and 70 °C with the lower moisture content as 50 % were comparatively superior in the treatment processes for both *E.coli* and *Enterococcus*. Lower moisture content showed differences in the inactivation rate coefficient values but were not

statistically significant. The rates of inactivation of *Enterococcus* was slower than *E.coli.* The inactivation of both *Enterococcus* and *E.coli* followed the first-order kinetics as indicated by a straight line on the log linear graph plots. The inactivation rate coefficients, *k,* were estimated from the results of inactivation experiments with Equation (2.2) and summarised in Table 2.4. The inactivation rate constants recorded higher values in all experimental conditions with moisture content at 50 %, followed by 60 % and finally 70 %. This same trend was observed in all conditions for both *E. coli* and *Enterococcus*. Our results are similar to several studies (Nakagawa *et al*., 2005; Kazama and Otaki, 2011), which indicated that, high temperature and lower moisture content are capable of rapidly inactivating *E.coli* and *Enterococcus*, however, there were not statistically significant. **Under high temperature, moisture content was not critical on the inactivation of *E.coli* and *Enterococcus.***

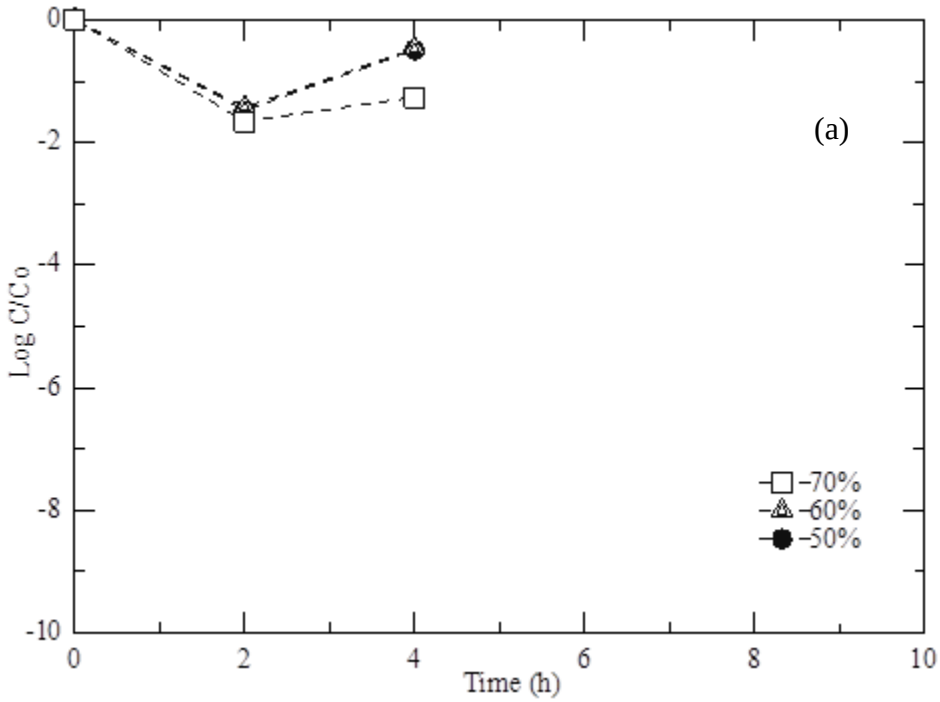

Figure 2.1a: *Inactivation of E.coli at 37 °C with varying Moisture Levels. Concentrations were measured in (CFU/g-dry solid).*

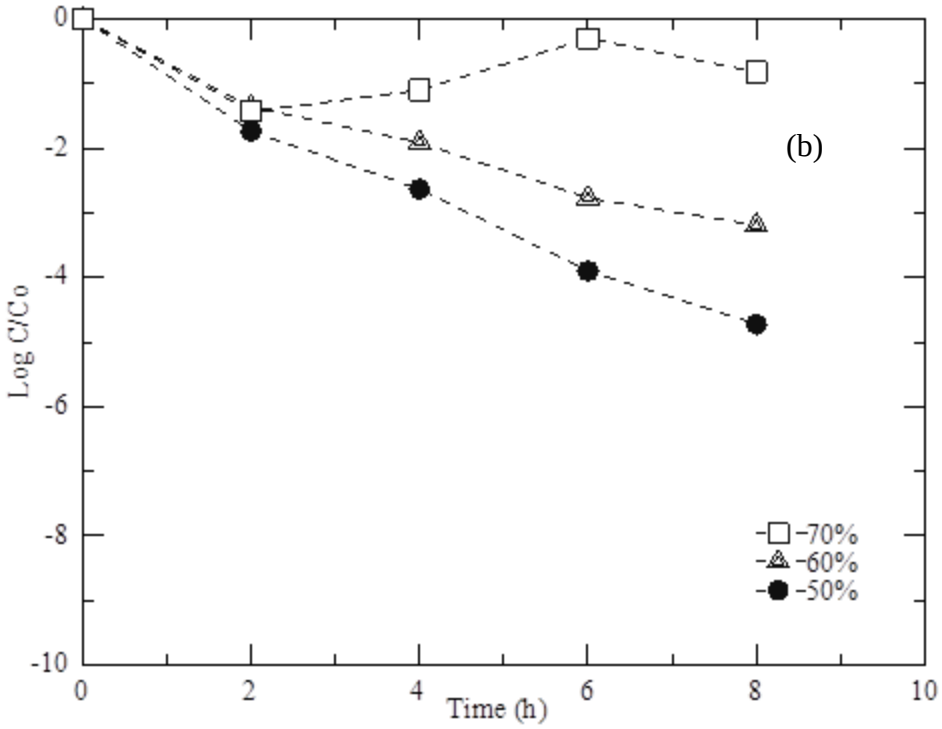

Figure 2.1b: *Inactivation of E.coli at 50 °C with varying Moisture Levels.*

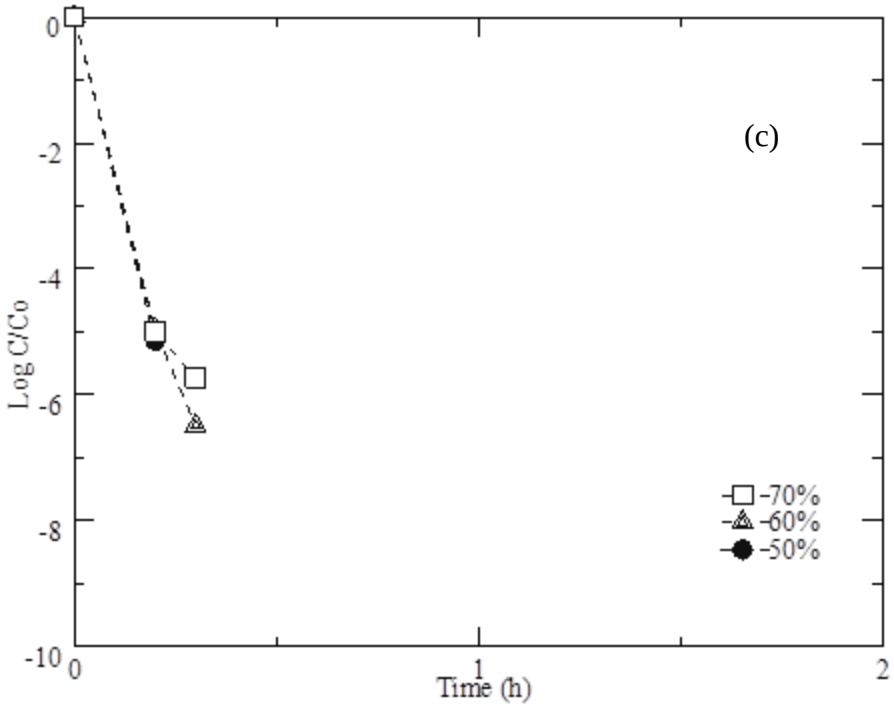

Figure 2.1c*: Inactivation of E.coli at 70 °C with varying Moisture Levels.*

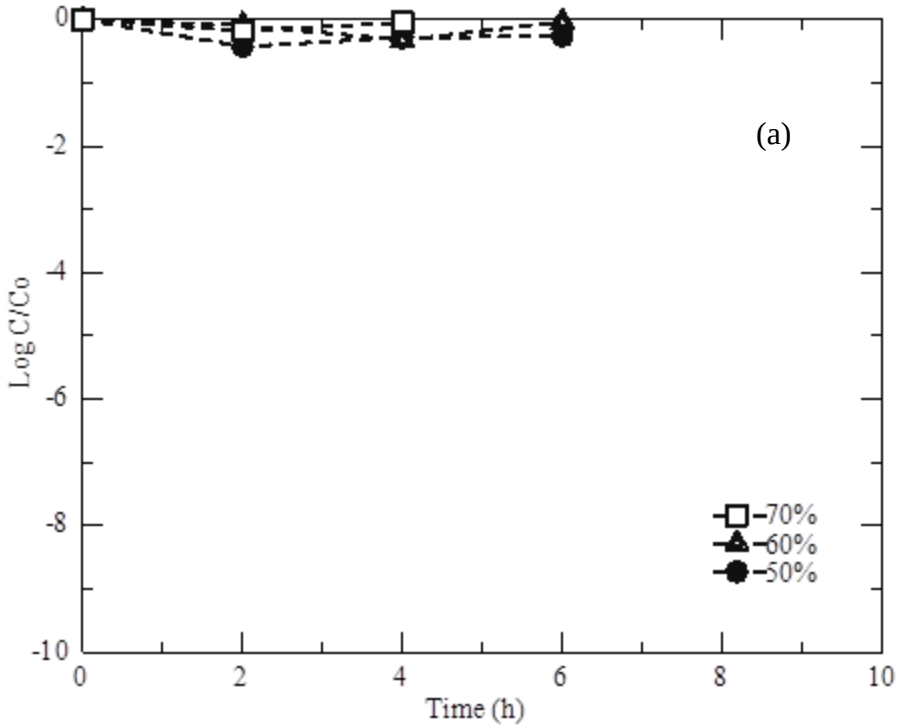

Figure 2.2a Inactivation of Enterococcus at 37 °C with varying Moisture Levels.

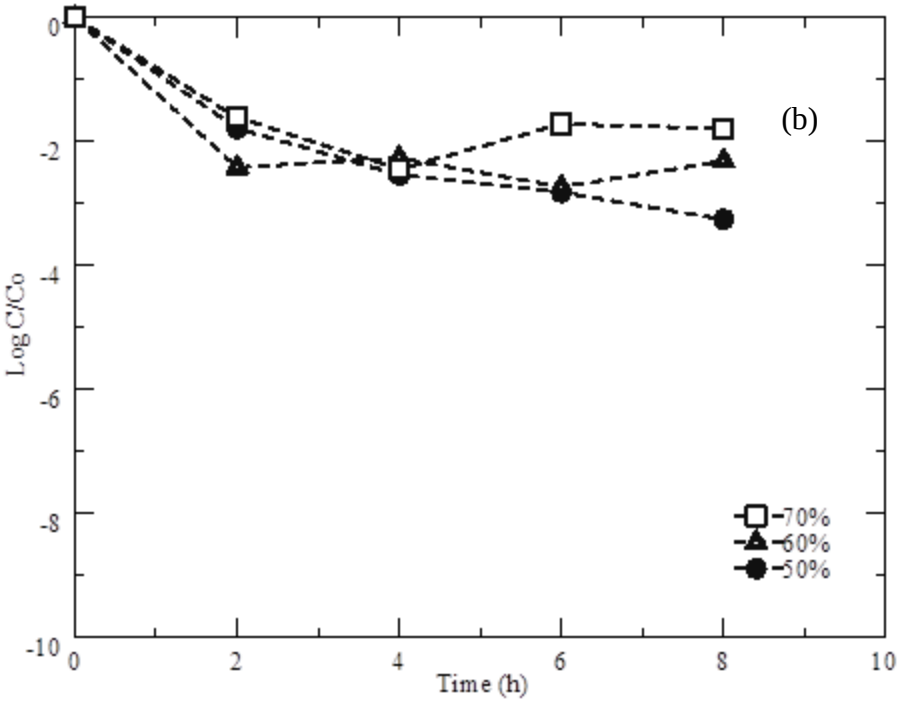

Figure 2.2b: *Inactivation of Enterococcus at 50 °C with varying Moisture Levels*

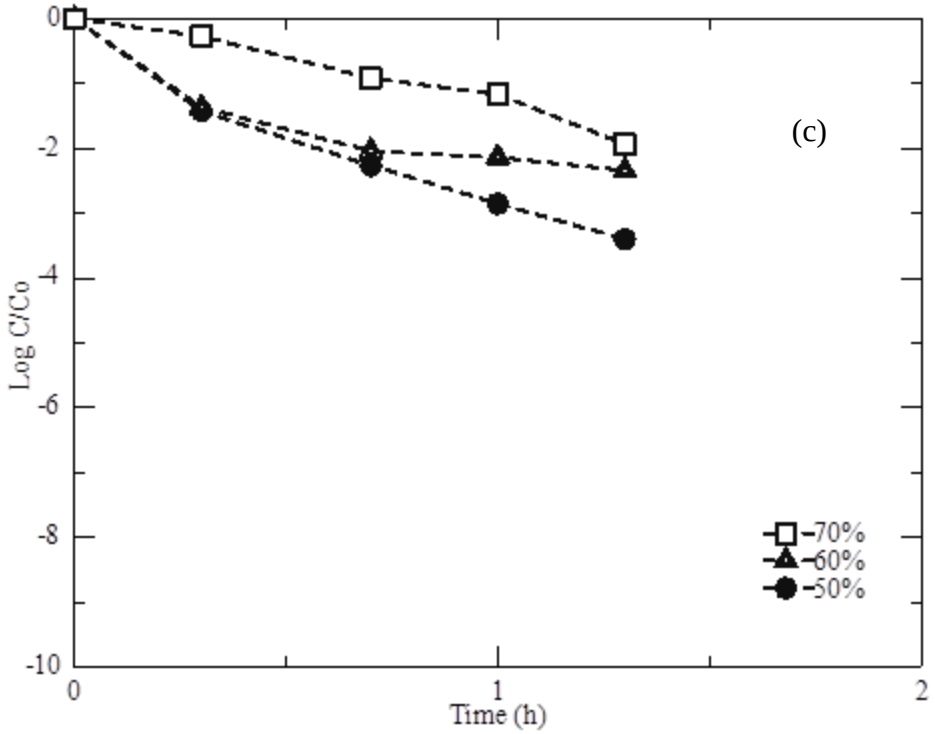

Figure 2.2c: *Inactivation of Enterococcus at 70 °C with varying Moisture Levels.*

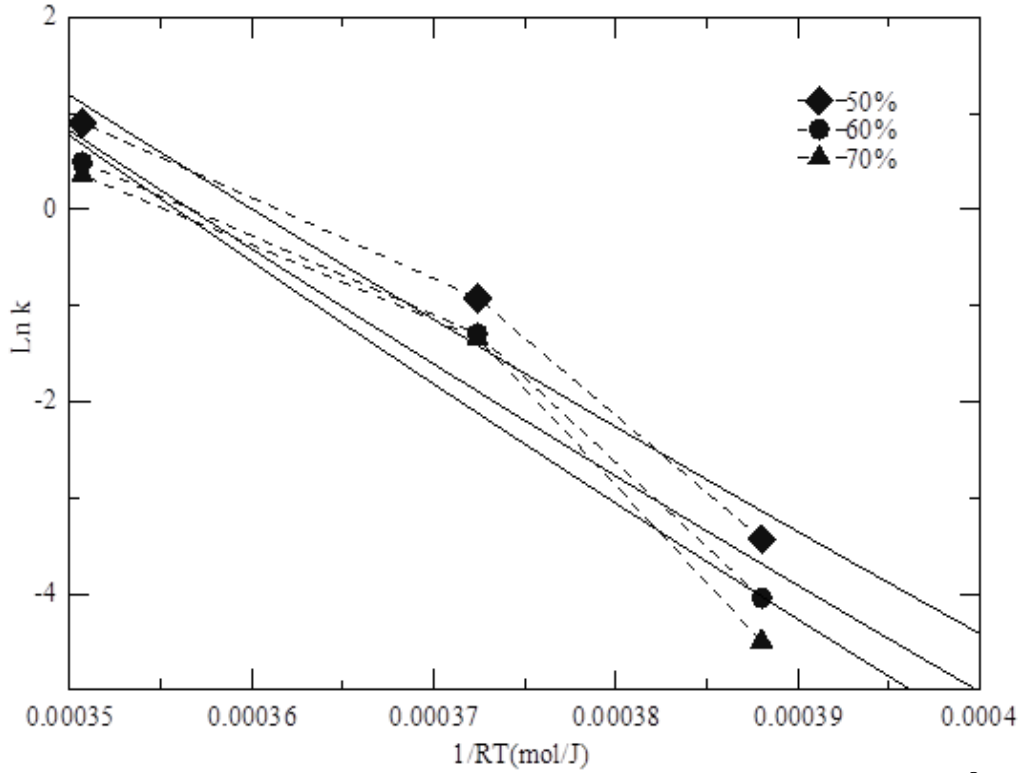

Figure 2.3: *Effect of Temperature on E.coli die-off with varying Moisture Levels. R^2 values for the trend of M C 50 %, 60 % and 70 % are 0.99, 0.98 and 0.97 respectively.*

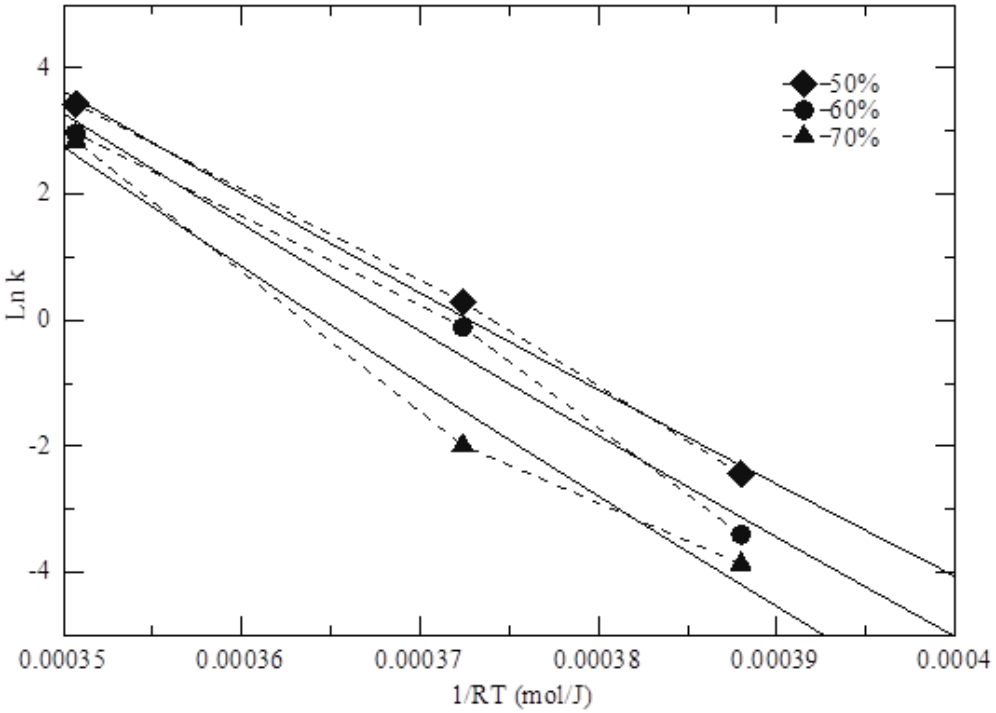

***Figure 2.4**: Effect of Temperature on Enterococcus die-off with varying moisture Levels. R^2 values for the trend of M C 50 %, 60 % and 70 % are 0.96, 0.95 and 0.93 respectively.*

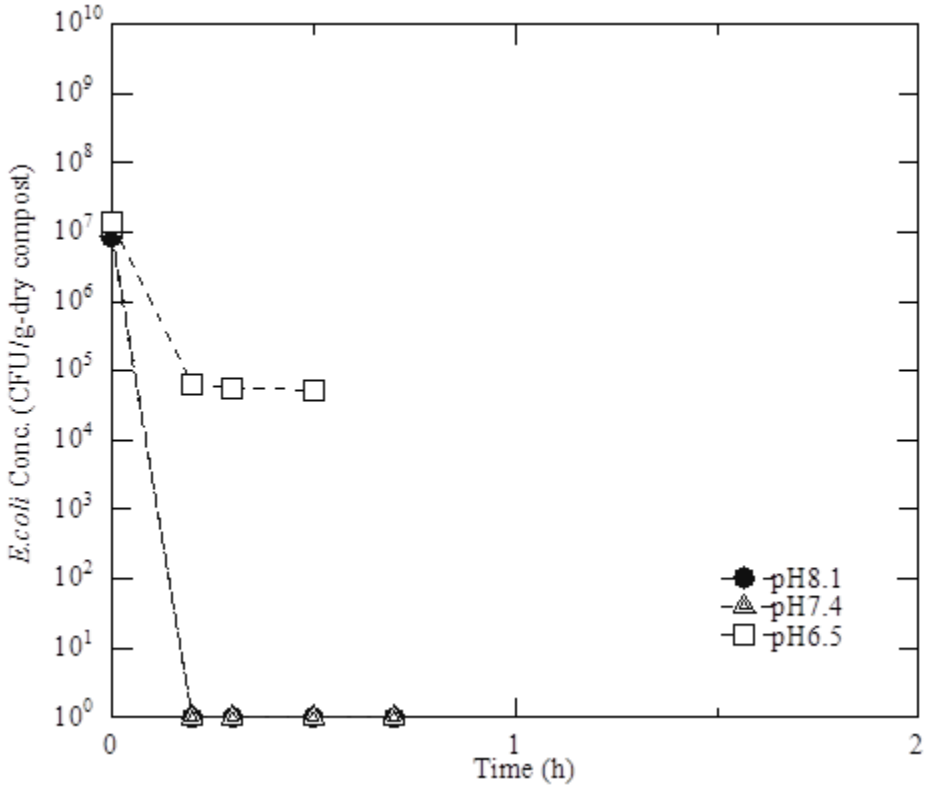

Figure 2.5 Change in Concentration of E.coli with pH

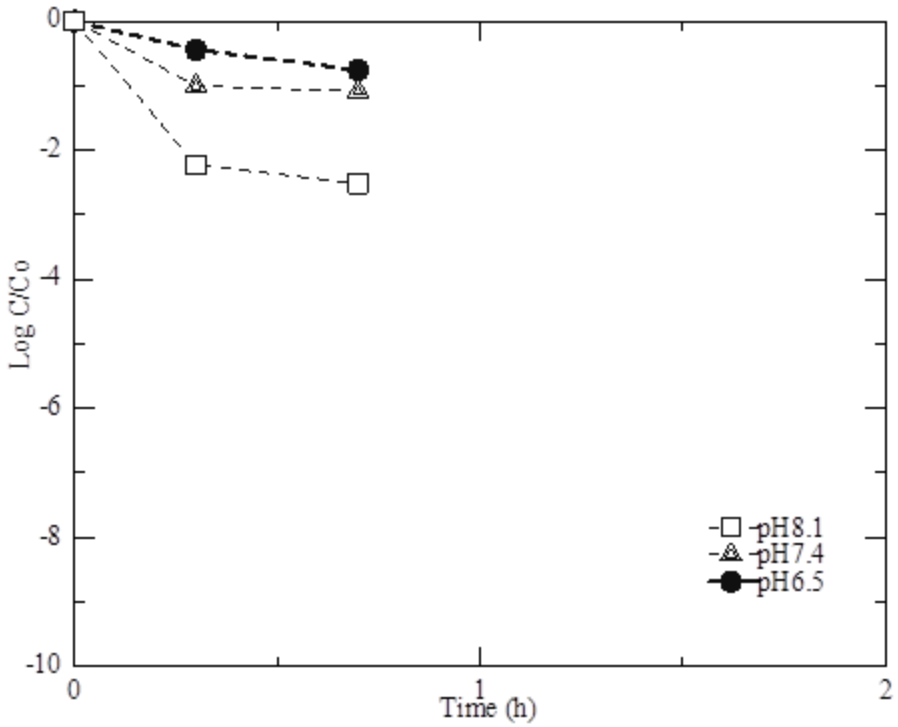

Figure 2.6 *Change of Enterococcus with pH*

2.3.4 Effect of the Post-treatment Conditions on Bacteria

Inactivation rate coefficients, *k*, vary under the same conditions with different microorganisms or the same organism in different conditions. Therefore, determining the inactivation rate coefficient, *k* with different organism under different conditions is necessary. During the post-treatment relevant data were gathered for the inactivation rate coefficient. The rate coefficients, for *Enterococcus* in these studies were all lower than those for *E. coli* which indicates that *Enterococcus* was more resistant to thermal inactivation than *E.coli*. The study of Reed *et al*. (1997) reported the same trend in an experiment on solar inactivation of faecal bacteria in water and the critical role of oxygen. Arrhenius plots for *E.coli* and *Enterococcus* are shown in Figure 2.3 and Figure 2.4. The solid lines are trend lines expressing the effect of temperature on *E.coli and* E*nterococcus* at different moisture content. There were strong correlations with temperatures, thus the effect of temperature on the inactivation of *E.coli* and *Enterococcus.* Carrington *et al.* (1991) observed the same trend when they conducted a study on the inactivation rate of agricultural waste during thermophilic aerobic digestion. They indicated that inactivation of *Enterococcus* was less rapid than the case of *E.coli.* The study also stated that, inactivation

rate constants could not be estimated, because rapid inactivation took place within the first 20 mins at both 55 °C and 60 °C representing a 3 log units reduction. Kelly *et al.* (1993), Koné *et al.* (2007), and Singh *et al.* (2011) have reported anomalous and thermoduric tendencies in the inactivation behaviour of *Streptococcus spp.* in waste, with numbers of indicator bacteria in the treated waste exceeding those in the feed waste even though thermophilic temperatures (68 °C) were achieved. The same trend of resistance was demonstrated in this study for thermal treatment.

Addition of 1.00 g, 0.50 g and 0.10 g dose of $Ca(OH)_2$ raised the pH of compost to 8.1, 7.4 and 6.5 respectively. The initial pH value without $Ca(OH)_2$ was 5.6. The addition of 1.00 g and 0.50 g of $Ca(OH)_2$ rapidly inactivated all *E. coli* within 10 mins. *Enterococcus* showed tolerance for 0.10 g $Ca(OH)_2$ addition at pH 6.5. In the pH experiment while *E. coli* could not be detected after 10 mins for the addition of 1.00 g and 0.5 g of $Ca(OH)_2$ *Enterococcus* recorded ≤ 1 log reduction after 40 mins at all pH levels. The results indicated that higher dose of $Ca(OH)_2$ can reduce the concentration of bacteria. During the alkaline treatment, *Enterococcus* was more resistant to high pH than *E.coli* comparatively. In the observation of Nakagawa et al. (2005), a small amount of CaO, about 100 -150 mg, was used to change the pH of sawdust and it reduced *E.coli* lethally and rapidly. Figure 2.5 and 2.6 shows the change in concentration of addition of $Ca(OH)_2$ dose. In Figure 2.5, inactivation was too fast and hence it was impossible to present it on a log graph. The *k* values during the thermal and alkaline treatments are summarised in Table 2.4 and 2.5, while the *k* values for pH 8.1 and pH 7.4 were too fast to measure (TFTM). The coefficient of determination, R^2, for the linear fit of the inactivation rate of the bacteria was in the range of 0.8 - 0.9.

Table 2.4 Summary of Inactivation Rate Coefficient Obtained in the post-treatment Study

Bacteria	**37°C**			**50°C**			**70°C**		
	Moisture Content, MC (%)								
	50	**60**	**70**	**50**	**60**	**70**	**50**	**60**	**70**
E.coli	0.087	0.033	0.020	1.336	0.896	0.137	30.865	19.474	17.172
Ent.	**0.032**	**0.017**	**0.011**	**0.398**	**0.274**	**0.263**	**2.469**	**1.636**	**1.437**

Table 2.5 Effect of pH on inactivation rate coefficient at 37°C, 60 % MC

$Ca(OH)_2$ addition (g/100g-wet compost)	**1.00**	**0.50**	**0.10**
pH	**8.1**	**7.4**	**6.5**
E.coli	TFTM	TFTM	10.13
Enterococcus	3.773	1.632	1.137

Previous studies reported that, high thermophilic temperatures above 55 °C can effectively inactivate pathogens (Tønner-Klank *et al.,* 2007; Niwagaba *et al.,* 2009; Sanguinetti *et al*., 2009 Germer *et al.,* 2010). They also reported that the use of ash in raising the pH can disinfect faeces. Vinneras *et al*. (2003) indicated that, apart from low moisture content contributing to successful inactivation of pathogens, high pH (addition of lime) >12 helps in the inactivation process. Post-treatment of compost will allow a rapid reduction of all pathogens surviving after the treatment process without any unfavourable effect on the process. In this study, high temperature conditions with lower moisture contents rapidly reduced the concentrations of *E. coli*. The concentration of *Enterococcus* reduced, although it was slower than the rates of *E.coli*. The behaviour of *Enterococcus* in this study and other published works emphasises the need for caution in the interpretation of indicator inactivation data and the need to choose indicator pathogen carefully in waste treatment. Considering the WHO guidelines for excreta reuse, faecal material requires a 6 log units reduction. Therefore the best performing post-treatment condition was at the lower moisture content 50 % i.e. 50 °C 50 % M C and 70 °C 50 % M C. Setting compost safe target at 6 log, the time required to achieve the 6 log unit reduction was estimated. The first order kinetic equation was used for the estimation. Post-treatment condition of 50 °C, 50 % MC requires over 4.5 h and 70 °C 50 % M C with a contact time of over 20 mins are required for *E.coli*. For *Enterococcus*, post-treatment condition 50 °C 50 % MC with a contact time of 15 h or 70 °C 50% with a contact time of over 2.5 h are required. The studies were designed to understand the inactivation efficiency of the post-treatment conditions. It would be assumed that post-treatment conditions (50 °C 50 % MC, 70 °C 50 % MC) with prolonged contact time could perform better during the treatment process. The results of this study indicate that to enhance the

inactivation rates it may be necessary to characterise pathogens inactivation under various post-treatment conditions, and eventually invest into developing a manual comprising inactivation rate coefficients, *k*, with moisture content in consideration. This will be a primary information to design a cost effective, energy saving technology for the treatment of compost. In addition, this study investigated only *E. coli* and *Enterococcus* as models of pathogenic bacteria. Other pathogens, especially virus, parasites, or eggs may not have the same inactivation rates during the post-treatment. Therefore further studies were carried out on parasites and viruses to have a complete post-treatment manual.

2.4 Summary

E. coli and *Enterococcus* were used as indicators for pathogenic bacteria when exposed to extreme post-treatment conditions of compost. It was found that, temperature and pH affected the inactivation rates, but results also depended on temperature and humidity for relatively low temperature but not under high temperatures. In general, bacteria decreased as temperature increased; moisture reduced and the length of time increased. However, under high temperature the effect of moisture was not significant. With regards to the relative resistance of bacteria *Enterococcus* was the most resistant to temperature. The addition of high dose of $Ca(OH)_2$ to increase the pH of compost to ≥ 8.1 is capable of reducing the concentration of pathogenic bacteria.

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Chapter 3

Effect of Temperature on the Inactivation of Helminth eggs (*Ascaris suum*) after the composting process

3.1 Introduction

Rural communities of Burkina Faso still practice open defecation resulting in oral contamination of faecal matter in water and food as previously mentioned in Chapter 1. In fact, faeces contain concentrations of microorganisms per gram of dry matter (Lentner and Wink, 1981). Some of the microorganisms are pathogenic and cause illness. Millennium Development Goals promised to improve sanitation in the area, but the rate of improvement is not enough because of lack of research (UN MDG report, 2014). On the other hand, Burkina Faso is one of the poorest countries in the world. The population is mainly rural (80 %) and primarily employed in agriculture. Most of the rural people cannot buy fertiliser to increase agricultural productions. People in the rural areas of the country use bio-solids and manure of their livestock for soil fertilisation and amending. Indeed, excreta from animals and humans are rich in nutrients therefore can be used to fertilise crops.

Nematodes dwelling in the intestines are the most widespread parasitic infections worldwide (Koné *et al*., 2007). *Ascaris lumbricoides* (roundworm), *Necator americanus, Ancylostoma duodenale* (hookworms) and *Trichuris trichiura* (whipworm) are among the most common worms associated with excreta (Koné *et al*., 2007). *A. lumbricoides* and *A. suum* are helminthic parasites infecting humans and pigs respectively (Dold and Holland, 2011). *A. lumbricoides* infection remains common with 1.2 billion infections globally (de Silva *et al*., 2003). Their infections are most commonly documented in Sub-Saharan Africa (WHO, 2006). The spectrum of the disease associated with *A. lumbricoides* infection is known as ascariasis, and morbidity assessed as disability-adjusted life years (DALYs) are approximately 10.5 million per year. Furthermore, morbidity with serious health consequences as a result of ascariasis is observed in 122 million cases per year (Chan, 1997). The infection is one of the major causes of disease burden among children in Sub-Saharan Africa, typically in rural communities and urban slums (World Bank, 1993). This high infection mirrors severe shortage in health care, education, and chronic poverty (Crompton, 1999). *Ascaris* and *Taenia* are persistent in the environment and are therefore regarded as an indicator and index of hygienic quality (WHO, 2006). *Ascaris* is considered the biological particle which is the most resistant to treatment and environmental conditions (Feachem *et al*., 1983). *A. lumbricoides* and *A. suum* are closely related ascarid species and both species constitute natural host-parasite relationships with similar life cycles,

while the eggs of *A. suum* are commonly used as model for *A. lumbricoides* eggs, because they are easier to obtain in large quantities (Cruz *et al*., 2012; Johnson *et al*., 1998).

As mentioned earlier, from preliminary experiments, *Ascaris* eggs were still detected in the compost samples taken from the composting toilets after three months of operation. This indicates that the composting process was not enough to inactivate pathogens because a small amount of fresh faeces remained in the reactor from daily usage. This fact motivated minimisation of the health risk with additional post-treatment to make multi-barrier for certain treatments. Several studies reported that high temperature, high pH, long treatment time and dry conditions decreased the viability of *Ascaris* eggs (Feachem *et al*., 1983; Maya *et al*., 2010; Hawksworth *et al*., 2000). Therefore, this rural model composting toilet requires a heat source like electricity to increase temperature in the reactor or an alkaline source to increase the pH. These requirements would add to the operation cost and would not be affordable in the rural areas. Some studies have investigated conditions required for inactivating *Ascaris* eggs in sludge and urine diversion toilet products (Hawksworth *et al*., 2000 ; Aitken *et al*., 2005), but in practice these cannot be used effectively for inactivation of *Ascaris* eggs in compost because of differences in the matrix (Aitken *et al*., 2005; Popat *et al*., 2010). Jensen *et al*. (2009) reported that the maturation of the eggs exposed to the external environment is likely to make those eggs more resistant to environmental stress than eggs collected directly from the uterus of adult female worms. Setting the required environmental conditions for the inactivation of *Ascaris* eggs from human faeces is important for the safe utilisation of compost in rural areas. The design of the post-treatment process requires data of kinetic inactivation considering important parameters such as temperature, pH, treatment time and moisture content to manage the compost from faecal matter for beneficial agricultural utilisation. However, none of the previous studies (Maya *et al*., 2010; Hawksworth *et al*., 2000) reported the inactivation rate coefficient, *k*, of *A. suum* eggs considering the effect of temperature and moisture. The hot and semi-arid climate in Burkina Faso could be sufficient to inactivate *Ascaris* eggs because the compost can be kept at high temperature by exposing it to solar energy for several days (Andreev and Samoil, 2009). To sanitise compost withdrawn from the composting toilet for food production by setting post-treatment conditions, the objectives of this study are: (1) to determine the kinetic inactivation parameters of *A. suum* eggs as an indicator for helminth eggs during a

post-treatment of compost, and (2) to evaluate the effect of temperature and moisture content of the compost on the inactivation rate.

3.2 Materials and Methods

3.2.1 Parasite eggs preparation

A. suum eggs were supplied by the Department of Infectious Diseases, Division of Parasitology, Faculty of Medicine, University of Miyazaki in Japan. A single batch of eggs was used for all inactivation experiments. The eggs were obtained from the intestine of affected pigs and suspended in an incubation solution of 0.1 N H_2SO_4, and the suspension was kept at 4°C. This was designed to result in minimal loss prior to use.

3.2.2 Compost preparation and inoculation

The compost was prepared in the laboratory by loading 500 g-wet of pig manure to 3 kg of rice husks in a composter with a mixing operation. The mixing was done every day for 30 days. After composting, it was kept in the composter for 1 week to reduce the moisture. The moisture content, *MC* (%), of the compost reached 23 ± 2 %. Here, around 5 g of compost samples was taken on a ceramic dish, then kept at 105°C for 1 night to estimate the moisture content. The moisture content is calculated by the following equation ;

$$MC = \frac{W_{wet} - W_{dry}}{W_{wet}} \times 100 \qquad (3.1)$$

where, W_{wet} and W_{dry} are wet and dry weights of compost sample (g).

One hundred grammes (100 g) of the compost was weighed into a sterilised bottle. Ultra-pure water was added to adjust the moisture content of the compost. The combinations of temperature and moisture content of the experiments are summarised in Table 3.1. The bottles were inoculated with 4 ml *A. suum* eggs concentration of 2.7 x 10^3 eggs/ml for 30 °C, 40 °C and 50 °C, while inoculating with 8 ml *A.suum* eggs concerntration of 6.4 x10^3 egg/ml for 60 °C, since the inactivation rate was too high to obtain valid data at high temperature. The compost was agitated for 1 min with a spatula to ensure that *A. suum* were uniformly mixed. The bottles were tightly closed to avoid any change in moisture content during the experiments. The samples were

then immediately placed into incubators (THE-051FA, Advantec Co. Ltd.) to keep the temperature constant. The duration for each experiment was three hours. Twelve grammes (12 g) of the compost sample were taken every hour for all experimental conditions.

Table 3.1 Experimental Condotions for thermal treatment of *A. suum* eggs

No. of sample	Temperature (°C)	Moisture content
1	30	50%
2		60%
3		70%
4	40	50%
5		60%
6		70%
7	50	50%
8		60%
9		70%
10	60	50%
11		60%
12		70%

3.2.3 Analytical methods for *A. suum* eggs

The USEPA protocol modified by Schwartzbrod (2003) was used to analyse *A. suum* eggs in the samples. A maximum of 3 g of compost was weighed into a 50 ml Falcon tube. Four samples were used for each condition. The *A. suum* eggs were extracted with 5 ml of 0.1 % Tween 80 (Polyoxyethylene sorbitan monooleate 0.1 %, Wako Pure Chemical Industry) solution from the sample in the tube and the tube was vigorously mixed by a vortex. The tube was then filled with 40 ml of the Tween 80 solution. The extraction of eggs was done repeatedly by vigorously mixing the tube with a vortex mixer and resting for 5 mins to prompt the detachment of eggs from the compost. The sample was centrifuged at 1389 G for 3 mins, then the mixture of the

compost and the solution was separated into two distinct phases to form a pallet in the bottom of the tube. After discarding the supernatant, the sample was re-suspended in deionized water, vigorously mixed to wash off Tween 80, centrifuged at 1389 G for 3 mins and the supernatant was discarded. The sample was re-suspended in 5 ml of (0.57 g/ml) $ZnSO_4$ (super grade, Wako Pure Chemical Industry Co. Ltd.) solution whose specific gravity, *SG*, was 1.29 and vigorously mixed. The tube containing the sample was filled with 40 ml more of $ZnSO_4$ solution, and then was vigorously mixed, and centrifuged at 617 G for 3 mins. This process allows the eggs to float on top of the high density solution from the heavier solids. The tube was carefully removed from the centrifuge to avoid spillage and subsequent loss of eggs. The supernatant in the tube was transferred to 4 different tubes with a Pasteur pipette. These tubes were filled with distilled water to reduce the specific gravity of the $ZnSO_4$ solution in the tubes to allow the eggs to deposit upon centrifuging. The tubes were centrifuged at 964 G for 3 mins and the supernatant was discarded. The residues in the tubes were combined into one tube and distilled water was added to fill it. These samples were centrifuged at 964 G for 3 mins and the supernatant discarded, leaving a volume of about 5 ml. The recovery rate of the eggs was 31.48 %.

3.2.4 Viability test and egg count

Viability of *A. suum* eggs was determined using the Safranine O dying method developed by de Victorica and Galván (2003). After the final centrifuge and supernatant removal, the eggs in the sample were stained by adding two drops of Safranine O (2.5 % in H_2O, Wako Pure Chemical). After 10 mins, the tube was filled with water to wash-off safranine O and then centrifuged for 5 mins at 800 G and the supernatant was discarded. This washing process was repeated 3 times to remove excess Safranine O, leaving a final volume of about 5 ml. The sediment was then diluted with 5 ml of 0.1 N H_2SO_4. The particles in the tube were allowed to settle down and the supernatant poured-off leaving a final volume of 1ml. Care was taken so as not to pour-off the eggs. For enumeration, a volume of 0.02 ml of a well-mixed final sample was placed on a glass microscope slide with a coverslip and the eggs counted under the microscope and viable and non-viable eggs were observed. If the dye had penetrated the eggs, they were counted as non-viable (Figure 3.1). The number of eggs in the tube was obtained by dividing the count by the

volume on the slide, and then multiplying by the final volume of 1ml. In case of small counts, another slide was counted to increase the count of eggs on the slides.

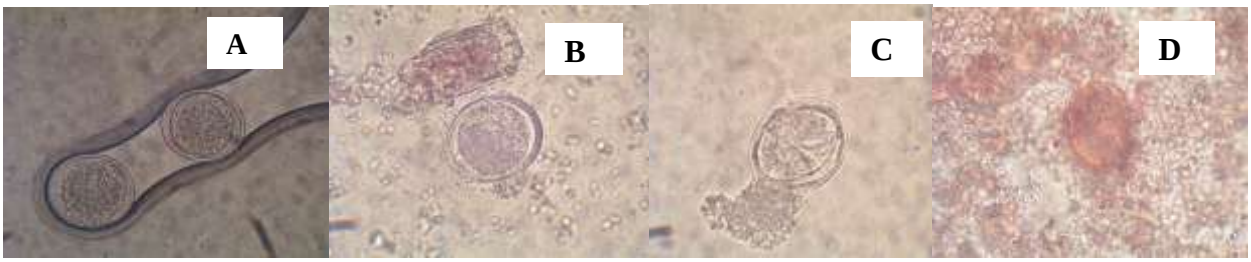

Figure 3.1 *Damage observed when eggs were subjected to heat A) A. suum eggs before temperature treatment B) viable (below) damaged (above) C) damage A. suum eggs and D) non-viable A. suum eggs stained with safranine O with red colour*

3.2.5 Data Analysis

The time course of the concentration of *A. suum* eggs in the compost obtained from the inactivation experiments were fitted to a first order kinetic model expressed as (Nakagawa *et al*., 2005):

$$\ln k = C/C_o = -kt \qquad (3.2)$$

where, C is the concentration of the *A. suum* eggs in the compost sample on dry basis at time t (eggs/g-dry solid), C_o is the initial concentration of the *A. suum* eggs in the compost sample on dry basis (eggs/g-dry solid), k is the inactivation rate coefficient (h^{-1}), and t is the treatment time (h). After the estimation of the inactivation rate coefficients, evaluation of the effect of the temperature was done with Arrhenius equation described as follows;

$$k = A\exp\left(-\frac{E_a}{RT}\right) \qquad (3.3)$$

where, A is the pre-exponential factor (h^{-1}), E_a is the activation energy (J/mol), R is the universal gas constant (J/mol/K), T is the temperature (K). Here, the absolute value of E_a does not have any meaning in the inactivation process, but the Arrhenius plot is useful for understanding the effect of the temperature on the inactivation rate coefficient. A statistical analysis using ANOVA was carried out to determine the significant effects ($p \leq 0.05$) of the temperature and moisture on the inactivation of *A. suum* eggs. The analysis was performed with R (open source coding software) using the packages "stats" for the ANOVA and Shapiro test.

3.3 Results and Discussion

3.3.1 *Ascaris suum* eggs Inactivation Efficiency in Compost

The characteristics of *A. suum* eggs at 30 °C with the variable moisture contents are shown in Figure 3.2a. At this temperature, the values of the inactivation rate coefficient were almost zero, indicating no reduction in the concentration of the eggs at 30 °C within the 3 hour treatments period. Figure 3.2b shows the change in the time course of the concentration of the *A. suum* eggs with the varying moisture content, 50 %, 60 % and 70 % at 40 °C. The inactivations rate coefficients were slightly higher than at 30°C and lower moisture content showed the higher value. Treatment at 50 °C with moisture content of 50 %, 60 %, and 70 % respectively resulted in 2.00 ± 0.14 log units, 1.00 ± 0.18 log units and 0.8 ± 0.14 log units reductions in 2 hours as shown in Figure 3.2c. At 3 hours, eggs were not observed due to the high temperature and extended contact time, therefore, could not be presented on the log graph. The change in concentration of *A. suum* eggs at 60 °C is shown in Figure 3.2d. The log unit reduction at moisture contents of 50 %, 60 %, and 70 % were 3.60 ± 0.21 log units, 3.00 ± 0.14 log units and 2.00 ± 0.14 log units respectively in 3 hours. These characteristics of *A. suum* during thermal inactivation were also observed by previous studies (Hawksworth *et al*., 2000; Pecson *et al*., 2007). The first order rate coefficient for the inactivation of *A. suum* for the data collected during the inativation experiment are listed in Table 3.2. The coefficient of determination, R^2, for the linear fit of the inactivation rate of the eggs by eq. (3.2) was in the range of 0.8 - 0.9.

Table 3.2 Summary of inactivation rate coefficient of *A. suum* eggs

		Inactivation rate constants of *A. suum* eggs, (k h^{-1})		
		Moisture Content, MC (%)		
		(50)	**(60)**	**(70)**
Temp. (°C)	30	0.16	0.13	0.09
	40	0.22	0.19	0.12
	50	0.92	0.76	0.42
	60	1.22	0.99	0.74

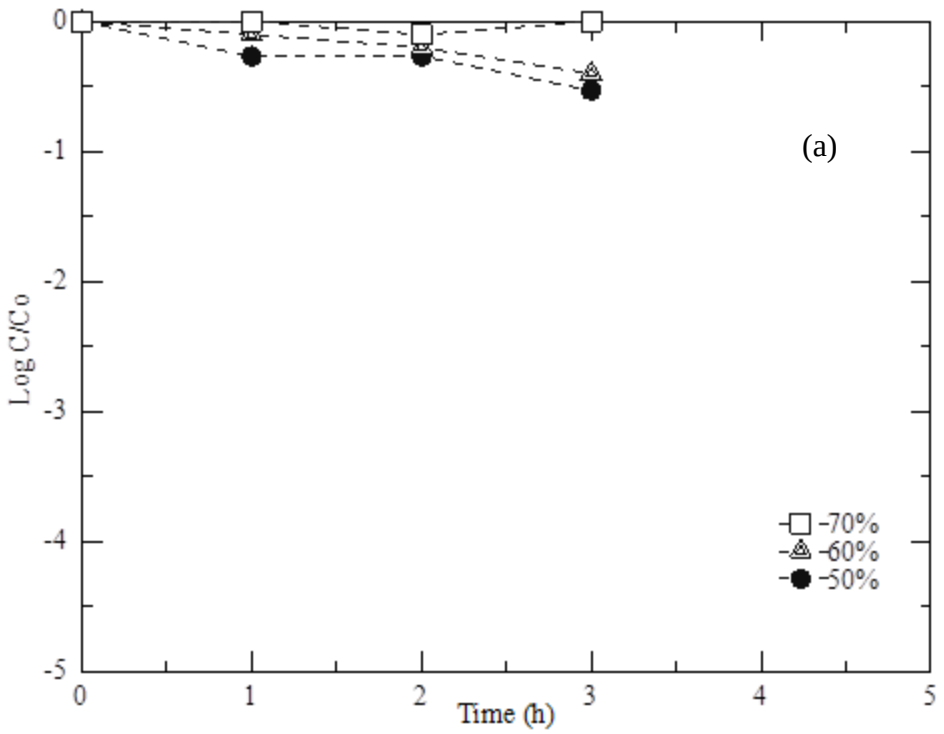

Figure 3.2a: *Inactivation of A. suum at 30 °C. Concentrations were measured in (eggs/g-dry solid).*

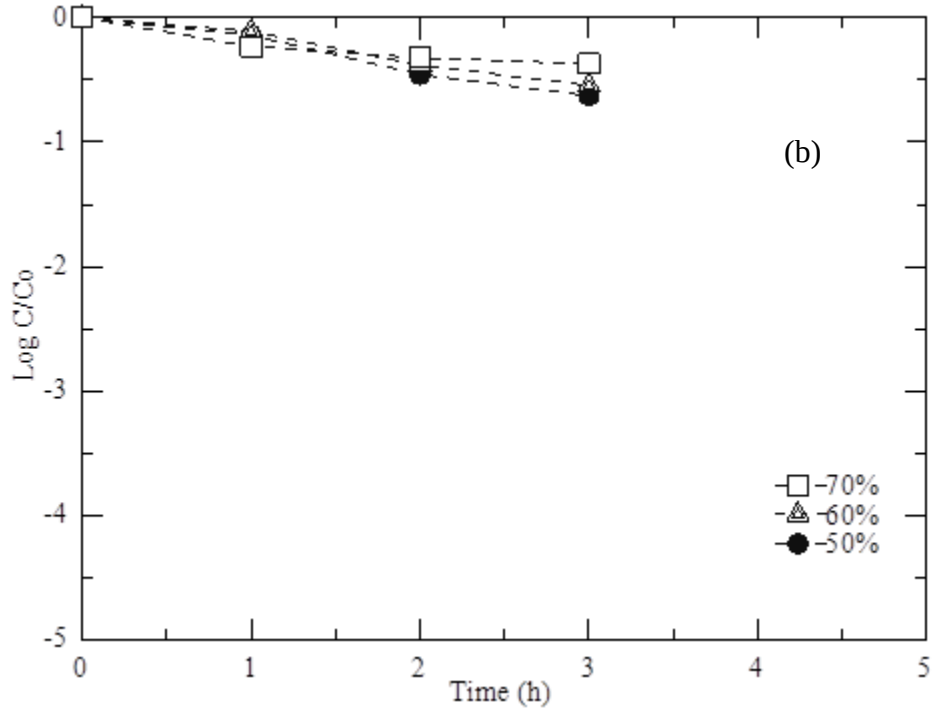

Figure 3.2b: *Inactivation of A. suum at 40 °C. Concentrations were measured in (eggs/g-dry solid).*

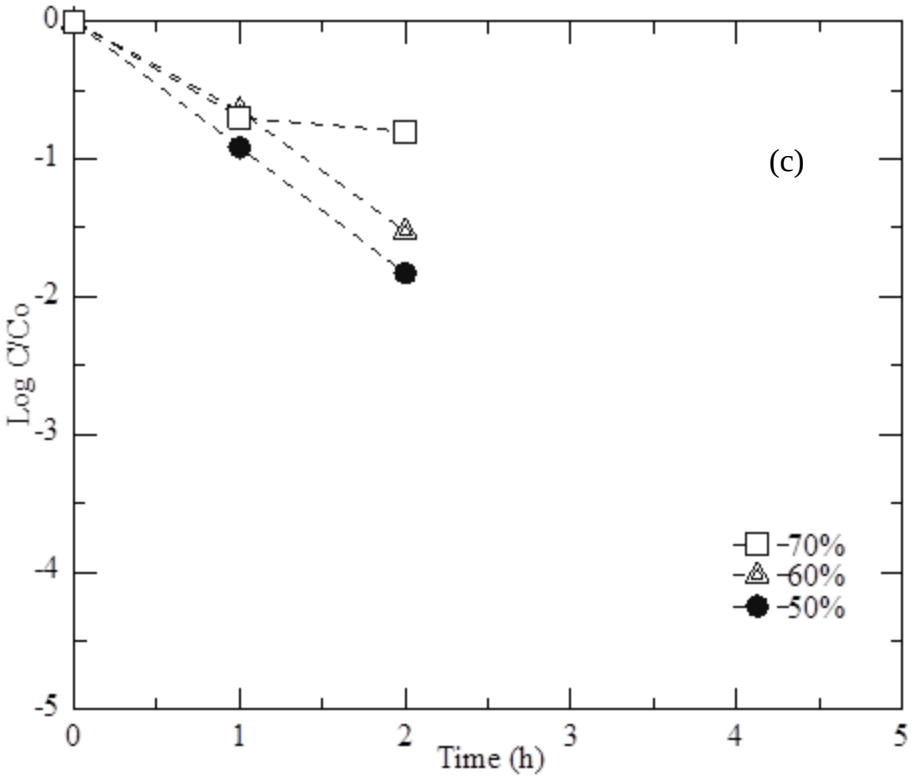

Figure 3.2c: *Inactivation of A. suum at 50 °C*

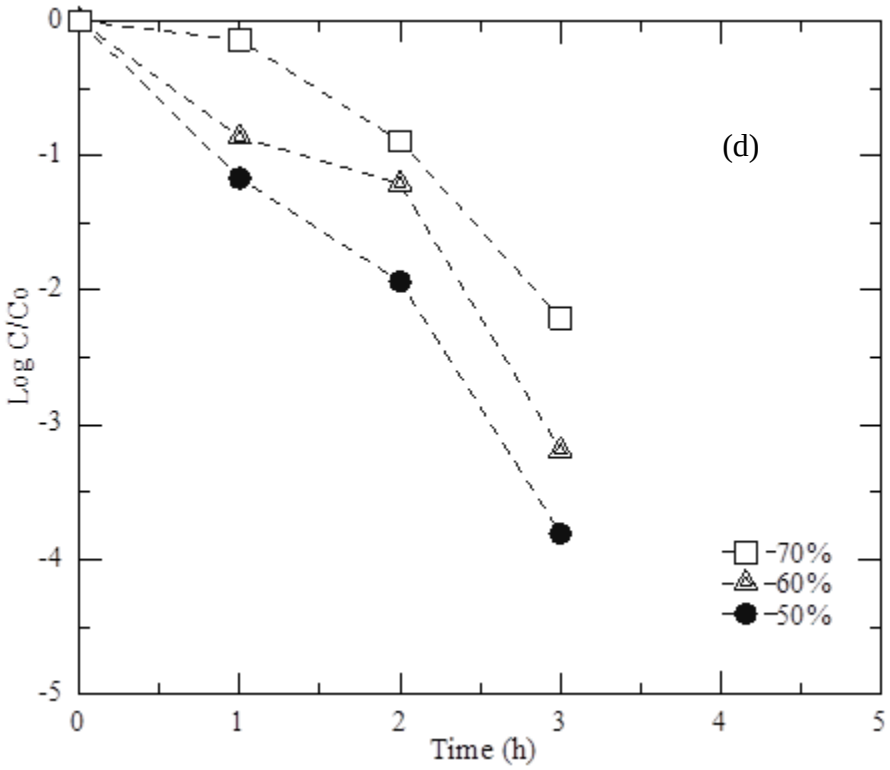

Figure 3.2d: *Inactivation of A. suum at 60 °C*

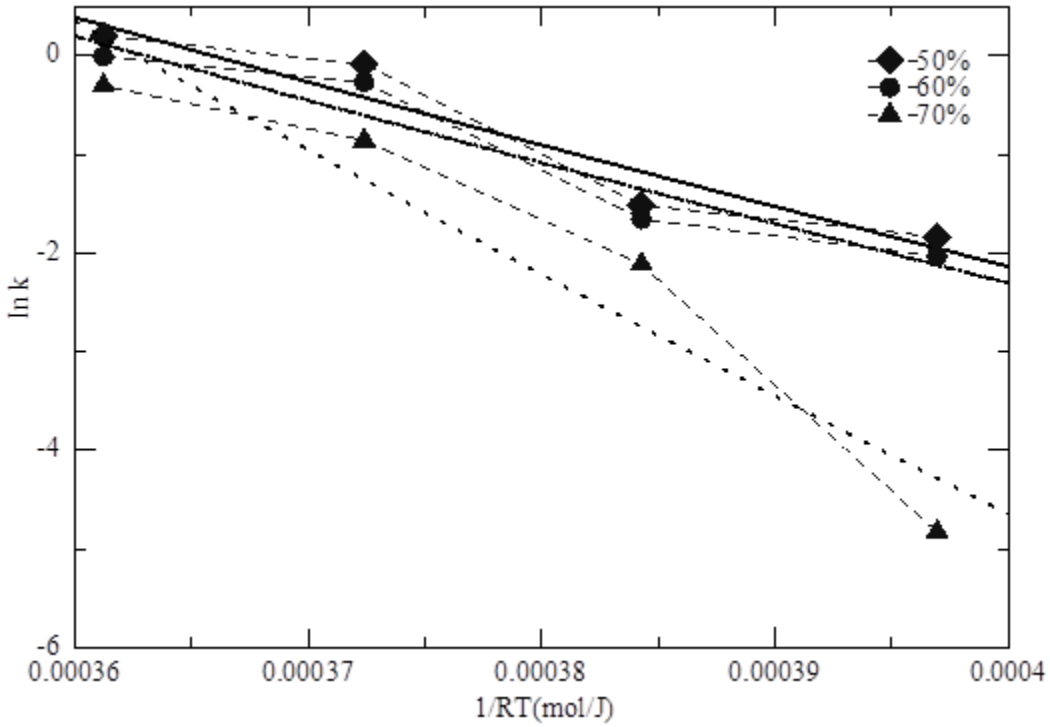

Figure 3.3: *Effect of temperature on A. suum eggs die-off with varying moisture content*

The lines were fittings from Arrhenius plots of In K vs 1/RT (mol/J) of the inactivation rate values at different moisture levels i.e 50 %, 60 % and 70 %. The lines were made to express the effect of temperature at different temperature level. R^2 *values for the trend of M C 50 % , 60 % and 70 % are 0.91, 0.92 and 0.91 respectively.*

3.3.2 Effect of Temperature and Moisture on *Ascaris suum* Inactivation

To design an equipment for preparing biologically safe compost, the inactivation rate of *Ascaris* eggs after withdrawal from the reactor of the composting toilet should be estimated. A number of studies have been conducted to evaluate the thermal inactivation of helminth eggs, but very few have been designed to quantify inactivation rates under conditions in which temperature and moisture have been precisely controlled or well characterised. A common end point in earlier studies on thermal inactivation of helminths is the amount of time required for safe levels at a given temperature (Aitken *et al*., 2005). Such information is important because it helps define treatment conditions to achieve essentially safe levels of bio-solids.

The effect of temperature and moisture on the inactivation of *Ascaris* eggs studied here show that inactivation rates increased with increasing temperature. High temperature increases

the rate of inactivation by speeding up the desiccation rate of *Ascaris* cells and subsequently destroying the cells. (Feachem *et al*., 1983; Pecson *et al*., 2007; Capizzi-Banas *et al*., 2004). The effect of temperature and moisture on the inactivation of eggs can be seen when comparing all temperatures, 30, 40 , 50 and 60°C at 50 %, 60 % and 70 % moisture content. The inactivation rate increased as the moisture decreased. At 30 °C, there was no change in concentration of the eggs after three hours of treatment at all moisture levels which indicates that lower temperature afforded more protection by slowing down the eggs desiccation rate. Temperatures above 40 °C with 3 hours contact time inactivated the eggs. The Arrhenius plot for *A. suum* inactivation is shown in Figure 3.3. The lines are trend lines expressing the effect of temperature on eggs at different moisture content. The lines were fitting lines from Arrhenius plots of In K vs $1/RT$ of the inactivation rate coefficients at the different moisture levels. There are strong correlations with temperatures and inactivation rates, thus the effect of the temperature on the inactivation of the *A. suum* eggs are shown by the trend lines in Figure 3.3. The results show that, the effect of temperatures on the *A. suum* inactivation were statistically significant ($p < 0.05$).

Table 3.3: Analysis of Variance for *A. suum* eggs

Source of Variation	*Sum sq.*	*DOF*	*Mean Sq.*	*F*	*p-values*
Anova Temp.	1.552	3	0.517	36.978	0.00029
Anova Moisture	0.167	2	0.083	5.960	0.03752
Residuals	0.084	6	0.014		

Lower moisture conditions increased the inactivation rate coefficients, in this experiment. Moisture content of compost below 50 % can enhance the inactivation process. Hawkworth *et al.* (2000) studied the inactivation of *A. lumbricoides* in a urine diversion toilet product and indicated that the die-off of *A. lumbricoides* was high at the low relative humidity. The study reported 0 % relative humidity resulted in a higher inactivation rate of *A. lumbricoides* in comparison to 100 % of relative humidity. Kone *et al*. (2007) confirmed that a decrease in moisture content in the surrounding environment increases the die-off. The statistical results of this experiment showed that there was an effect on the performance between the three different

levels of moisture content. The results showed that, the effect of moisture content on the *A. suum* inactivation were statistically significant ($p < 0.05$). The statistical analysis also showed that the temperature effects on the coefficients were stronger than that of the moisture content. Moreover, the results showed that the interaction between the temperature and the moisture content has a significant effect on the inactivation rate of the eggs.

Other treatments such as pH above 12.5 can inactivate *Ascaris* egg (Maya *et al*., 2010). Further studies on the effect of pH should be done as an optional post-treatment for *Ascaris* eggs. Concentration of eggs could vary from community to community and infestation would not be the same. Therefore, the study recommends that treatment should be done by assuming a high concentration of eggs in the compost before reuse as fertilizer. The data presented here could be a guide to perform field studies to determine the inactivation rate of *Ascaris* under solar thermal heat in rural Burkina Faso. These results would allow researchers and farmers to better understand the behaviour of *Ascaris* eggs during a post-treatment.

3.4 Summary

Treatment of Helminth eggs in a possible additional post-treatment after the composting process was tried in the laboratory by studying the effect of temperature (solar thermal heat) and moisture content on the rate of inactivation. As a result, (1) the inactivation rates of *A. suum* were 0.74 - 1.22 h^{-1}, 0.42 - 0.92 h^{-1} , 0.12 - 0.22 h^{-1} and 0.09 - 0.16 h^{-1} for 60, 50, 40 and 30 °C respectively at moisture content 50 % - 70 %. (2) inactivation rates of *A. suum* in the compost depended on temperature and moisture content. The best combination of post-treatment conditions for the efficient inactivation of Helminth eggs under solar thermal heat would be 50 °C 50 %. The reduction rate of helminth eggs in the compost under solar thermal sanitisation would be 0.42 - 0.92 h^{-1}. This study demonstrates that post-treatment is an efficient approach for inactivation of Helminth eggs. When sufficient heat is applied with low moisture of about 50 % and below, high efficiency of 3.60 ± 0.210 log units can be reached in 3 hours during post-treatment. This study would be a useful information for researchers and farmers to understand the behaviour of helminth eggs during post-treatment with solar thermal heat.

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Chapter 4

Effect of Temperature on the Inactivation of MS2 Bacteriophage as indicator for pathogenic viruses after the composting process

4.1 Introduction

In Chapter 1, the function of the composting toilet system was explained. The mechanism can enhance the efficient organic decomposition of faeces with little odour (Kazama *et al.,* 2011). This is a sustainable sanitation system to protect human health and the environment while reducing the use of water and recycling nutrients (Nakagawa *et al.,* 2006).

In the rural parts of Burkina Faso where the rural model of composting toilet filled with millet husk as a composting matrix has been designed and installed farming is the main occupation of the people in the area (Ito *et al.,* 2012). A family with six people earned 132 Euro in Zinaire from the reuse of compost, urine and grey water in their sanitary gardens (Hijikata *et al*., 2014). The people are encouraged to use the composting toilet to make compost from their faeces, and then reuse onto their farmland for cultivating vegetables and other food crops. Pathogenic viruses in the compost need to be assessed before reuse on the farmlands.

There is no information available for the treatment of compost in Burkina Faso using heat inactivation of pathogenic viruses. In spite of the importance of viruses as a pathogen causing a wide range of infectious diarrhoea, nausea, vomiting, hepatitis, and meningitis throughout the years in developing countries, attention has not been paid to inactivation of viruses in compost using an affordable sanitisation method such as the solar energy. *E.coli* has been used for the verification and monitoring of treated excreta (WHO, 2006). However, *E.coli* cannot be used to monitor viruses that have a strong tolerance (Kazama *et al.,* 2011). Certain coliphage are considered an appropriate indicator than coliform bacteria to monitor viruses in a water environment (Mocé-Llivana *et al.,* 2003). Enteric bacteriophages specifically, F^+ RNA bacteriophage infecting *Escherichia coli,* have been proposed as more reliable indicators of human viral pathogens (Nappier *et al.,* 2006) because they are similar to human enteric viruses in their physical structure, composition, and morphology, survivability in the environment, and persistence in treatment processes (Nappier *et al.,* 2006; Funderburg and Sorber, 1985; Havelaar, 1987). F^+ RNA coliphages can also be detected and quantified by simple, inexpensive, rapid, and reliable methods (Nappier *et al.,* 2006; Havelaar *et al.,* 1993). They are abundant in domestic wastewater, raw sewage sludge, and polluted waters (Nappier *et al.,* 2006) and originate almost exclusively from the faeces of humans (Sobsey *et al.,* 1995). The study specifically, studied the thermal inactivation of MS2 in the laboratory . The laboratory test is important to simulate the conditions

affecting MS2 under thermal heat. The main aim of the study was to sanitise compost withdrawn from the rural model of composting toilet by setting post-treatment conditions. The specific objectives of this research were (1) to determine the kinetic parameters of inactivation of MS2 bacteriophage (presumably viral pathogen) during a post-treatment of compost; and (2) to evaluate the effect of temperature and moisture content on the inactivation rate coefficients (k h^{-1}).

4.2 Material and Methods

4.2.1 Measuring MS2 bacteriophage

MS2 bacteriophage were obtained from the National Biological Resource Centre (NBRC), National Institute of Technology and Evaluation, Japan. Bacteriophage MS2 belongs to the family levividae and it was used as the reference bacteriophage for F-specific RNA bacteriophage (Hensen *et al.,* 2007). *E.coli* strains with number 13965 was used as host bacteria for MS2 bacteriophage. LB (Luria Bertani) broth was used as a growth medium for coliphage and *E.coli* strain. *E.coli* was incubated in a shaking water bath at 37 °C for 4 hours. In order to measure the concentration of microorganism in the compost, microorganism needs to be extracted into a solution (Kazama *et al.,* 2011). A 4 % (w/v) phosphate buffer solution was used as an extraction solution. The phosphate buffer was prepared by dissolving 42.5 g of KH_2PO_4 to 500 ml of pure water. pH was adjusted to 7.2 with 4 (w/v) of NaOH, then autoclaved. The compost at 4% (w/v) was then agitated for 3 minutes to extract MS2 bacteriophage in the Phosphate buffer solution. LB broth was used to dilute the extracted solution to a suitable concentration for measuring the organism. The LB broth was prepared by dissolving 10 g of polypetone, 5 g of Bacto Yeast Extract, 1.5 g of glucose, 5 g of NaCl, 0.2 g of $MgSO_4.7H_2O$ and 0.5 g $MnSO_4.5H_2O$ in 1000 ml of distilled water, then autoclaved. MS2 was enumerated by plaque assay with *E. coli* NBRC 13965 using a double agar overlay technique and using LB agar (Kazama *et al.,* 2011; Hensen *et al.,* 2007). Plaques were observed and enumerated following 18 to 24 hours of incubation at 37 °C ± 2 °C. The results were converted to the plaque weight of compost.

4.2.2 Compost preparation and Inoculation

Twenty grammes (20 g) of compost was weighed into 250 ml sterilised bottle. The compost was prepared by loading 500 g-wet of pig manure to 3 kg of rice husk in a composter with a mixing

mechanism for 30 days. After composting, it was kept in the composter for 1 week to reduce moisture. The moisture content measured before the experiment was 27 %. Ultra-pure water was added to adjust moisture content of the compost. The combinations of temperature and moisture content of the experiments are summarised in Table 4.1. To study the combined effect of temperature, moisture content and contact time, temperatures were set to 30 °C, 40 °C and 50 °C. In Burkina Faso, the average temperature is approximately 30 °C. The temperature of the compost can be increased when exposed to solar thermal heat. During a proper operation of the composting toilet, the moisture content is kept around 50 - 60 % (Lopez Zavala *et al*., 2004). Therefore, moisture contents were set to 50 % (normal condition), 60 % and 70 % (anaerobic conditions). Here, the moisture content is defined by the following equation below:

$$\text{Moisture content, } MC = \frac{W_{wet} - W_{dry}}{W_{dry}} \times 100 \qquad (4.1)$$

Where, W_{wet} and W_{dry} are wet and dry weights of compost sample, g. The bottles were inoculated with MS2 bacteriophage which contains 3.2 x 10^5 PFU/g-dry compost. The compost was agitated for 1 minute to ensure that MS2 bacteriophage was uniformly mixed. The bottles were tightly closed to control any change in moisture content during the thermal treatment. The samples were then immediately placed into the incubator to keep temperature constant. Moisture content did not change throughout the experiment for all thermal treatments. The total treatment time was 8 hours. One gramme (1g) of the compost sample was taken at 2 h interval for all experimental conditions.

Table 4.1 Experimental conditions for thermal treatment for MS2

No. of sample	Temperature °C	Moisture content
1	30	50%
2		60%
3		70%
4	40	50%
5		60%
6		70%
7	50	50%
8		60%
9		70%

4.2.3 Data analyses

To determine virus inactivation rates, the number of virus per gram (dry weight) of the compost were transformed into $\log_{10}$ PFU/gram (dry weight) of compost. Concentration versus time data obtained from the inactivation experiments were fitted to a first order kinetic model. This was done for thermal treatment of MS2 bacteriophage. Nakagawa *et al.,* 2006 indicated that inactivation of microorganisms follows a first order reaction and it is expressed as:

$$\ln k = C/C_o = -kt \qquad (4.2)$$

where, C is concentration of microorganism in compost sample in dry basis at time, t (PFU/g-dry solid), C_o is initial concentration of microorganisms in compost sample in dry basis (PFU/g-dry solid), k is inactivation rate coefficient (h^{-1}), and t is reaction time (h). After the estimation of inactivation rate coefficients (k h^{-1}), the effect of temperature was evaluated with Arrhenius equation described as follows;

$$k = A\exp\left(-\frac{E_a}{RT}\right) \qquad (4.3)$$

where, A is pre-exponential factor, E_a is activation energy (J/mol), R is universal gas constant (J/mol/K), T is temperature (K). ANOVA was done to determine the significant effect ($p \leq 0.05$) due to increasing temperature and moisture on the inactivation coefficient rate values of MS2.

The analysis was performed with R (open source coding software) using the packages "stats" for the ANOVA and Shapiro.test.

4.3 Results and Discussion

4.3.1 MS2 bacteriophage inactivation in compost

The concentration of MS2 bacteriophage decreased as the temperature increased, moisture content (MC) decreased and the length of time increased. The characteristics of MS2 at 30 °C for the conditions of moisture content (50 %, 60 %, and 70 %) is shown in Figure 4.2a. At this temperature the inactivation rate coefficients, k h^{-1}, values were almost zero. This indicates no reduction in the concentration of MS2 at 30 °C within the 8 hour treatment period. Figure 4.2b shows the change in concentration of MS2 at 40 °C with moisture content of 50 %, 60 % and 70 %. The inactivation rate coefficients, k h^{-1}, values were 0.252, 0.165, 0.088 for the respective moisture contents. At 40 °C, the log unit reduction recorded for 50 % was 2 log units, 60 % moisture was 1 log and 70 % recorded < 1 log unit in 8 hours. Figure 4.2c shows the change in concentration of MS2 at 50 °C. The total length of time for treatment was 8 hours at 50 °C; the point at the 8^{th} hour was not shown because there was no count. Presenting 0 count on a log plot was impossible and hence the omission. The inactivation rate coefficients, k, values at 50 °C at 50 %, 60 %, 70 % moisture content were 0.447, 0.308 and 0.100 respectively. Treatment at 50 °C with moisture 50 %, 60 %, 70 % recorded >3 log units, >1 log unit and < 1 log units reductions in 8 hours. Table 4.2 shows the summarised inactivation rate coefficients, (k h^{-1}) data during the post-treatment of MS2 bacteriophage with varying temperature and moisture contents. The coefficient of determination, R^2, for the linear fit of the inactivation rate of the MS2 was in the range of 0.8 - 0.9.

Table 4.2 Summary of inactivation rate coefficients, k h^{-1} MS2 bacteriophage

	Moisture content, MC (%)		
Temp. °C	k, h^{-1} (50 %)	k, h^{-1} (60 %)	k , h^{-1} (70 %)
30	0.093	0.038	0.020
40	0.252	0.165	0.088
50	0.447	0.308	0.100

4.3.2 Effect of temperature and moisture on MS2 inactivation

Thermal treatment is a method employed by many sludge generators to inactivate pathogens. The proposed thermal treatment is inexpensive and generate a product beneficial and acceptable for agricultural use. The evaluation of a cost effective solution for sufficient inactivation of pathogen provides valuable information for implementation of the technology. The study focused on the inactivation rate for MS2 bacteriophage in compost as affected by temperature and moisture content in a laboratory experiment. Enteric viruses are of importance with regards to land application of compost due to potential of run-off resulting in surface contamination and potential movement of viruses in the soil column into underground aquifers. Such movement is enhanced during rainfall events where the cation particulates are reduced and vary with soil composition. Inactivation rate of MS2 in a possible additional treatment after the composting such as drying up with air (Low moisture content) and heating by solar energy (high temperature) was tried with laboratory test. High temperature has been regarded as one of the most important critical factors governing the inactivation of MS2 bacteriophage (Nakagawa *et al*., 2006). It was found that, inactivation rates increased with increasing temperature from 40 °C to 50 °C (Figure 4.2b and Figure 4.2c) and this was statistically significant ($p \leq 0.05$). These findings conformed to the studies of Kazama *et al*. (2011) who indicated that, inactivation of MS2 are strongly dependent on temperature.

Table 4.3: Analysis of Variance for MS2 bacteriophage

Source of Variation	*Sum sq.*	*DOF*	*Mean Sq.*	*F*	*p-values*
Anova Temp.	0.082604	2	0.041302	8.0645	0.03949
Anova Moisture	0.056870	2	0.028435	5.5521	0.07013
Residuals	0.020486	4	0.005121		

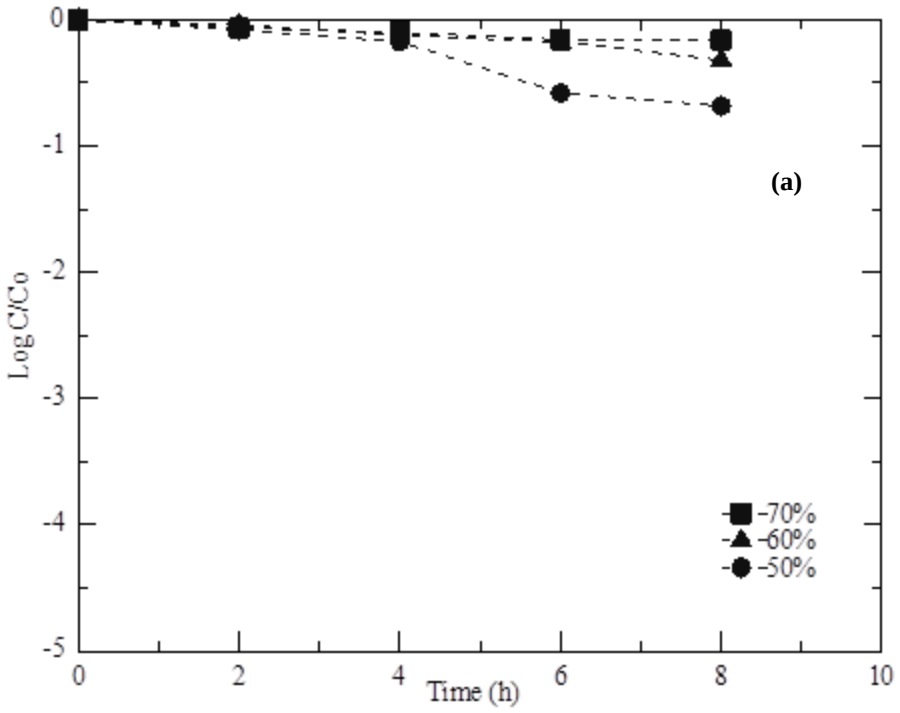

***Figure 4.1a**: Inactivation of MS2 bacteriophage at 30 °C*

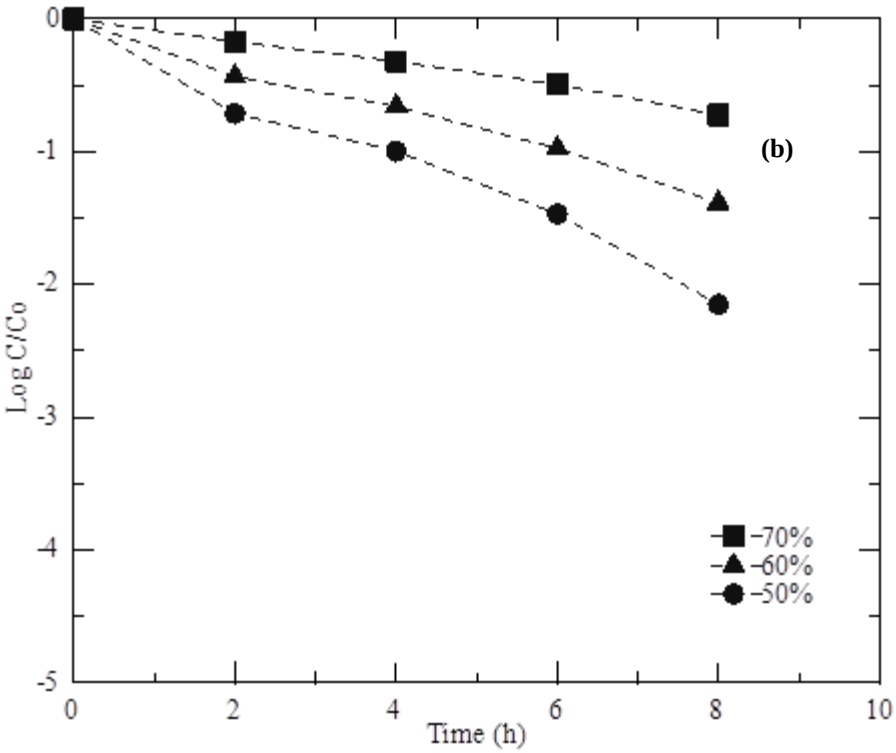

***Figure 4.1b**: Inactivation of MS2 bacteriophage at 40 °C*

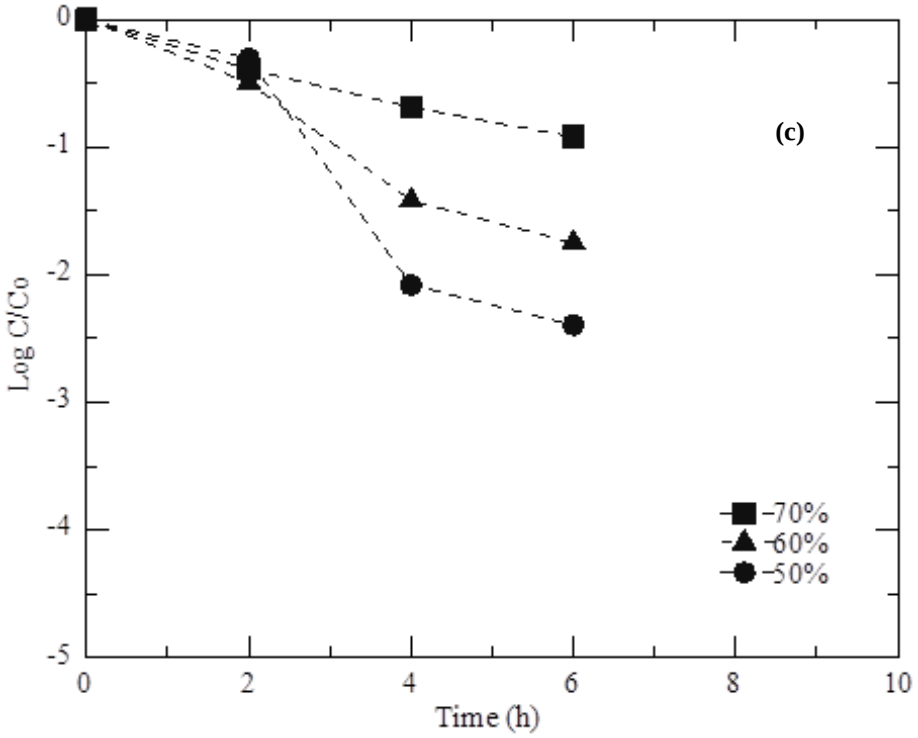

***Figure 4.1c**: Inactivation of MS2 bacteriophage at 50 °C*

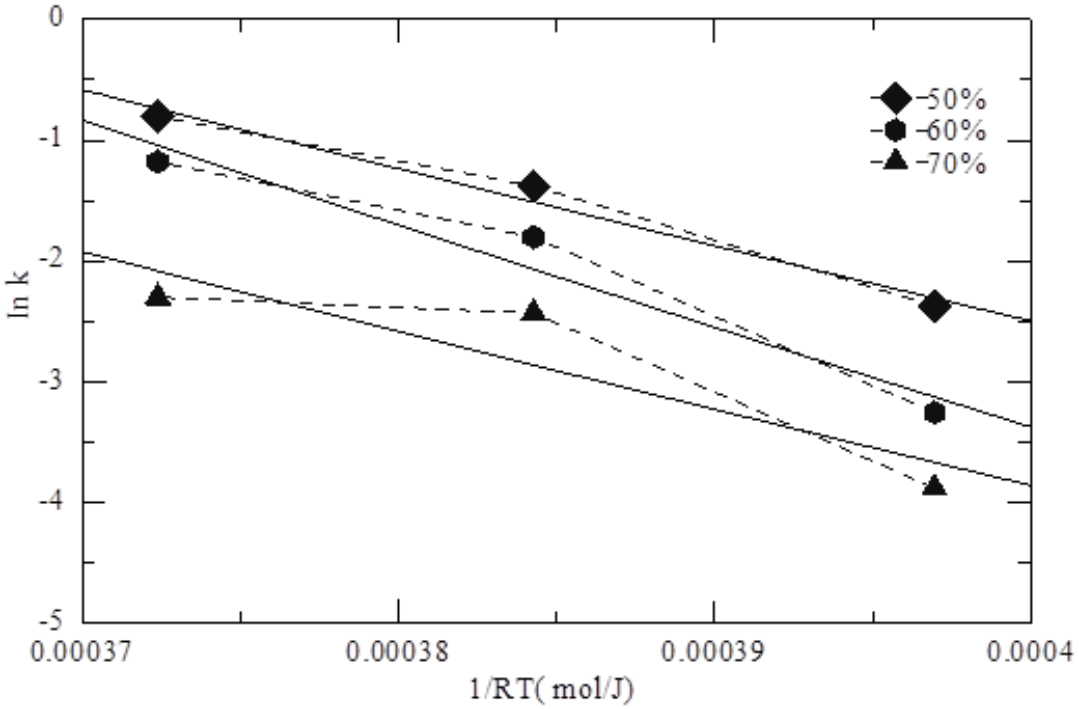

***Figure 4.2**: Effect of temperature on MS2 inactivation with varying moisture content. R^2 values for the trend of M C 50% , 60% and 70% are 0.98, 0.95 and 0.82 respectively.*

Results showed that, there was no change in concentration of MS2 after eight (8) hours treatment period at 30 °C which indicates that, lower temperatures facilitate the survival of MS2. Range of temperature from 40 - 50 °C and above are capable of inactivating MS2 as shown in these results. However, rates will obviously vary with varying temperature. Inactivation rate coefficients, k, h^{-1}, varied under different post-treatment conditions. During the treatment, relevant data were gathered for the inactivation rate coefficients. Arrhenius plots for MS2 inactivation is shown in Figure 4.3. The solid lines shows the correlation for the effect of temperature on MS2 at different moisture content, 50 %, 60 % and 70 %. The broken lines show the actual pattern of effect of temperature. There was a strong correlation between temperature 30 °C, 40 °C and 50 °C for all moisture levels. This indicates that, there is a greater effect of temperature for the various treatments.

Lower moisture conditions increased the inactivation rate coefficients, k, h^{-1} values in all our experiments. However, the difference in the k values were not statistically significant. These results are clearly shown in Figure 4.2. Conditions with lower moisture 50 % recorded higher inactivation rates indicating that compost with moisture content below 50 % can enhance the inactivation process during post-treatment. Inactivation rates are higher with increasing temperature and lower moisture content. Kazama *et al.,* (2011) showed that, inactivation rate coefficients, (k h^{-1}) at 50 °C 40 % moisture was 0.40 while the study reported 0.447 at 50 °C, 50 % MC (Table 4.2). The study observed that the rate of coliphage decreased more rapidly at high temperature because the inactivation rate coefficients, at 50 °C were higher than at 30 °C. This study reported the same trend. The most performing post-treatment condition was 50 °C 50 % MC which recorded a >3 log units reduction in 8 hours. The experimental data were important to allow simulation of inactivation of pathogens under solar thermal heat.

4.4 Summary

A composting toilet system is supposed to inactivate pathogens in compost before reuse as fertilizer. There was an insufficient heat buit-up in the composting process in the rural model of composting toilet. A post-treatment of the compost was necessary to increase the health and safety of farmers. Inactivation of MS2 bacteriophage (viral pathogens) in a possible additional post-treatment after the composting process by dying-up with air (lower moisture) and temperature (solar thermal heat) was investigated in the laboratory test. As a result, the

inactivation rate coefficient (*k*) values of MS2 bacteriophage depended on higher temperature but not on moisture content. The inactivation rates during the post-treatment were 0.093 - 0.020 h^{-1}, 0.025 - 0.088 h^{1}, 0.447 - 0.100 h^{-1} at 30 °C, 40 °C and 50 °C respectively.

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Chapter 5

Design of Post-Treatment Unit for Compost from a Composting Toilet with Microbial Risk Assessment

5.1 Introduction

Compost of human faeces used as fertiliser can be harmless and useful because it becomes part of nutrient recovery. A pilot model of a composting toilet was installed in a rural region of Burkina Faso to perform a source recycling system which makes compost from human faeces. Initial experiments were performed on some samples taken from the composting toilet. Results showed that pathogens such as bacteria and parasites still remained in the compost after withdrawal from the rural model of composting toilet after three months of operation. Therefore, post-treatment of the collected compost is required to minimise the health risk when recycling the faeces as fertiliser on farmland. For the inactivation of pathogens, several methods of treatments are proposed, including heating, drying, chemical treatments, treatment by worms, long storage times, etc. In low income countries like Burkina Faso, people cannot pay materials for post-treatment, however, they have abundant solar energy. Therefore; this study proposes a solar disinfection unit to inactivate the pathogens. The operation condition to inactivate pathogens should be designed based on the risk assessment by setting a safe level of pathogens concentration in the compost after post-treatment.

Norovirus, *Ascaris* eggs and *Salmonella* were selected reference pathogens in this study. Noroviruses are a major cause of human gastroenteritis, and they are frequently associated with food, water contamination (Teunis *et al*., 2008) and accidental ingestion. *Ascaris* infections are very common in developing countries. One fertile egg can cause infection of Ascarisis to humans. The carrier state of *Salmonella* typhi is defined as persistant shedding in faeces for greater than 12 months (Berger, 2015).

These enteric infections can be transmitted through the compost from faeces to the human body with pathogenic species. Quantitative microbial risk assessment (QMRA) has been widely used to establish the health risks associated with wastewater reuse in both developed and developing regions under different scenarios. The QMRA-Monte Carlo techniques (QMRA-MC) based on the work of Haas *et al*. (1999) was used to estimate risk in this study.

The objectives of this study are to perform risk assessment for the design of the post-treatment unit by using the QMRA-MC techniques and to determine the treatment time to reach the safe level of pathogens in the compost.

5.2 Material and Methods

5.2.1 Post-treatment Unit

People would collect the compost from the rural model of composting toilet with urine diversion (Figure 5.1) in the pilot families and used it in their gardens as fertilizer. Application of the post-treatment would be achieved by spreading the compost evenly on the steel box as shown in Figure 5.2. The box was fabricated with a length of 60 cm, a width of 40 cm, and a depth of 10 cm. The total volume of the box is 24 L. The steel box has steel septa which facilitate deep penetration of heat to compost. The steel box is painted black in colour to aid in the absorption of heat. The steel box does not have a solar concentrator (Adreev and Samoil, 2009; Darimani *et al*., 2015a). The temperature distribution of the compost in the box was measured at 3 positions which were 1 cm, 5 cm, and 10 cm from the surface.

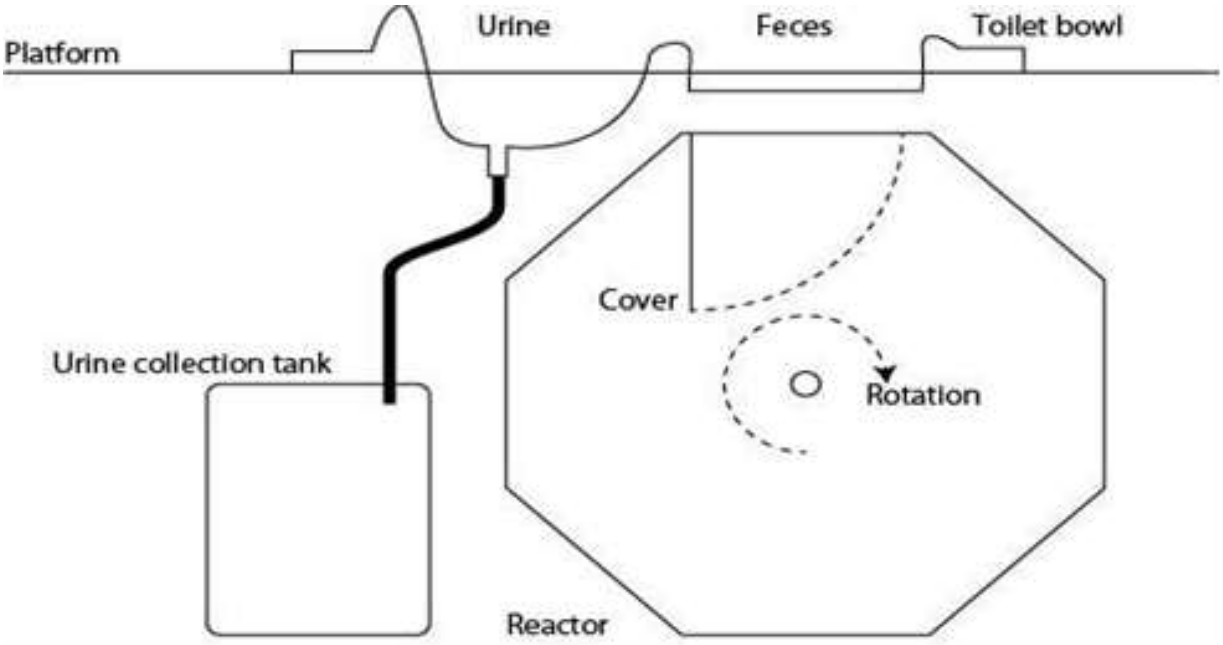

Figure 5.1: *Arrangement of the composting toilet*

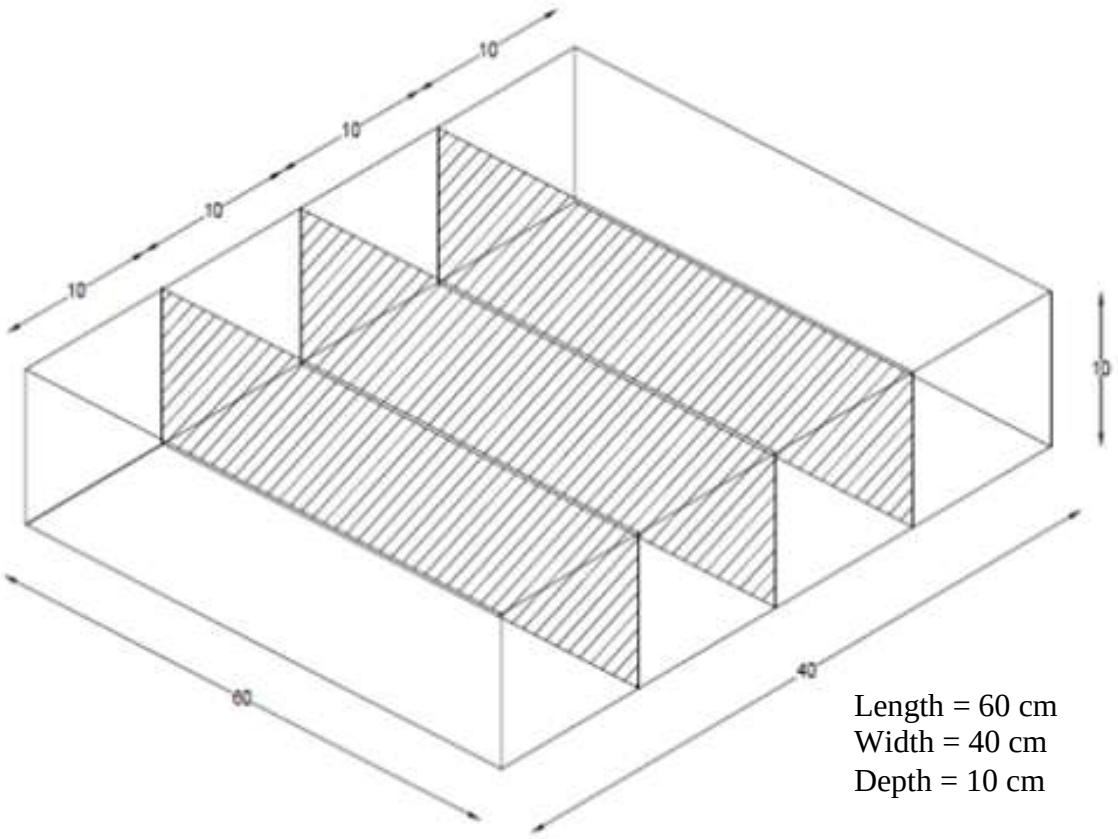

Figure 5.2: *A proposed compost solar sanitisation installation that could reduce the heat loss (Adreev and Samoil, 2009)*

5.2.3 Scenarios for reuse of compost

During the utilisation of the compost, people may accidentally ingest compost with the pathogens orally. The people exposed to the pathogens would have diseases with a probability estimated by risk assessment. Four (4) scenarios were set, temperature at 3 positions (1cm, 5 cm and 10 cm) in the steel box (S-1) as a post treatment for the assessment. Lower temperatures were set for minus five, minus ten and minus fifteen of the steel box temperature distribution. These lower temperatures were derived from the assumption that the temperature measured in the steel box in Burkina Faso is minus five, minus ten and minus fifteen degree celcius lower than the actual temperatures measured. These are to account for the lower temperature during the year. These corresponds to the temperature at different positions (1 cm, 5 cm and 10 cm) i.e. Lower temperature (-5 °C) as S-2, lower temperature (-10 °C) as S-3 and lower temperature (-15 °C) as S4. The different depths are defined as bottom, middle and top. For the calculation of

concentration in the compost, the inactivation rates coefficient from the previous measurement were used (Darimani *et al*., 2015b). The details of the ingestion model are as follows:

- To consider the worst case, 50,000 eggs/g in wet faeces is excreted from a heavily infested person (Hotez *et al*., 2003). The value of the initial concentration of *Ascaris* eggs was 336 eggs/g-dry compost. This number was estimated by multiplying the number of eggs excreted per gram (50, 000 eggs/g) by the 100 g of compost dividing by the bulk density of the compost (14,881 kg/m^3).
- Highly infested person of viral infection excretes a maximum of 10^{11} viral copies/g in faeces from highly infected person (Teunis *et al*., 2008 ; Aoki *et al*., 2010; Hall, 2012) was used for the risk assessment taking account of the highest risk. Assuming this concentration, the initial concentration was estimated at 6.72 *x* 10^8 viral copies/g-dry compost. This number was estimated by multiplying the number of norovirus excreted per gram (10^{11} viral copies/g) by the 100 g of the compost and dividing by bulk density of the compost (14,881 kg/m^3).
- Concentration of *Salmonella* spp in faeces is 10^4 - 10^{10} per gram of faeces (Haas *et al*., 1999). Assuming this concentration, the initial concentration was estimated at 6.72 *x* 10^7 CFU/g-dry compost. This number was estimated by multiplying the number of *Salmonella* excreted per gram (10^{10} CFU/g) by the 100 g of the compost and dividing by bulk density of the compost (14,881 kg/m^3).
- Ingestion rate of compost is 150 - 800 mg/event. This is used in the risk assessment of dioxin in soil ingestion rate (Nakata *et al*., 2003).
- Post-treatment would be done every 4 months.
- The concentration of pathogens in the compost after the post treatment was estimated using the first-order kinetic model from the earlier studies on *Ascaris* eggs and indicator MS2 bacteriophage inactivation and *E.coli*. The data from these experiments were used to re-estimate the inactivation rate co-efficient (Darimani *et al*., 2015).
- The moisture content of all treatments was 50 %.

5.2.4 Hazard identification

Farmers performing post-treatment would be exposed to pathogens in the compost. There are several groups of pathogens, but the pathogens of considerable interest in the study area are *Ascaris* eggs, viral infections (norovirus) and *Salmonella* because *Ascaris* and norovirus are also known to be the most resistant to treatment processes (Feachem *et al*., 1983; Kazama *et al*., 2011). Burkina Faso recorded 32.8 % of bacteremia among febrile children admitted to hospital (non-tyhpoid *Salmonella*) between 2012 - 2013 (Berger, 2015) and it is also reported that the carrier state of *Salmonella* typhi is defined as persistent shedding in faeces for greater than 12 months (Berger, 2015). Accidental ingestion of a small dose consequently implies a high risk of infection compared to many other pathogens (Nakata *et al*., 2003).

5.2.5 Dose-response assessment

The QMRA-MC was used to estimate risks of *Ascaris* and norovirus and *Salmonella* infections. The study by Navaro *et al*. (2009) found that *Ascaris* infection data best fitted the β-Poisson dose-response equation (Navaro et al., 2009):

$$P_I(d) = 1 - \left[1 + \left(d / N_{50}\right)\left(2^{1/\alpha} - 1\right)\right]^{-\alpha} \qquad (5.1)$$

where P_I (d) is the probability of infection in an individual (infection/event), d is the ingested number of *Ascaris* eggs on one occasion (eggs/event), N_{50} is the mean infective dose number of *Ascaris* eggs (eggs), I means considerable spice for calculation of probability (-) and α is an infectivity constant of *Ascaris* (-). They found the values of N_{50} and α to be 859 and 0.104, respectively. Since they were working with epidemiological data on *Ascaris* prevalence rather than conducting human *Ascaris* dose-challenge studies, the value found for N_{50} is not a measure of the actual median *Ascaris* infective dose, but rather an empirical value arising from their statistical analyses (Mara *et al*., 2007).

The annual probability of infection, $P_{I(A)}(d)$ (pppy), is given by:

$$P_{I(A)}(d) = 1 - \left[1 - P_I(d)\right]^n \qquad (5.2)$$

Where n is number of events per year to the single *Ascaris* dose (-) (Mara *et al*., 2007). For norovirus, the dose response data set of Teunis *et al*. (2008) was used in place of the β-Poisson

equation (Mara *et al*., 2007). The β-Poisson equation was used to assess the dose response of salmonellosis. The N_{50} and α used are 17700 and 0.23475 respectively.

5.2.6 Exposure assessment

The human exposure assumed to take place is an event when farmers work on compost. Practically, one egg is enough to cause an infection. Norovirus has an extremely low infectious dose (Hall, 2012) and salmonellosis is a public health concern in Burkina Faso (Berger, 2015).

5.2.7 Risk characterisation

The Monte Carlo technique has been used to evaluate the infection risk. The random number is applied for estimation of variables with distributions for simulation of Eqs. 5.1 and 5.2. The simulation was repeated 10,000 times (Mara *et al*., 2007). Then, 95 percentile of the probability was estimated as the infection risk.

5.2.8 Temperature Distribution

Considering actual practices, solarisation is one of the main processes for disinfection of enteric pathogens, because sunlight is available in the study region. The solarisation relates to the ambient temperature, while the temperature is not constant as shown in Figure 5.3. One week temperature was measured during February, 2015 in the post-treatment unit with the aid of ThermoManager sensors in Ouagadougou, Burkina Faso. They were placed in the compost at the bottom, middle and top at 10 cm, 5 cm and 1cm respectively. The sensors recorded temperature data every five mins during the week. Figure 5.3 shows the temperature pattern in the post-treatment unit. The maximum and minimum temperatures recorded from the bottom were 51°C and 10.5 °C. The middle recorded 50 °C and 9.5 °C for maximum and minimum temperatures while the top recorded maximum of 78.5 °C and a minimum of 6.5 °C. Obviously, the lower temperatures were recorded in the night and high temperature during the day.

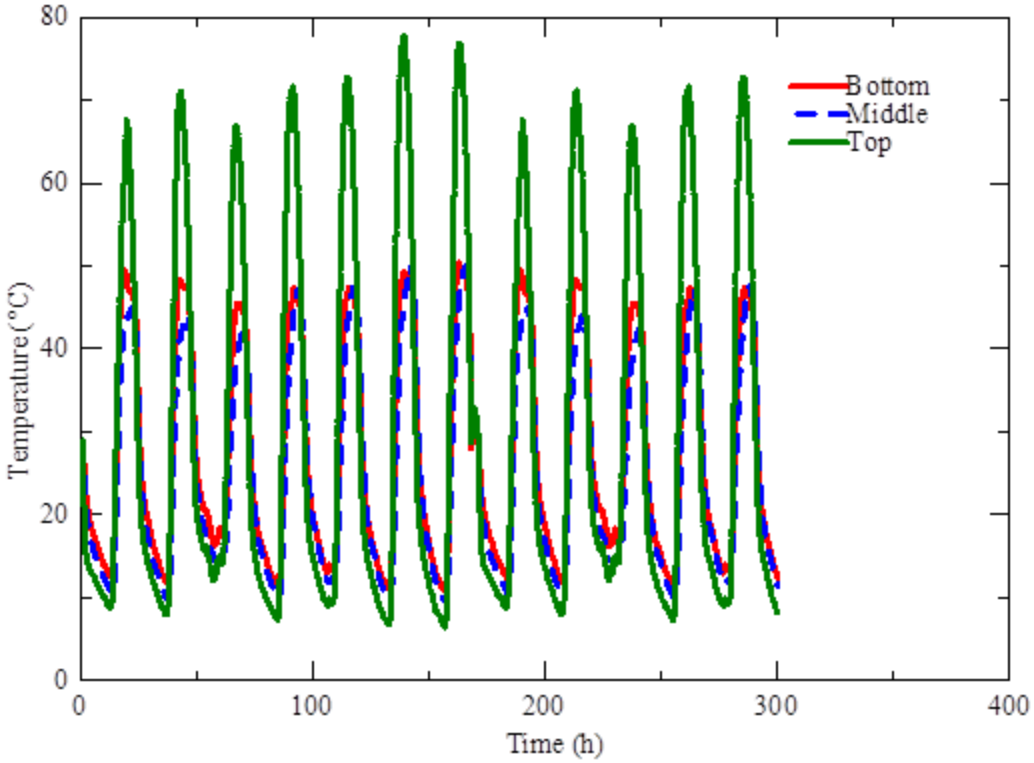

Figure 5.3: *Temperature distribution assumed in the risk estimation*

5.3 Results and Discussion

The change in concentration of *Ascaris* for the steel box and lower temperatures (-5,-10,-15 °C) is shown in Figure 5.4. The concentration declined from the initial value of 336 eggs/g dry-compost. High temperature gives high decline rate of the concentration due to high inactivation rate coefficient. High and low reduction rates are found in the figures. This is because high temperature at day time and low temperature at night respectively give high and low reduction. All scenarios for *Ascaris* obtained reduction of eggs in 295 h and the differences of the temperature resulted in the differences in concentrations. The change in concentration of norovirus with elapsed of time under all scenarios are shown in Figure 5.5. The concentration declined from the initial of 6.72 x 10^8 copies/g-dry compost. Higher temperature condition also gives higher decline rate. The reduction rate of norovirus concentration had difference among four scenarios like the *Ascaris* case. The concentration varied due to the varied temperature especially at night. As expected, the day time recorded higher temperatures and lower temperatures were recorded at night. All the scenarios achieved safe level at 845 hours. The change in concentration of *Salmonella* with elapse time under all scenarios are shown in Figure

5.6. The concentration declined from the initial of 6.72 *x* 10^7 CFU/g dry-compost. Higher temperature condition also gave higher decline rate. The reduction rate of *Salmonella* concentration had difference among four scenarios like the *Ascaris* and norovirus case. All scenarios achieved safe level at 969.5 h.

The 95-percentile annual risk of *Ascaris*, norovirus and *Salmonella* infections for the all scenarios are shown in Figures 5.7, 5.8 and 5.9. The risk of the pathogens are almost 1 at the initial for all scenarios. This means the people who use the compost would be heavily polluted by the pathogens. They would be infected if the composting reactor fails to reduce the pathogen concentration and also if they do not apply the post-treatment. Schönning *et al*. (2007) also reported a 95-percentile risk of rotavirus and *Ascaris* for 0 months' storage in a worst case as 1. The results show the risks for the *Ascaris* for the steel box. The lower temperature -5, -10 and -15 °C reduced concentrations and reached a safe level at 97.5 h, 138 h, 190 h and 295 h respectively.

The volume of the composting reactor is 100 L. Taking account of the temperature distribution with depth of the unit. The top and bottom temperature would achieve a safe level before the middle because that is the lowest temperature zone in the post-treatment unit. It should be noted that about 25 % of the volume of the composting reactor was used for the design of the unit. This is to ensure that the unit is not too deep to reduce the efficiency of the unit. The unit is considered as a batch reactor (BR) where concentration of the compost would change with time. The expected concentration can be obtained by adjusting the reaction time. The temperature distributions in the steel box recorded a shorter time than the lower temperatures. The treatment time can be reduced if the unit is improved. During the day, there is a sufficient increase in temperature, but it suddenly decreases towards the evening and at the nights. This phenomena causes sufficient inactivation by the balance of the high inactivation rate at high temperature and the low inactivation at low temperature. To reduce treatment time, one needs to improve the post-treatment unit increasing the maximum temperature and keeping temperature during the night.

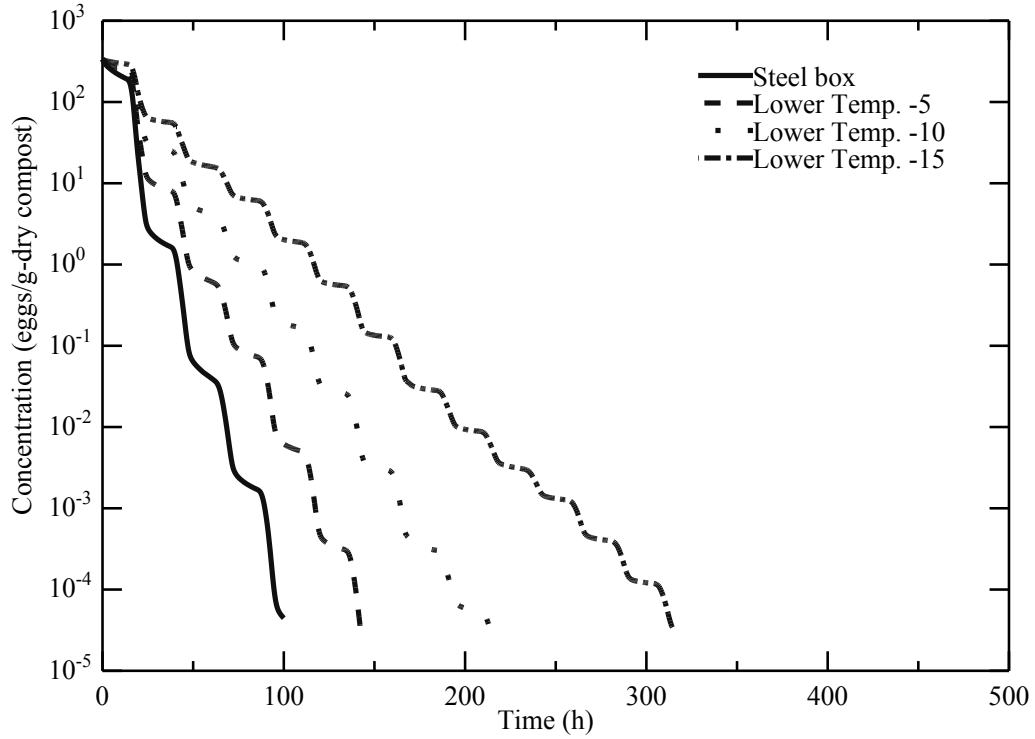

***Figure 5.4**: Change in Ascaris eggs concentration for the post-treatment*

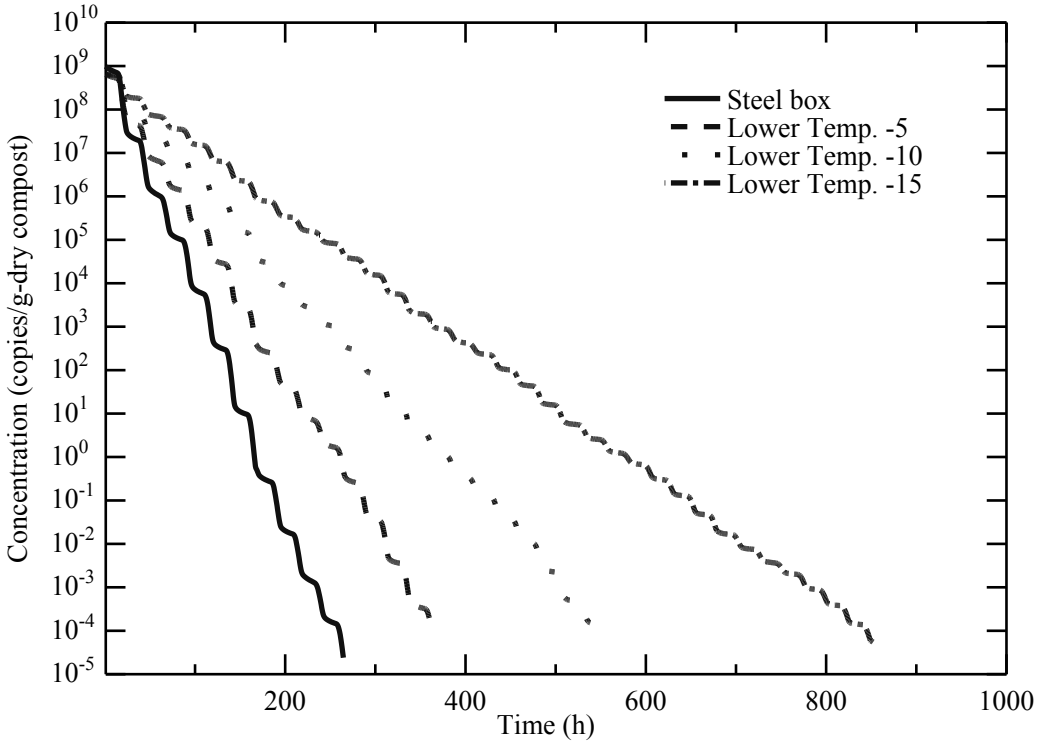

***Figure 5.5**:. Change in Norovirus concentration for post-treatment*

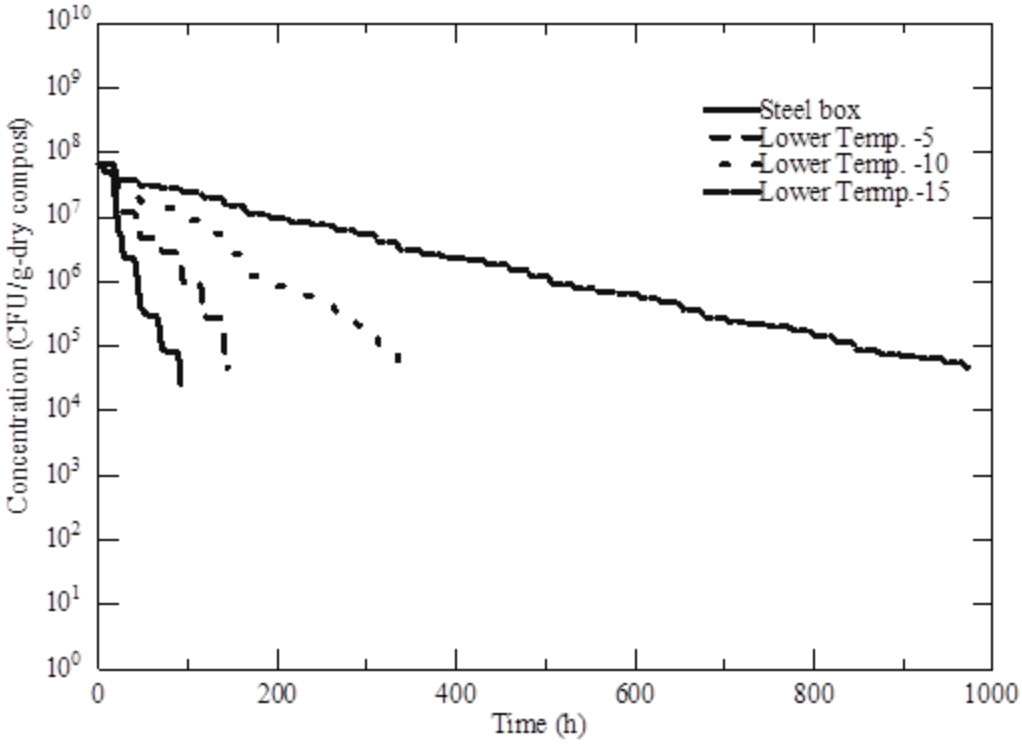

Figure 5.6: *Change in Salmonella concentration*

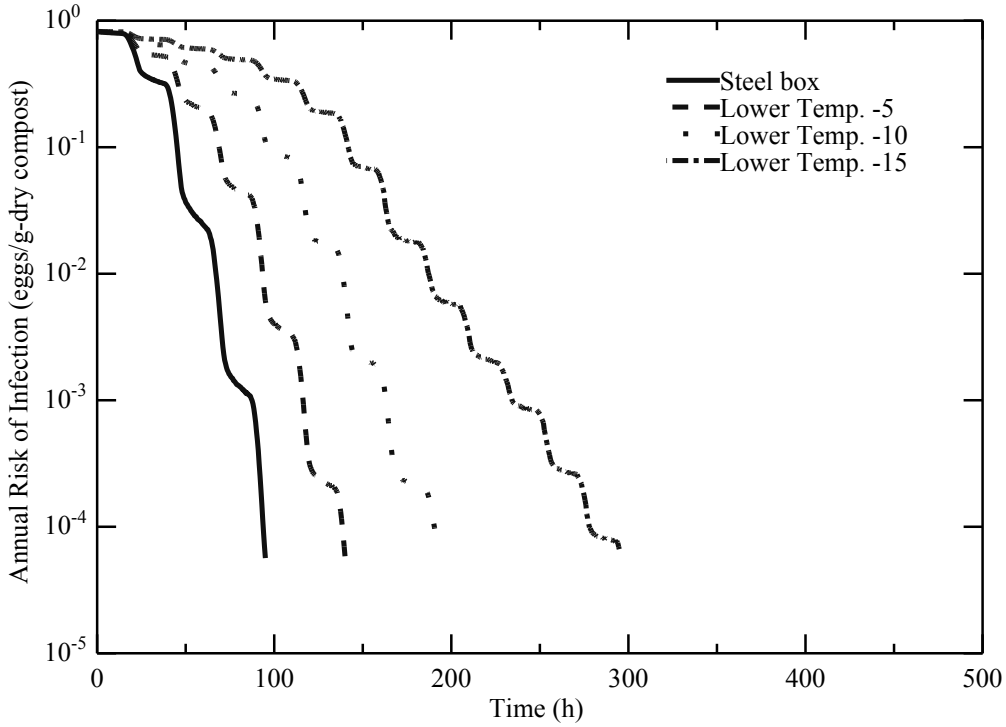

Figure 5.7*: Ascaris annual infection risk associated with post-treatment*

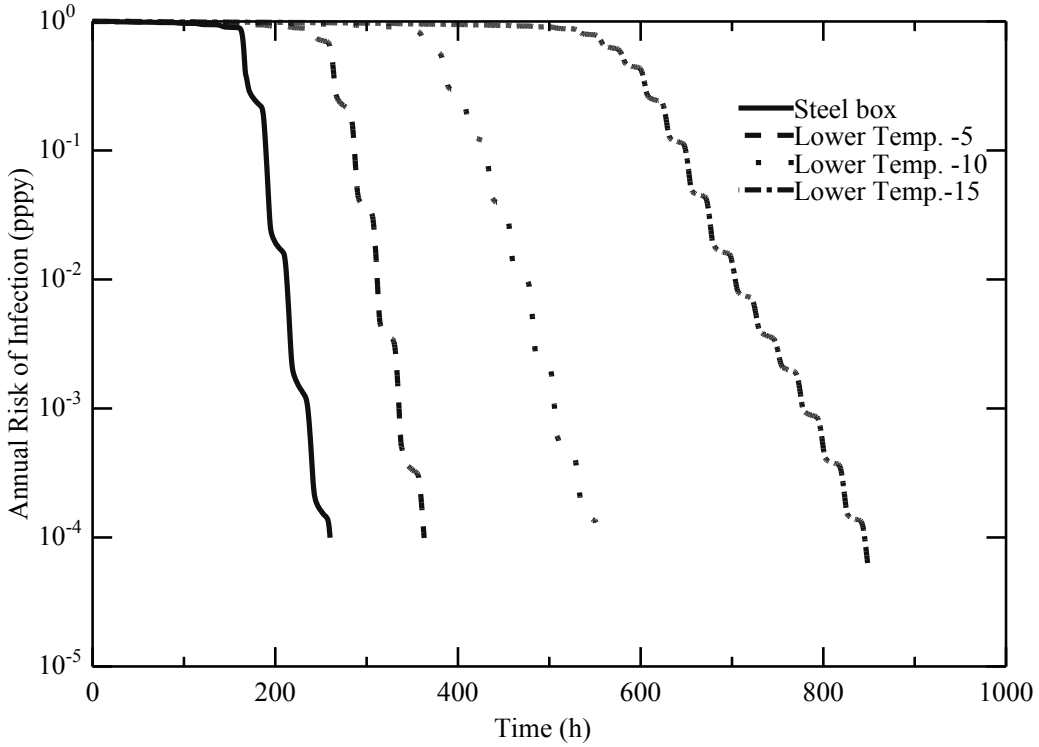

Figure 5.8: *Norovirus annual infection risk with post-treatment*

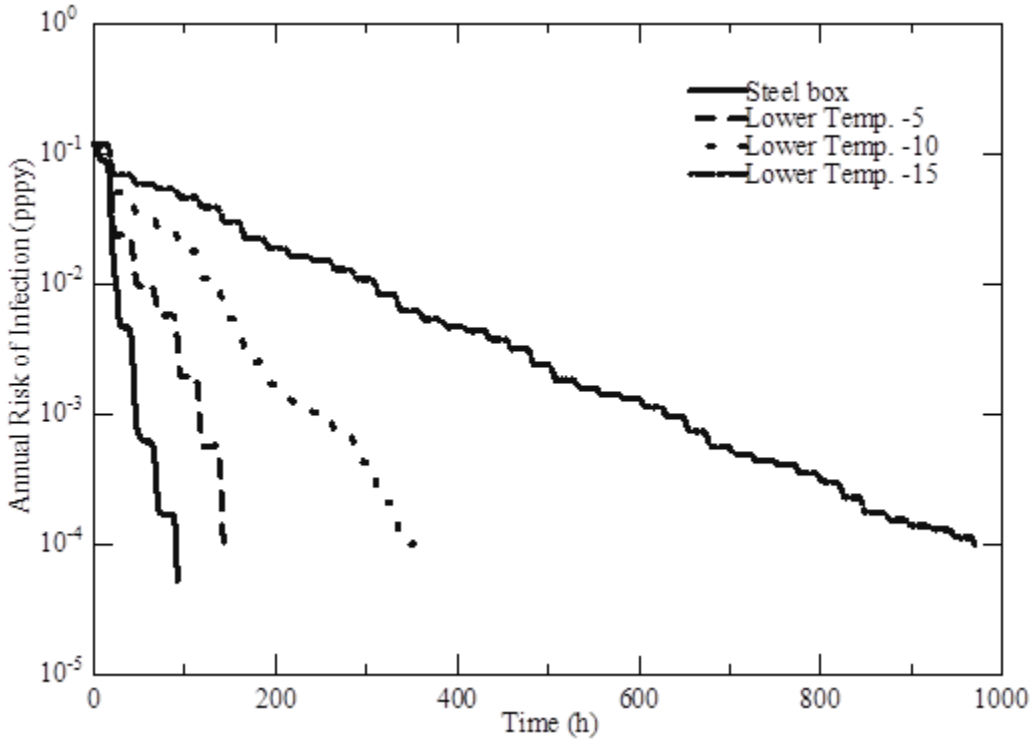

Figure 5.9: *Salmonella annual infection risk associated with post-treatment*

The required times to reach the safe level for norovirus for the steel box at the lower temperatures -5, -10 and -15 °C were 264 h, 362.5 h, 554 h and 845 h respectively. The time required to reach the safe level for *Salmonella* in the steel box, the lower temperature -5, -10 and -15 °C were respectively 90.5 h, 143 h, 356.5 h and 969.5 h respectively. Comparing *Ascaris*, norovirus and *Salmonella*, *Salmonella* requires more time at lower temperatures than *Ascaris* and norovirus to reach safe level of 10^{-4} per person per year (pppy) (Regli *et al*., 1988). This is probably due to the fact that lower temperature are favourable conditions for bacteria. Therefore, *Salmonella* is more important indicator for the design of the unit, even though *Ascaris* eggs have possibility to survive several months in a soil system (Seidu *et al*., 2008).

Risk assessments for post-treatment of compost have received very little documentation. Seidu *et al*. (2008) reported increased levels of *Ascaris* and rotavirus infection for farmers due to accidental ingestion of contaminated soils. The estimated median risk values for farmers were 0.99 and 7.2 x 10^{-2} pppy for Ascariasis and rotavirus. The study indicated that the elevated hazard posed by the soils on the farm could be attributed to the persistence of *Ascaris* in the soils. This implies that compost must be treated properly before reuse as fertilizer so as not to pose even greater risk in the soils. However, in semi-arid regions where the compost is expected to be used, inactivation of *Ascaris* occurs in soils rapidly (Hall, 2012) which indicates that post-treatment in these regions could be feasible. The results of this study indicate that high temperature with prolonged treatment time could reduce the hazard considerably. Mara *et al*., (2007) reported risk of fieldworkers' involuntary ingestion of 100 mg of waste-water contaminated soils. The median of norovirus infection risk for an ingestion of 100-1000 mg, 10-100 mg, 1-10 mg of contaminated soil were 0.98, 0.32, and $3.7x10^{-2}$ pppy respectively. The study also reported the median *Ascaris* infection risk for ingestion of 100-1000 mg, 10-100 mg, and 1-10 mg of contaminated soils as 0.14, 1.5 x 10^{-2}, and 1.5 x 10^{-3} pppy respectively. In this study, the risk associated with the exposure of *Salmanella* at lower temperature was estimated to be the highest, thus, this level of pathogen reduction will provide sufficient protection against *Ascaris* and norovirus infections.

5.4 Summary

Higher temperature is efficient in reducing the risk of pathogens. The temperature distribution in the steel box and the lower temperatures although reached a safe level, the time required for the safe treatment is too long and hence the steel box need an improvement. Therefore, to efficiently reduce pathogens during the post-treatment and also reduce the time of treatment, the steel box needs an insulator to maintain the temperature. The guidelines for the design of the post-treatment facility are: For *Ascaris*, the steel box and the lower temperature -5, -10 and -15 °C, post-treatment requires approximately 295 h to achieve the safe level of 10^{-4} pppy. For norovirus, post-treatment requires approximately 845 h for scenarios to achieve a safe level. *Salmonella* requires 969.5 h, for all scenarios to reach a safe level. The evaluation of the performance of post-treatment unit for risk assessment of the targeted pathogens has been achieved with the developed mathematical model.

5.5 References

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Chapter 6

Inactivation of *Enterococcus* in Compost-Amended Soils

6.1 Introduction

The rural model of composting toilet has been designed and installed in consideration of local material availability and affordability (Ito *et al*., 2012). Although the composting process reduces enteric pathogens (Sossou *et al.*, 2012; Darimani *et al*., 2015) 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 (Otaki *et al*., 2006). Reuse guidelines for human excreta has been published by WHO (2006) and also optional post-treatments such as solar heating, drying (Redlinger *et al*., 2001; Darimani *et al*., 2015) and increasing pH with alkaline treatment by lime or ash (Kazama and Otaki, 2011; Tezuka *et al*., 2012) has been investigated to ensure pathogen inactivation (Chapter 1). Increasing temperature of the compost-amended soil layer by solarisation also occur slight drying of the surface of the layer due 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 contain high levels of enteric bacterial indicators as previously observed (Redlinger *et al*., 2001). When the improperly treated compost is amended to the field, the soil, possibly provide intermediate 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 risk 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 (Person *et al*., 1975). The die-off periods for these organisms ranges from 30 mins to several years (Reddy *et al*., 1981). 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 (Person *et al*., 1975). 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 (Reddy *et al.*, 1981). *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 (Bendixen *et al*., 1999). It is often used to determine water quality and waste product quality (Jiang *et al*., 2002).

The study by Berger (2015) indicated that Burkina Faso recorded 32.8 % of bacteremia among febrile children admitted to hospital (non-tyhpoid *Salmonella*) between 2012-2013 (Berger, 2015) and it is also reported that the carrier state of *Salmonella* typhi is defined as persistent shedding in faeces for greater than 12 months. These reports indicates that salmonellosis could pose a major public health risk in Burkina Faso. This underlines the necessity for a joint and coordinated surveillance and monitoring programmes for salmonellosis in Burkina Faso. In the previous chapter, the health risk of *Ascaris*, Norovirus and *Salmanella* were estimated under different scenarios. Although *Salmanella* reached a safe level faster at the top temperature distribution, it 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 farmland. The solarisation relates to the ambient temperature, while the temperature is not constant as shown in Figure 6.1. 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 (i) to characterise the die-off represented by the kinetic inactivation rate coefficient of *Enterococcus* in the soil system amended with compost from the composting toilet, (ii) to determine the effect of temperature, compost-to-soil ratio and soil type on the inactivation rates of *Enterococcus.* (iii) to determine the health risk of *Salmonella* in the soil system after the amendment of compost.

6.2 Materials and Methods

6.2.1 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 6.1. Moisture contents, *MC* (%), on 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:

$$MC = \frac{W_{wet} - W_{dry}}{W_{dry}} \times 100 \qquad (6.1)$$

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 (Kelly *et al*., 1993).

Table 6.1 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±0.0l	NM
C	0.54±0.02	NM
SOM	0.93±0.03	NM

**NM , not measured

Table 6.2 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		

6.2.2 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 6.2. The pH of the compost-amended soils and the compost were checked 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.

6.2.3 Bacteria extraction and measurement

Enterococcus was cultured following the modification of method 9215 A in Standard Methods for the Examination of Water and Wastewater (APHA, 1998). 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, Disodium Phosphate Anhydrous 3.5, and Potassium Dihydrogen Phosphate 1.5. Ten grammes (10 g) 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.

6.2.4 Data Analysis

Concentration versus time data obtained from the inactivation experiments were fitted to a first order kinetic model. Nakagawa *et al.* (2005) indicated that inactivation of microorganisms follows a first order reaction and it is expressed as:

$$\ln k = C/C_o = -kt \qquad (6.2)$$

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 (h^{-1}), evaluation of the effect of temperature was done with the Arrhenius equation described as follows;

$$k = A\exp\left(-\frac{E_a}{RT}\right) \qquad (6.3)$$

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).

6.2.4 Estimation of risk of Salmonella during amendment in the soil

Quantitative microbial risk assessment 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 17700 and 0.235, 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 (Mara *et al.*, 2007). Then, 95 percentile of the probability was estimated as the infection risk.

6.2.5 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 shown in Figure 6.1 below. The fluctuation of the temperature distribution is observed for the bottom, middle and top positions respectively. This rise and fall of the temperature is as a result of the day and night. At the top, the maximum and minimum temperatures recorded are 66°C and 21°C, the middle recorded 61°C and 20°C, while the top recorded 69°C and 15.5°C respectively.

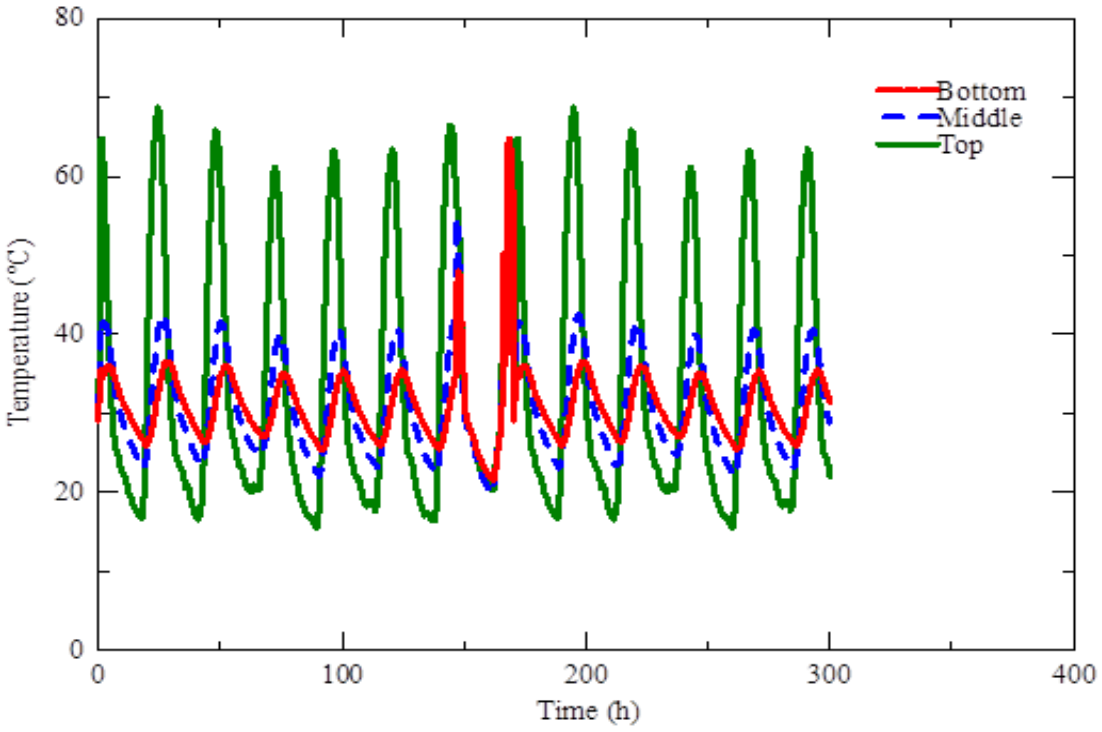

***Figure 6.1**: Temperature distribution in the soil system in Ouagadougou, Burkina Faso.*

6.3 Results and Discussion

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 6.2a. Figure 6.2b 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 unit, 1:25 recorded 1.2 log units, 1:50 and 1:100 recorded 1 log unit 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 6.2c, and the ratio 1:10 and 1:100 recorded 1.5 log units reduction, while the ratio 1:50 and 1:100 recorded 1.8 and 2 log units 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 unit reduction in 8 h at 30 °C for all ratios, as shown in Figure 6.3a. Decline in concentration of *Enterococcus* was observed at 40 °C by 1 log unit, 1.2 log units , 1.5 log units and 1.6 log units reductions in 8 h for compost amended sandy loam soil formulations 1:10, 1:25, 1:50, and 1:100, respectively (Figure 6.3b). 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 6.3c indicates *Enterococcus* decreased at 50°C by 1.4 log units, 1.8 log units, 2.4 log units and 2.5 log units in 8 h 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 6.3 summarises the inactivation rate coefficients. Arrhenius plots for *Enterococcus* inactivation for both clay soil and sandy loam soil are respectively shown in Figure 6.4 and Figure 6.5. Other factors influencing the die-off of pathogenic bacteria in the soil are soil composition and pH (Fenlon *et al*., 2000). Under field conditions, other variables, such as solar radiation and dryness, may also affect the survival of pathogens (Kelly *et al*., 1993).

Increase in temperature increased the inactivation rate of all soil types. The first order kinetics inactivation rate coefficient, *k,* of *Enterococcus* increased with temperature as summarised in (Table 6.3). Several studies reported the effect of temperature on bacterial inactivation rate (Reddy *et al*., 1981; Jiang *et al*., 2002; Gerba *et al*., 1975), indicating that, higher temperature decreased the survival time of faecal bacteria. The results showed that inactivation rate

coefficient *k*, values increased as temperature increased from 30 °C to 50 °C. The Kruskal-Wallis test 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 6.4 and Figure 6.5. 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.

Table 6.3: Kruskal-Wallis test for Enterococcus

	Temperature			Compost-to-soil ratio			
	30 °C	40 °C	50 °C	1:10	1:25	1:50	1:100
Type of soil	Mean	Mean	Mean	Mean	Mean	Mean	Mean
Clay	0.024_a	0.343_b	0.504_c	0.219_a	0.306_a	0.328_a	0.307_a
Sandy loam	0.091_a	0.388_b	0.569_c	0.293_a	0.339_a	0.362_a	0.402_a

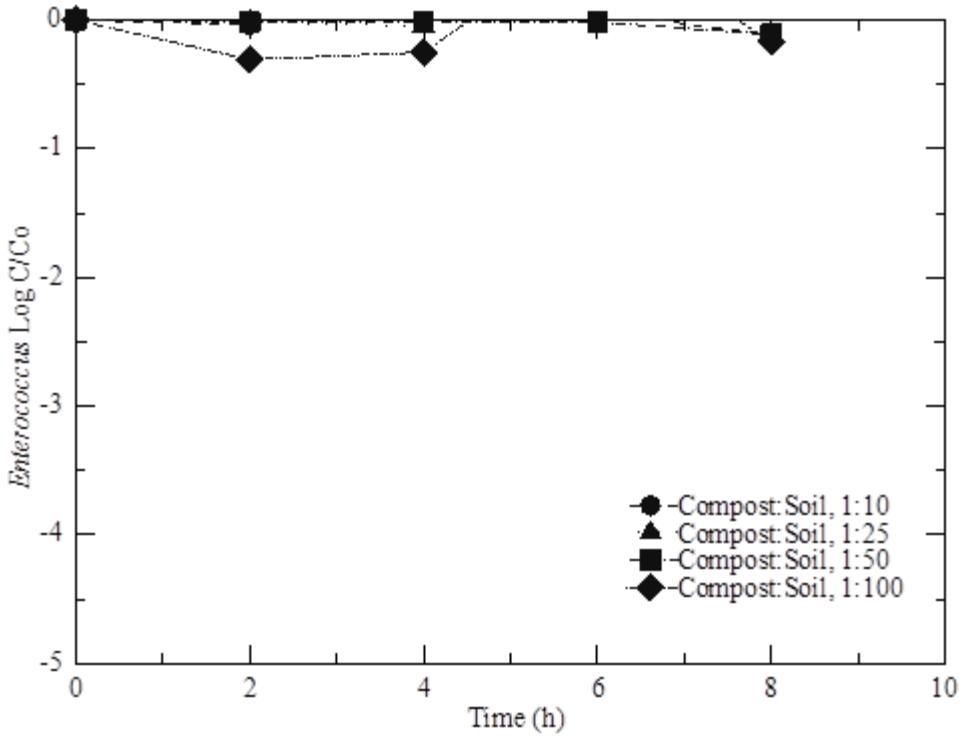

Figure 6.2a: *Inactivation of Enterococcus in compost amended clay soils at 30 °C*

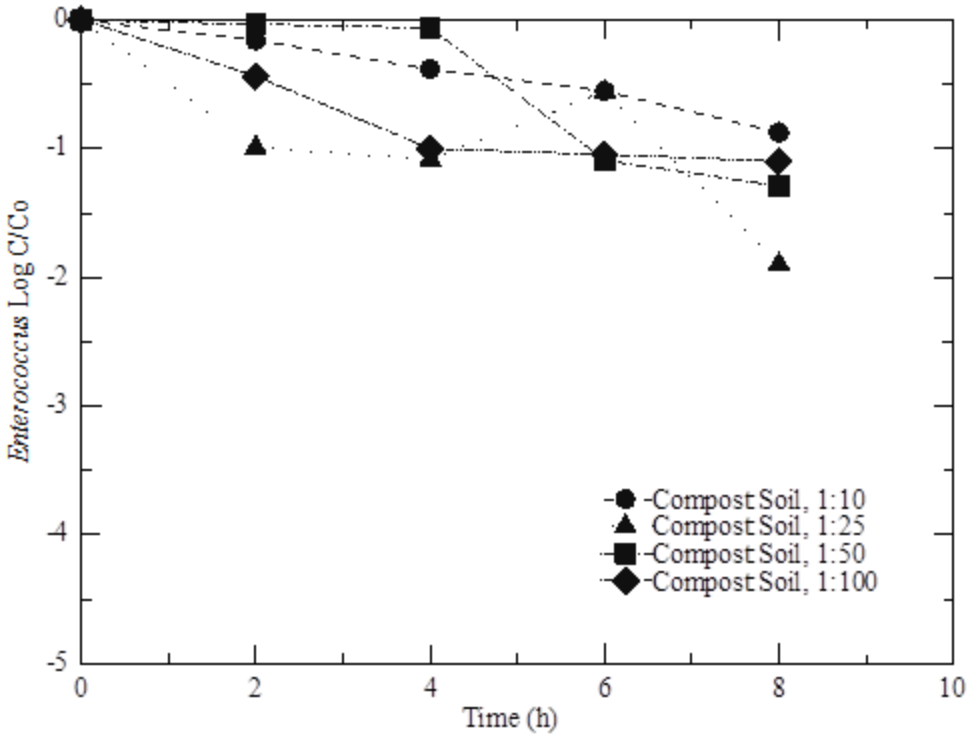

***Figure 6.2b**: Inactivation of Enterococcus in compost amended clay soils at 40 °C*

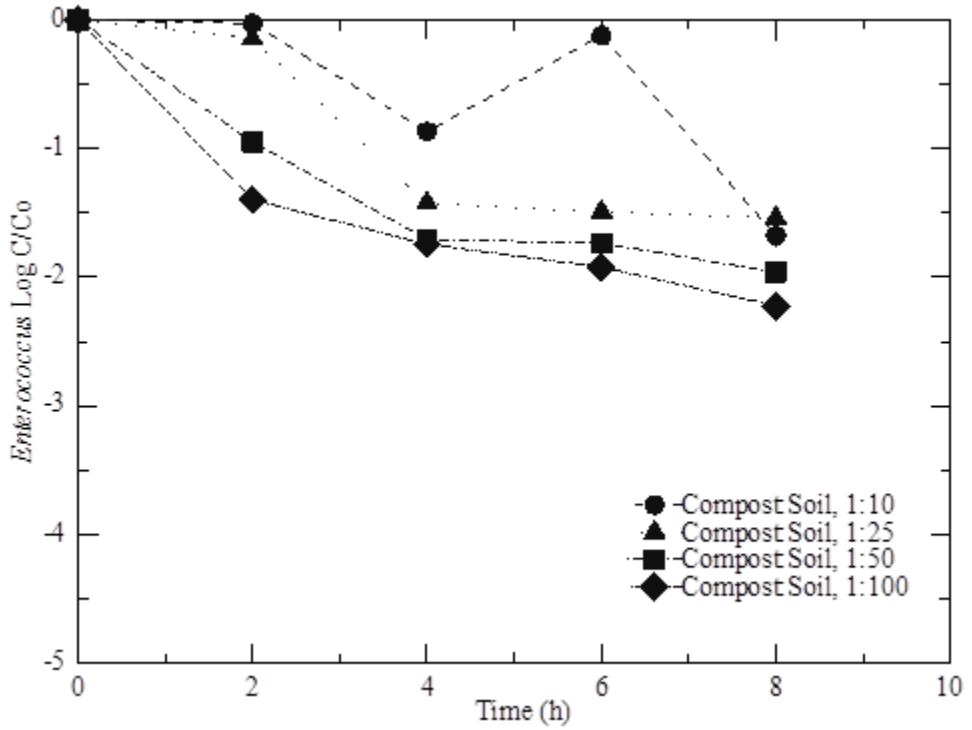

***Figure 6.2c:** Inactivation of Enterococcus in compost amended clay soils at 50°C*

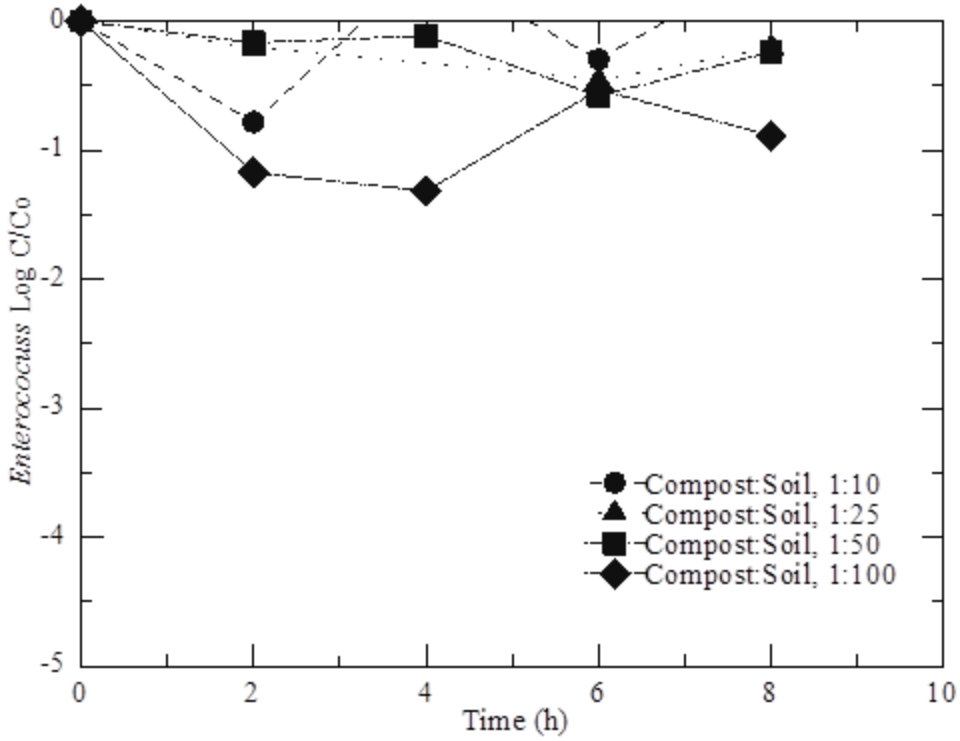

Figure 6.3a: *Inactivation of Enterococcus in compost amended sandy loam soils at 30 °C*

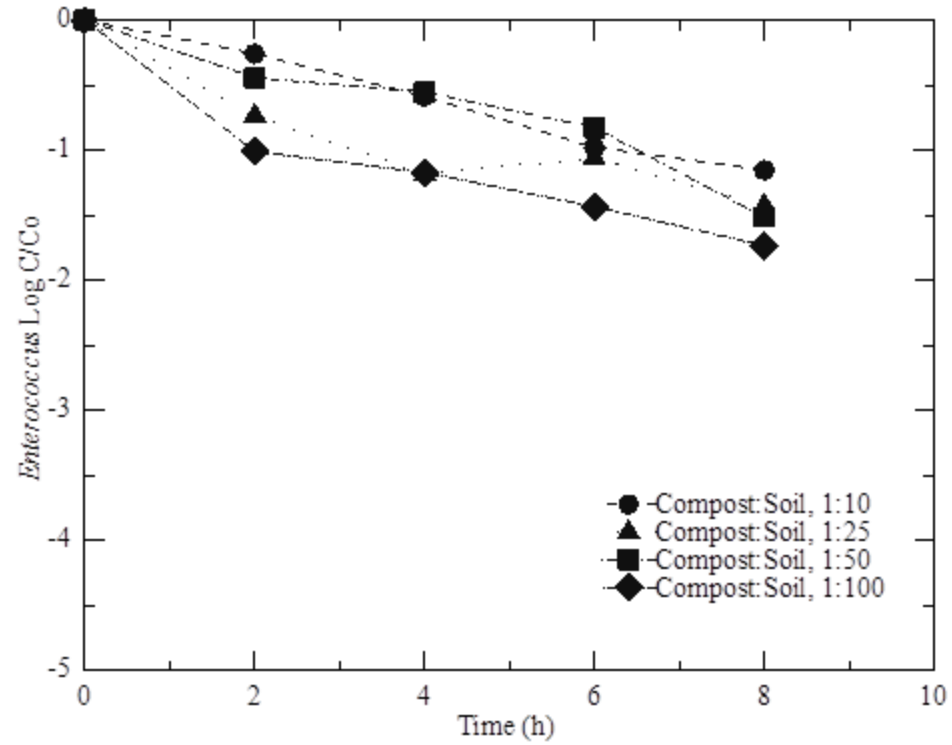

Figure 6.3b: *Inactivation of Enterococcus in compost amended sandy laom soils at 40 °C*

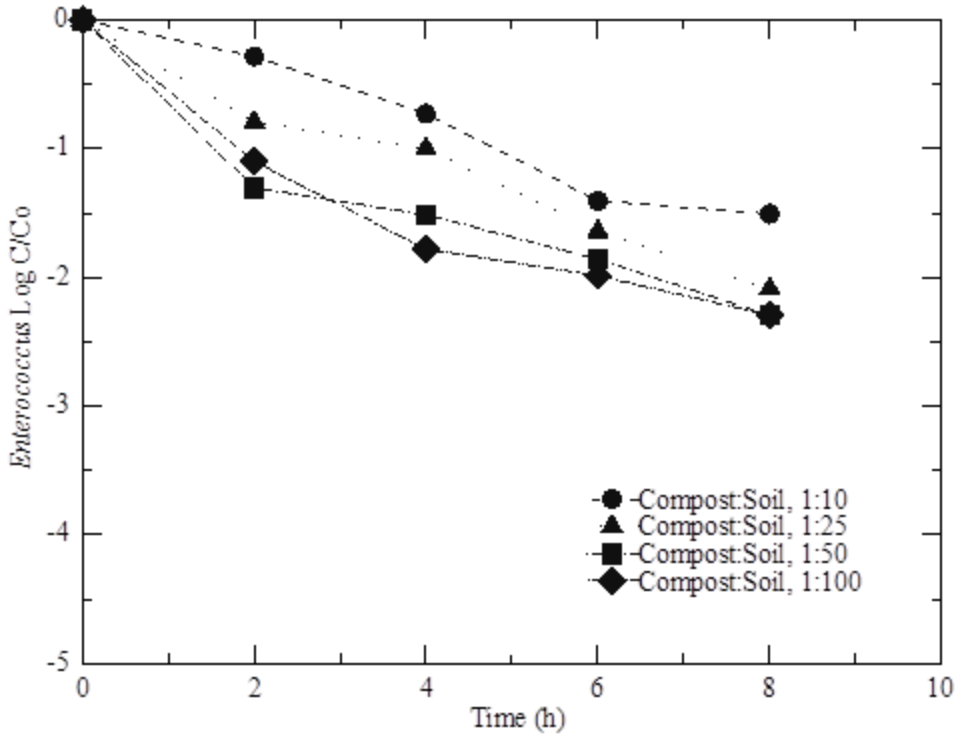

***Figure 6.3c**: Inactivation of Enterococcus in compost amended sandy loam soils at 50 °C*

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 acidic soil conditions (Reddy *et al*., 1981). In this 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 were 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 (Crane *et al*., 1983; Gerba and Bitton, 1984). 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) (Chauret, 2011). 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 less than sandy loam (Kruskal-Wallis test, $p < 0.05$; $p = 0.007$) for all temperatures. Jamieson *et al* (2002)

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 retention capacity for bacteria (Gerba *et al*., 1975). Retention of bacteria increased with an increase in clay content, cation exchanged capacity of the soil and specific surface area (Jimieson *et al*., 2002). Another study by Mubiru *et al.*[28)] (2000) 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.* (1981) reported that retention of organism is enhanced when the clay is present.

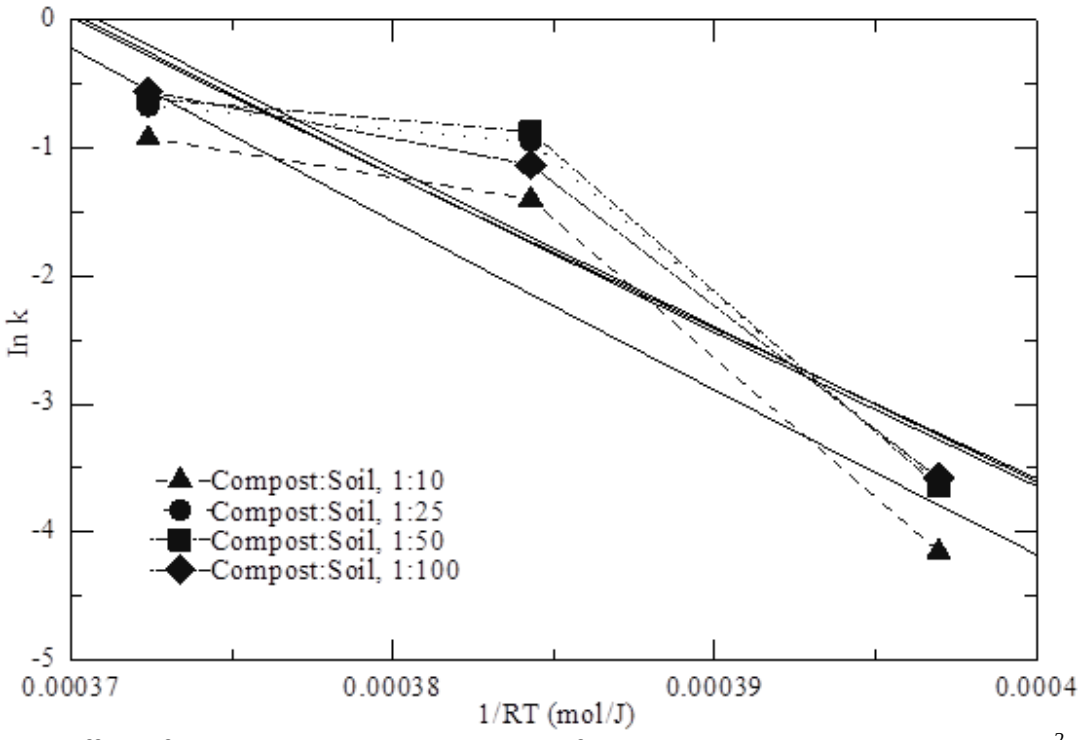

Figure 6.4: *Effect of Temperature on Enterococcus for compost amended clay soil. The* R^2 *values for the different ratios ranged from 0.80 – 0.9.*

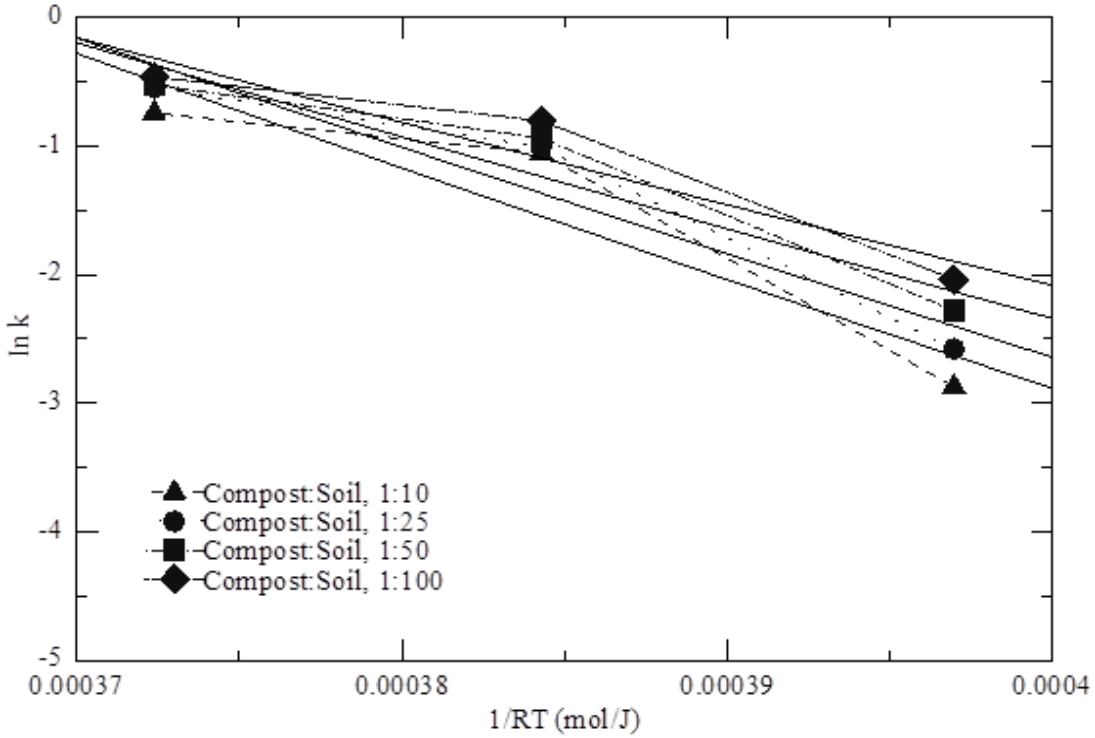

Figure 6.5: *Effect of temperature on Enterococcus for compost amended sandy loam soils. The R^2 values for the different ratios ranged from 0.86 - 0.92.*

Table 6.4 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

Compost-to-soil ratio showed a variation on the inactivation rate coefficient in this study. In the compost-amended clay soil formulations, the inactivation rate coefficient values were higher when the soil volume was increased (Table 6.4). This showed that high compost applications recorded lesser inactivation rate coefficient than low compost applications. It was found that the clay soil formulation 1:50 at 30 °C and 1:100 at 40 °C values 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.* (1981) 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, confirming the results of this study.

The temperature measured in Ouagadougou 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 (Palacios et al., 2001). Temperature and humidity changes over time due to strong sunlight under field conditions which can influence field results. Therefore, care should be taken in interpreting the laboratory 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 6.6 and Figure 6.7 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 positions were 2.8×10^{5}, 6.37×10^{6} and 4.03×10^{-15} respectively. Amending of the soil with compost was 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 bottom and middle 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.

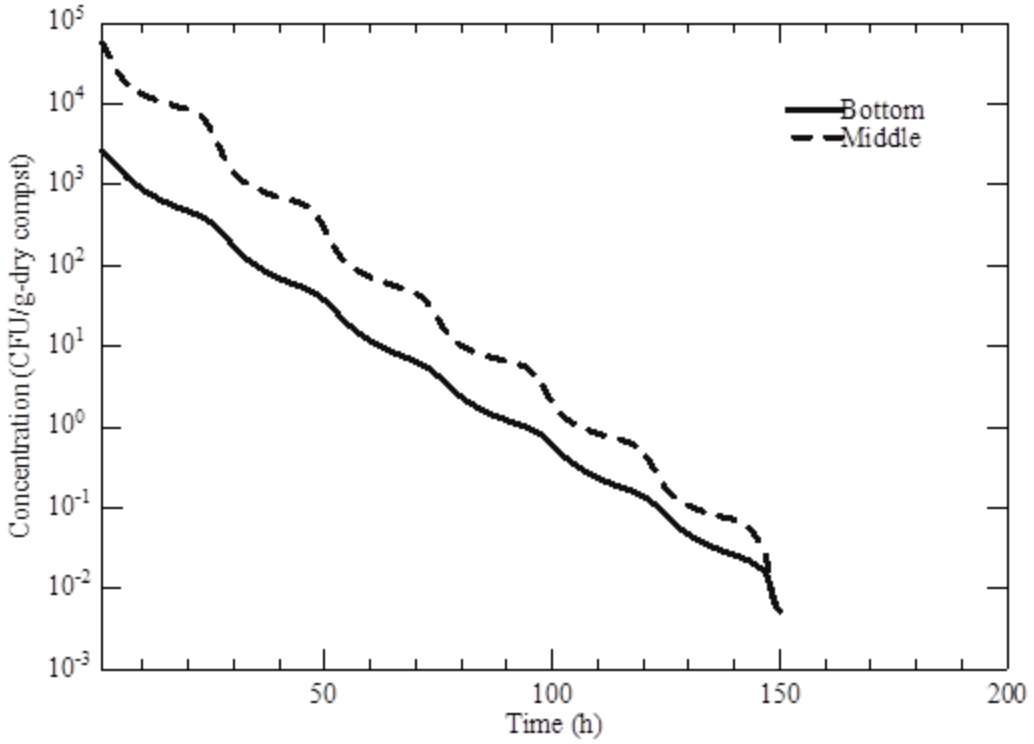

***Figure 6.6**: Change in concentration of Salmonella at different depth in the soil*

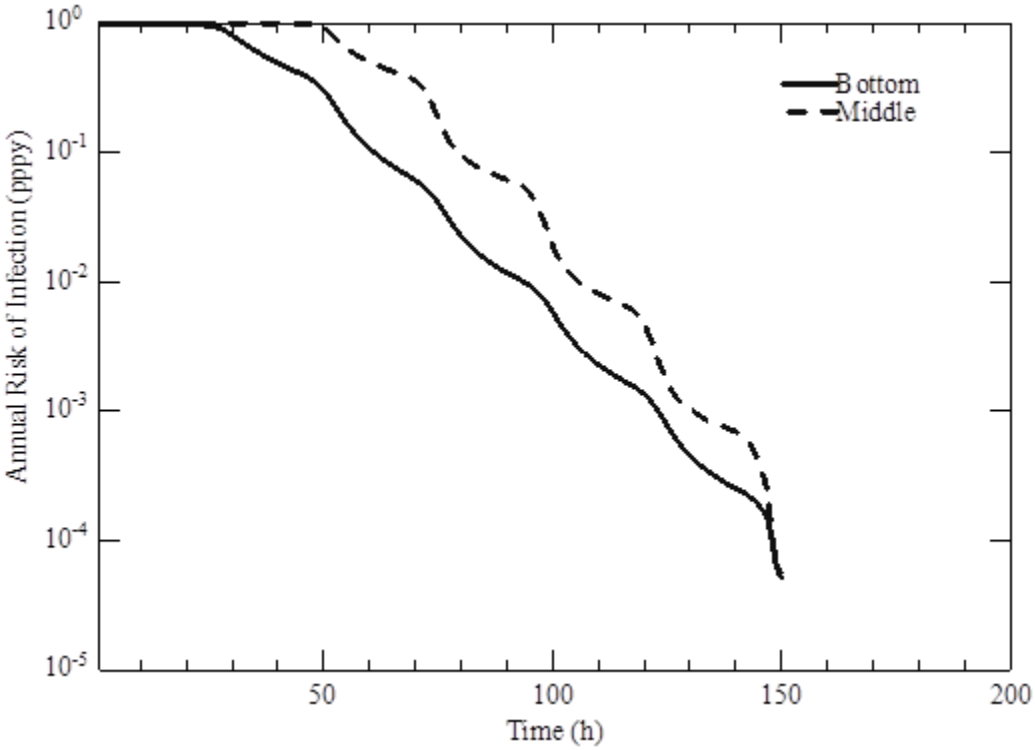

***Figure. 6.7:** Annual risk of Salmonella at different depth in the soil*

6.4 Summary

Inactivation of *Enterococcus* (pathogenic bacteria) in the soil with high temperature under different compost application rates was attempted 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, 40 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, 40 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 laboratory experimental conditions in this study are different from the real field situation because of temperature and humidity changes 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.

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Chapter 7

Conclusion

Conclusions

From this research, the thermal sanitation of compost with a post-treatment unit was investigated. For a sustainable reuse of compost by farmers for food production; the following conclusions were made:

- To successfully thermally inactivate pathogenic bacteria in compost *E.coli* requires a post-treatment condition of 50 °C, 50 % MC and a contact time over 4.5 h or post-treatment condition of 70 °C 50% MC with a contact time of over 20 mins to achieve a 6 log units reduction. For *Enterococcus*, to achieve a 6 log reduction, post-treatment condition 50 °C 50 % MC with a contact time of 15 h or 70 °C, 50 % with a contact time of over 2.5 h are required. Temperatures (>50 °C) with prolonged time will successfully inactivate bacteria. The inactivation of bacteria at 37, 50 and 70 °C with MC 50, 60 and 70 % resulted in the equations below. Inactivation rates and log removal ratio of *E.coli* and *Enterococcus* can be estimated using the following equations:
  - *E.*coli at 50%; $\ln k = -156601/RT + 52.426$
  - *E.coli* at 60%; $\ln k = -168936/RT + 62.386$
  - *E.coli* at 70%; $\ln k = -182521/RT + 66.594$
  - *Enterococcus*, at 50%; $\ln k = -114172/RT + 41.135$
  - *Enterococcus*, at 60%; $\ln k = -119070/RT + 42.483$
  - *Enterococcus*, at 70%; $\ln k = -126725/RT + 45.110$
- Inactivation rates of *A. suum* in the compost depends on temperature and moisture content. The best combination of post-treatment conditions for the efficient inactivation of Helminth eggs under solar thermal heat was 50 °C, 50 % MC. The reduction rate of helminth eggs in the compost under solar thermal sanitisation would be 0.42 - 0.92 h^{-1}. When sufficient heat is applied with low moisture about 50% and below, high efficiency of 3.60 ± 0.210 log unit can be reached in 3 hours during post-treatment. The rates of *A. suum* inactivation at 30, 40, 50 and 60 °C with MC 50, 60 and 70 % resulted in the equations below. Inactivation rates and log removal ratios of *A. suum* can be estimated using the following equations:

- at 50%; ln k = -63196/RT + 23.122
- at 60%; ln k = -62564/RT + 22.702
- at 70%; ln k = -125499/RT + 45.501

- Inactivation rate coefficients of MS2 bacteriophage depends on higher temperature but not on moisture content. Temperatures (>50 °C) with prolonged time will successfully inactivate viral pathogens. The rates of MS2 (indicator viruses) inactivation at 30 °C, 40 °C, and 50 °C with MC 50 %, 60 % and 70 % resulted in the equations below. Inactivation rates and log removal ratios of viruses can be estimated using the following equations:
  - at 50% ; ln k = -63932/RT + 23.066
  - at 60% ; ln k = -848665/RT+ 30.556
  - at 70% ; ln k = -64780/RT + 22.041

- The temperature characterised in the unit and in the soil varied. The non-uniformity might prolong the treatment time. However, the maximum temperature recorded (50 -78 °C for the unit; 61 - 69°C for compost) in Burkina Faso could be used to disinfect pathogens in the compost and in the soils.

- Results from the risk assessment for a 25 % volume of compost from a composting toilet, showed that the initial risk value was 10^0 pppy for all scenarios. This indicates that farmers are at a higher risk of pathogens at the initial stages of post-treatment. To efficiently reduce pathogens during the post-treatment and also reduce the time of treatment, the steel box needs an insulator to maintain the temperature. The guidelines for the design of the post-treatment unit are: for *Ascaris*, the steel box and the lower temperature -5 °C, -10 °C and -15 °C, post-treatment requires approximately 295 h to achieve the safe level of 10^{-4} pppy. Norovirus requires approximately 845 h, while *Salmonella* requires 969.5 h to reach a safe level.

- The longest time for the post-treatment unit (steel box) to reach a safe level was 264 h for Norovirus. From a health risk perspective, farmers should treat the compost for two weeks

before amendment onto the soils. In the case of lower temperatures more time is required to treat the compost.

- Inactivation rates of *Enterococcus* in soils amended with compost from the composting toilet depends on temperature and soil type, but not on the compost to soil ratios. The inactivation rates of *Enterococcus* in clay soils at 30, 40 and 50 °C resulted in the equations below. Inactivation rates and log removal ratios of *Enterococcus* in clay soils can be estimated using the following equations:
  - at ratio 10 ; $\ln k = -132320/RT + 48.725$
  - at ratio 25 ; $\ln k = -120515/RT + 44.599$
  - at ratio 50 ; $\ln k = -124457/RT + 46.149$
  - at ratio 100; $\ln k = -123590/RT + 45.77$

  The inactivation rates of *Enterococcus* in sandy loam soils at 30, 40 and 50 °C resulted in the equations below. Inactivation rates and log removal ratios of *Enterococcus* in the soil can be estimated with the equations below:

  - at ratio 10 ; $\ln k = -87256/RT + 31.997$
  - at ratio 25 ; $\ln k = -83088/RT + 30.574$
  - at ratio 50 ; $\ln k = -71780/RT + 26.356$
  - at ratio 100 ; $\ln k = -64430/RT + 23.675$

- The amount of water had influence on the die-off rates of some pathogens, lower moisture contents are recommended during the post-treatment. There is sufficient solar energy and hence drying-up occurs quickly. Therefore, moisture content should not be of any importance during the dry season. Handling of compost should be avoided during the rainy season. For a successful inactivation of pathogens the moisture content should not exceed 60 %.

- The health risk of the soil amended after 24 h post-treatment of the compost requires a further 147 h to reach a safe level. Compost amended to soils would be safe after one week

considering the temperature measured in Burkina Faso. Lower temperatures would require 969.5 h (40 days) to be safe.

- Protocol/mathematical models to design post-treatment unit from the perspective of microbial risk assessment were developed. Application of these models would guide farmers to treat compost to a safe level before reuse for food production.

Annexes

Annexes 1: Life cycle of Helminth eggs

The helminths are a very large and varied group of multicellular parasitic worms. Some infect humans, others animals or plants, while many may be free-living in the soil. Some produce male and female worms, others are hermaphrodites. All have differentiated organs, and their life cycles include the production of ova (eggs) or larvae as well as the complex alternation of generations which can include up to three different hosts (Moodley *et al*., 2008). There are three major groups of helminths containing members that have man as their host viz. flukes (Trematoda), tapeworms (Cestoda), and roundworms (Nematoda). Helminth infections are spread through the ingestion or inhalation of their ova, some of which can survive outside the host for long periods of time, or via larvae/cercariae penetrating skin exposed to infected soil/water. Once inside the body, helminth ova hatch and many undergo maturation in the tissues before re-entering the gut and lodging in the intestines. Here they grow and undergo sexual reproduction, resulting in the production of eggs or larvae which are passed out via the faeces to the environment (Moodley *et al*., 2008).

Helminths may damage tissues (e.g. visceral larva migrans caused by the dog and cat round worm, genus Toxocara), cause blood-loss (e.g. hookworm species) and result in more serious effects like epilepsy when man becomes an accidental intermediate host (e.g. the pork tapeworm, Taenia solium). Contamination of crops with helminths can take place through direct faecal contamination with both human and animal excreta, or through the use of contaminated sludge or faeces-based compost for agriculture (Moodley *et al*., 2008). Figure A1-1, A1-3 and A1-4 shows the life cycle *Ascaris lumbricoides,* Hookworm and Taenia solium.

Life Cycle of *Ascaris lumbricoides*

Ascaris lumbricoides is the largest of the intestinal nematodes found in man. The shell layers of the egg provide a very resistant structure which can withstand many chemicals which make them ideal parasites of the intestine. Small burdens of worms in the intestine may cause no symptoms. In heavy worm burdens the adult worms actively migrate in the intestine resulting in intestinal

blockage, vomiting and abdominal pain but infections may also be asymptomatic. The worms can penetrate through the wall of the intestine, or into the appendix, travel up the common bile duct, which may become blocked or they may then enter the gal bladder or liver. A heavy worm burden in children may lead to severe nutritional impairment and retardation in growth (Moodley *et al*., 2008).

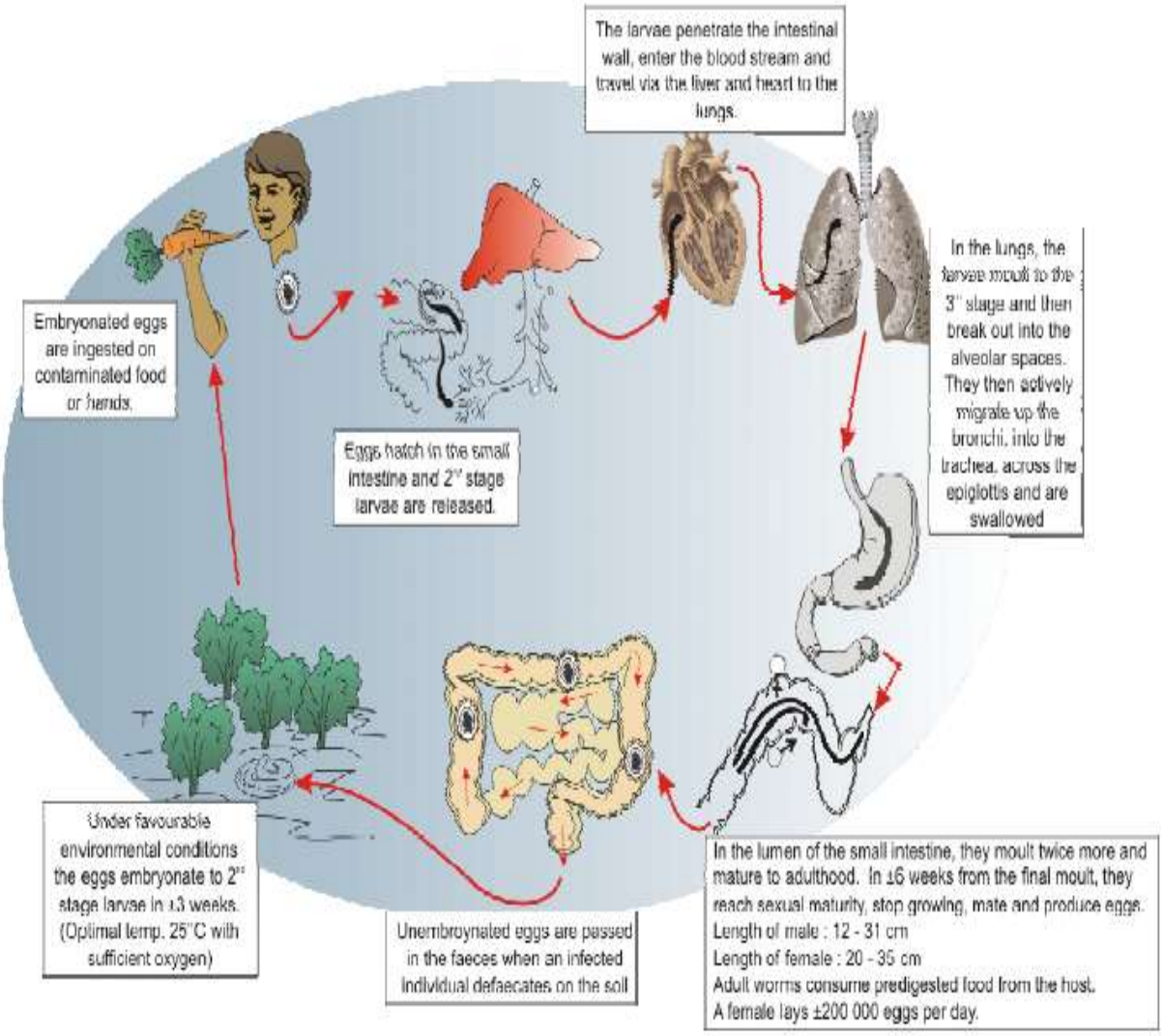

Figure A1-1*: Diagram of the life cycle of Ascaris lumbricoides, the common human nematodes infections world wide (Adapted from Moodley et al., 2008).*

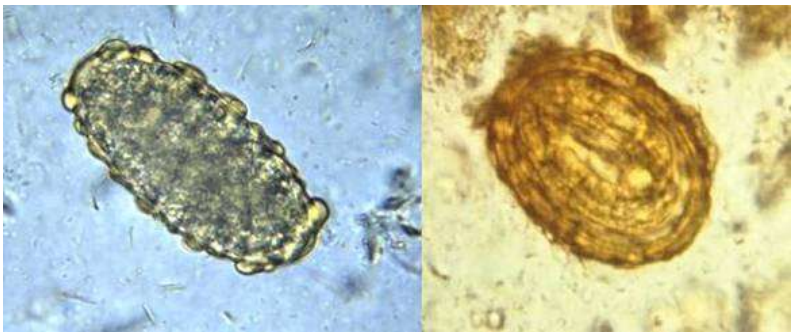

Figure A1-2*: Unfertilized (left) and fertilized (right) Ascaris lumbricoides eggs. The unfertilized eggs are longer and narrower than fertilized eggs, measuring 75-85µm by 35-50µm wide. The fertilized eggs have a thick and bumpy outer wall which is stained golden brown with bile. (Saline wet prep) (SOURCE: PHIL 411/4821-CDC/Dr. Mae Melvin)* http://www.phsource.us/PH/PARA/Chapter_5.htm

Life Cycle of Hookworm Species

Hookworms infective to man comprise of two species, *Necator americanus* and *Ancylostoma duodenale*. They are classed as one of the most destructive of human parasitic helminths. There is no intermediate host, with man being the only definitive host. It is estimated that there are some 900 million cases of infection world wide (Crompton, 1989). The infection is serious where the worms insidiously undermine the health of their hosts.

The adult worms live in the small intestine, attached firmly to the mucous membrane of the gut lining, and feed on blood and tissue. The adult females deposit their eggs whilst in the gut (they can produce up to 20,000 eggs per day), the eggs are then passed out in the feces. The rhabditiform larvae hatch in warm, damp soil (light sandy loam), feeding on bacteria. After about one week during which they have gone through two molts become infective and climb into a suitable position waiting for a suitable host to pass by. The larvae enter the host by penetrating unbroken skin (it is now recognized that *A. duodenale* can successfully enter man by oral ingestion, this may be more important for this species than skin penetration). The larvae then enter blood vessels and are carried to the heart, lungs and trachea. They are then swallowed and

develop into adult worms in the small intestine. Larvae that are initially swallowed may not show this migration (Berger, 2015).

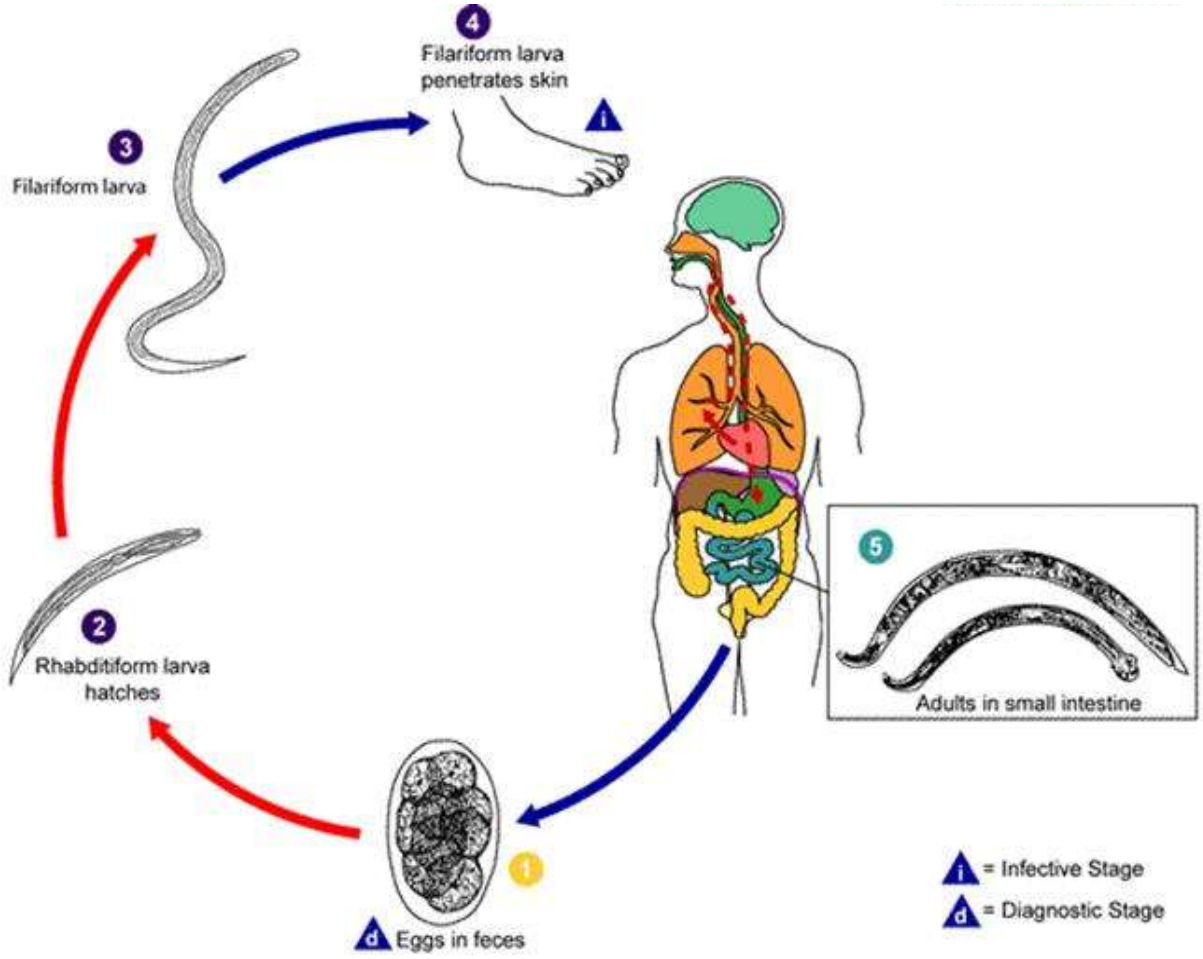

Figure A1-3 : *Eggs are passed in the stool* 1*, and under favorable conditions (moisture, warmth, shade), larvae hatch in 1 to 2 days. The released rhabditiform larvae grow in the feces and/or the soil* 2*, and after 5 to 10 days (and two molts) they become become filariform (third-stage) larvae that are infective* 3*. These infective larvae can survive 3 to 4 weeks in favorable environmental conditions. On contact with the human host, the larvae penetrate the skin and are carried through the veins to the heart and then to the lungs. They penetrate into the pulmonary alveoli, ascend the bronchial tree to the pharynx, and are swallowed* 4*. The larvae reach the small intestine, where they reside and mature into adults. Adult worms live in the lumen of the small intestine, where they*

attach to the intestinal wall with resultant blood loss by the host [5]*. Most adult worms are eliminated in 1 to 2 years, but longevity records can reach several years. Some A. duodenale larvae, following penetration of the host skin, can become dormant (in the intestine or muscle). In addition, infection by A. duodenale may probably also occur by the oral and transmammary route. N. americanus, however, requires a transpulmonary migration phase. (SOURCE: CDC)* (Berger, 2015).

Available at: http://www.phsource.us/PH/PARA/Chapter_5.htm

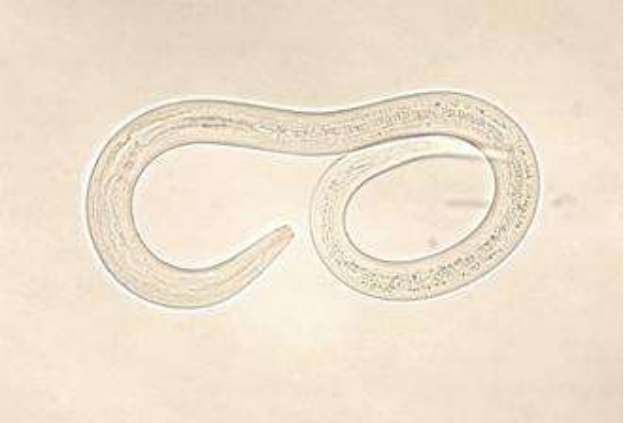

Figure A1-4: *Hookworm larva. Both Ancylostoma duodenale and Necatoramericanus larvae have similar general morphology and measuring approximately 10-13µm for females and 8-11µm for males. (SOURCE: PHIL 1513–CDC/Dr. Mae Melvin) Available at: http://www.phsource.us/PH/PARA/Chapter_5.htm*

Life cycle of Trichuris trichiura

Trichuris trichiura, more commonly known as the Whip Worm, due to the whip-like form of the body. Their Life cycle eggs require a warm, moist environment with plenty of oxygen to ensure embryonation, but once they have embryonated they are extremely resistant to environmental conditions (Berger, 2015).

Adult worms are found in the cecum and upper part of the colon of man. In heavy infection they can be found in the colon and the terminal ileum. They attach to the mucosa by the anterior end

or by embedding the anterior portion of the body in the superficial tissues, obtaining nutrition from the host tissues.

Once fertilized the female worms lay several thousands of eggs, which are unsegmented at the oviposition and are passed out in the feces. Once they have been passed out they require an embryonation period in the soil which may last from two weeks to several months, after which they become infective (Berger, 2015).

When embryonated eggs are swallowed by human hosts larvae are released into the upper duodenum. They then attach themselves to the villi lower down the small intestine or invade the intestinal walls. After a few days the juveniles migrate slowly down towards the cecum attaching themselves to the mucosa, reaching their final attachment site simultaneously (Berger, 2015).

The larvae reach maturity within three weeks to a month after infection, during which they undergo four molts. There is no lung migration and the time from ingestion of infective eggs to the development of adult worms is about three months (Berger, 2015).

Infection is achieved by swallowing soil that contains embryonated eggs. Therefore, children are most commonly seen to possess the infections, as they are more likely to swallow soil (Berger, 2015).

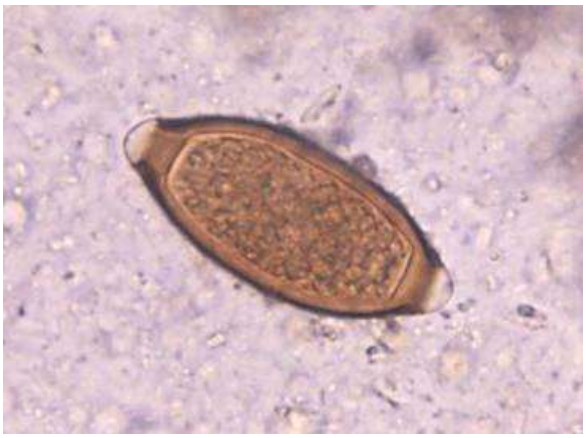

Figure A1-5: *Trichuris trichuria ovum with its opercular plugs, shown as white gaps at either end of the egg (Saline wet prep) (SOURCE: PHIL 652 – CDC/Dr. Mae Melvin).*

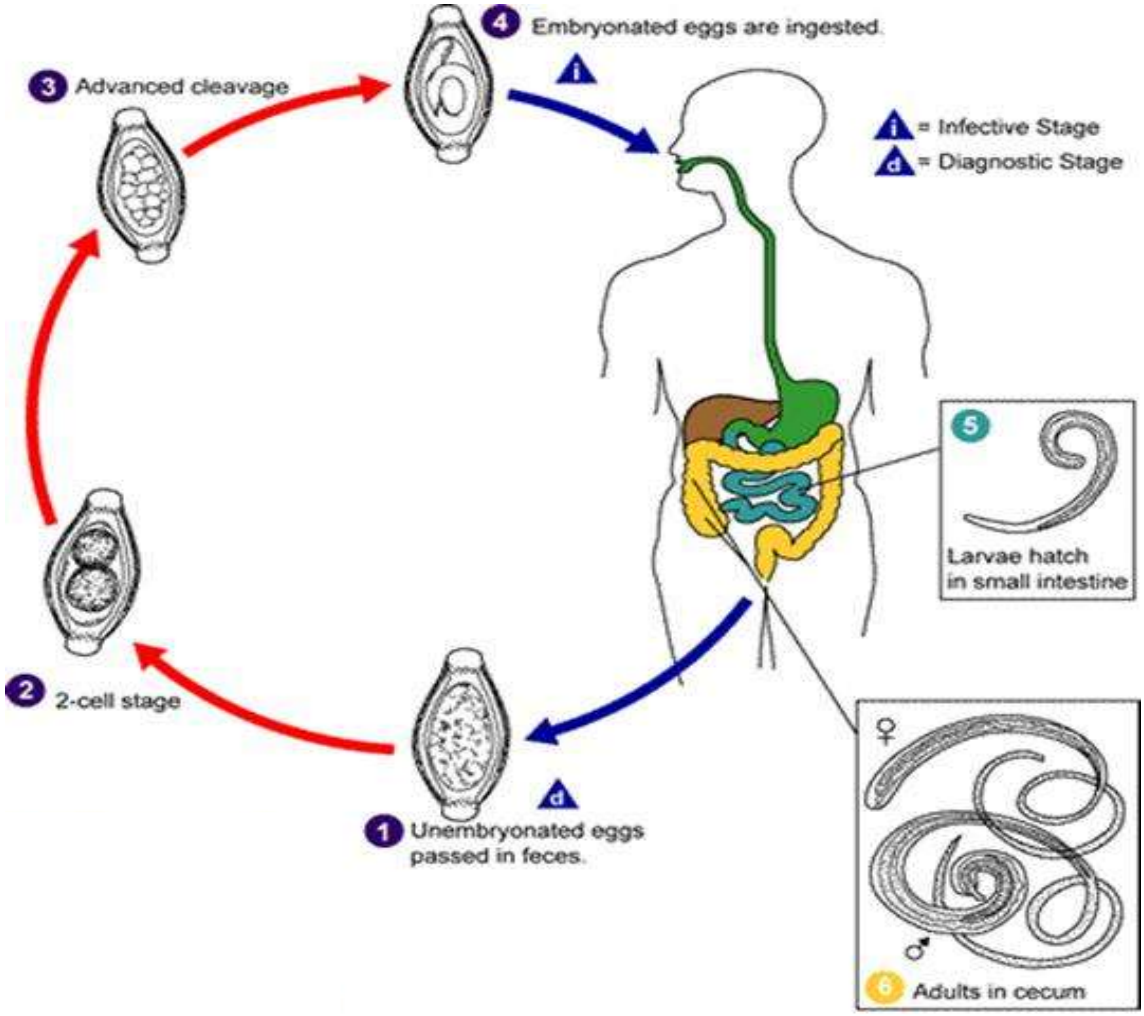

Figure A1-6: *The unembryonated eggs are passed with the stool* ❶*. In the soil, the eggs develop into a 2-cell stage* ❷*, an advanced cleavage stage* ❸*, and then they embryonate* ❹*; eggs become infective in 15 to 30 days. After ingestion (soil-contaminated hands or food), the eggs hatch in the small intestine, and release larvae* ❺*that mature and establish themselves as adults in the colon* ❻*. The adult worms (approximately 4 cm in length) live in the cecum and ascending colon. The adult worms are fixed in that location, with the anterior portions threaded into the mucosa. The females begin to oviposit 60 to 70 days after infection. Female worms in the cecum shed between 3,000 and 20,000 eggs per day. The life span of the adults is about 1 year. (SOURCE: CDC)* (Berger, 2015).

Life Cycle of *Taenia* Species

Taenia species are the most common cestode parasites of humans. More than 60 million people are infected with *T. saginata* (beef tapeworm) world wide and about four million are infected with *T. solium* (pork tapeworm). The life cycle of a *Taenia* species can be seen in Figure 7. *T. saginata* has a cosmopolitan distribution, but is more common in developing countries where hygiene is poor and the inhabitants have a tendency of eating raw or insufficiently cooked meat. *T saginata* is the most common adult tapeworm found in man (Berger, 2015).

Both humans and cattle or pigs are necessary to the complete life cycle of *Taenia* species. Cattle are the normal intermediate hosts, but in the tropics several other ruminants, e.g. goat, sheep may serve as the intermediate hosts.) Eggs ingested by the intermediate hosts usually contain oncospheres. The oncospheres then hatch out in the duodenum, pass into the intestine where they penetrate the intestinal wall and are then carried by the circulation to be deposited in tissues (usually muscle). There they develop into cysticerci larva which are white and ovoid, measuring approximately 8 x 5µm (Berger, 2015).

Humans become infected by ingesting inadequately, cooked beef or pork with cysticerci, containing an invaginated protoscolex. The protoscolexes evaginate and pass into the small intestine where they attach themselves to the mucosa and develop into adult worms (Berger, 2015).

Eggs and proglottids are passed out in the feces, and are then eaten by the intermediate host, thus, perpetuating the life cycle (Berger, 2015).

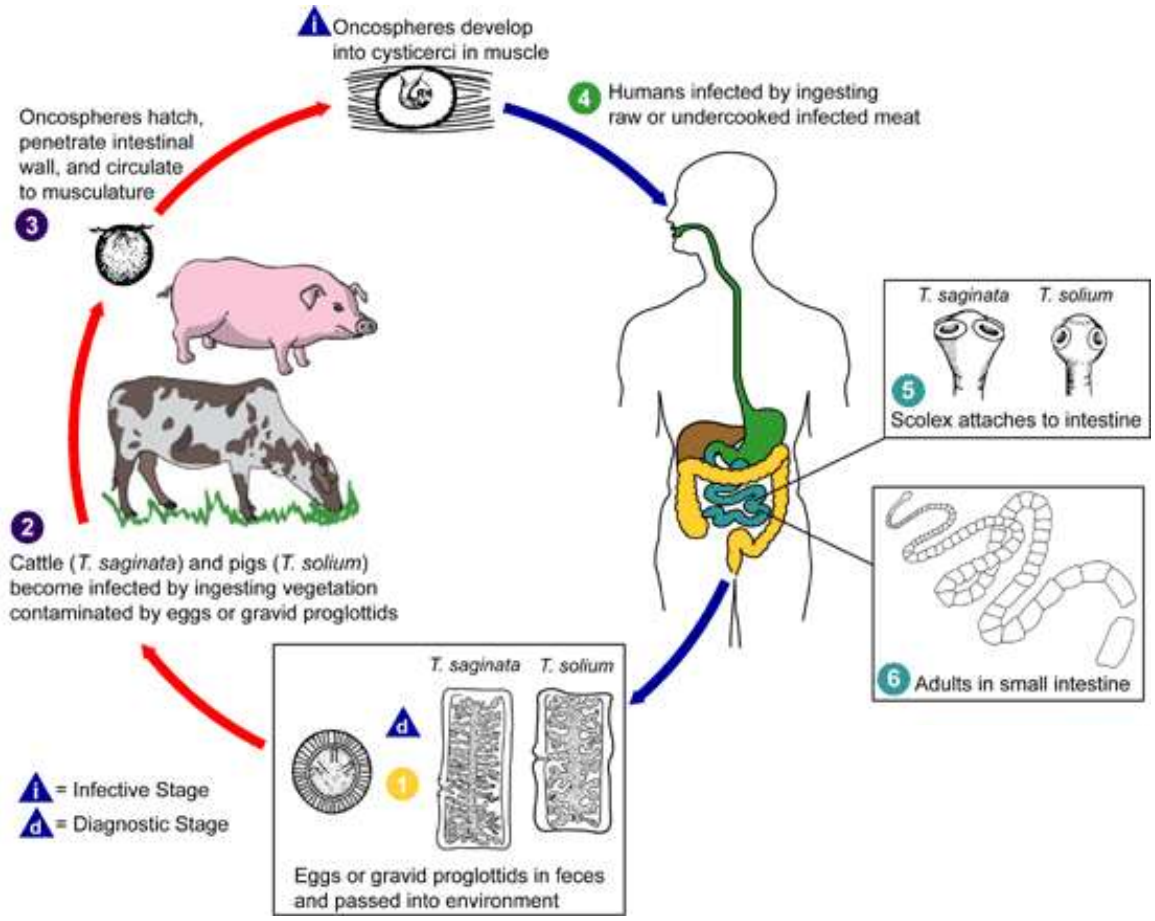

Figure A1-7*: Taeniasis is the infection of humans with the adult tapeworm of Taenia saginata or Taenia solium. Humans are the only definitive hosts for T. saginata and T. solium. Eggs or gravid proglottids are passed with feces* 1*; the eggs can survive for days to months in the environment. Cattle (T. saginata) and pigs (T. solium) become infected by ingesting vegetation contaminated with eggs or gravid proglottids* 2*. In the animal's intestine, the oncospheres hatch* 3*, invade the intestinal wall, and migrate to the striated muscles, where they develop into cysticerci. A cysticercus can survive for several years in the animal. Humans become infected by ingesting raw or undercooked infected meat* 4*. In the human intestine, the cysticercus develops over 2 months into an adult tapeworm, which can survive for years. The adult tapeworms attach to the small intestine by their scolex* 5 *and reside in the small intestine* 6*. Length of adult worms is usually 5 m or less for T. saginata (however it may reach up to 25 m) and 2 to 7 m for T. solium. The adults produce proglottids which mature, become*

gravid, detach from the tapeworm, and migrate to the anus or are passed in the stool (approximately 6 per day). T. saginata adults usually have 1,000 to 2,000 proglottids, while T. soliumadults have an average of 1,000 proglottids. The eggs contained in the gravid proglottids are released after the proglottids are passed with the feces. T. saginata may produce up to 100,000 and T. soliummay produce 50,000 eggs per proglottid respectively (Berger, 2015).

Annex 2: Composting Matrix Available in Burkina Faso and West Africa

There are several materials in Burkina Faso that can be used as an artificial soil composting matrix for the composting toilet. Some of these are rice husk, millet husk and sawdust. Sawdust is mostly used for composting toilets in Japan. Find below some of the pictures of composting matrixes

Figure A 2-1: Rice husk*; Source: https://www.google.com/search*

Figure A 2-2: Shea nut husk; *Source: https://www.google.com/search*

Figure A 2-3: Sawdust*; Source : H. Darimani*

Annex 3: Commercial Composting toilets

This type of composting toilet does not have urine diversion. All waste is directly put into the reactor. It uses electricity, where the required composting temperature is attained for bio-degradation of faeces. The moisture is controlled and the desired final pathogen removal achieved.

Figure A 3-1a: Commercial composting toilet available in Japan (Saiwa Denko).
Source : H. Darimani

Figure A3-1b: Composting reactor of the commercial composting toilet (Saiwa Denko).
Source : *H. Darimani*

Figure A3-2: Commercial quantities of composting toilet produced for sale in Japan (Saiwa Denko). *Source***:** *H. Darimani*

Annex 4a : Example of the calculation of concentrations for the estimation of health risk.

Concentrations were calculated using the temperature data collected in the soil and compost in Burkina Faso. Once the temperature was known, the following steps were used for the estimation:

1. $1/RT$ was estimated, where R is the universal gas constant (J/Mol/K) and T, the temperature.
2. The equation of the line determined (Chapter 7) from the inactivation experiments were used to re-calculate the inactivation rate coefficient at each temperature point measured in Ouagadougou, Burkina Faso.
3. Finally, the decline in concentrations from a worse case scenario estimated.
4. These concentrations are used for the assessment of the health risk and the length of time required to reach a safe period.

All 4 scenarios followed the above steps for the estimation of concentrations.

Annex 4b: Example of the calculation of concentrations (*Ascaris* eggs)

Elapse Time (h)	Real Time (h)	Temp. °C	Temp. °C (-5)	1/*RT* (Temp. 5)	In *k* (-5)	*k* (1/h) (-5)	Conc/copy per g (-5)
0.0	0:00						
0.5	0:30	27.7	22.7	0.000406796	-2.585860086	0.07533126	335.9989248
1.0	1:00	26.5	21.5	0.000408406	-2.687650357	0.068040623	323.5786899
1.5	1:30	23.1	18.1	0.000413198	-2.990441837	0.050265223	312.7555886
2.0	2:00	22.1	17.1	0.000414621	-3.08041235	0.045940309	304.9931773
2.5	2:30	21.0	16.0	0.000416175	-3.178582666	0.041644638	298.0672858
3.0	3:00	20.2	15.2	0.000417378	-3.254600304	0.038596244	291.9250039
3.5	3:30	19.4	14.4	0.000418466	-3.323392875	0.036030378	286.3454106
4.0	4:00	18.8	13.8	0.000419317	-3.377146795	0.034144738	281.2330324
4.5	4:30	18.3	13.3	0.000420049	-3.423395815	0.032601538	276.472471
5.0	5:00	17.8	12.8	0.000420906	-3.47755746	0.030882751	272.0022898
5.5	5:30	17.3	12.3	0.00042152	-3.516379862	0.029706783	267.8344616
6.0	6:00	17.0	12.0	0.000422013	-3.547519473	0.02879598	263.8856109
6.5	6:30	16.5	11.5	0.000422754	-3.594365634	0.027478109	260.1134097
7.0	7:00	16.1	11.1	0.000423374	-3.633530003	0.026422747	256.5641351
7.5	7:30	15.6	10.6	0.00042412	-3.68067908	0.025205852	253.1968626

8.0	8:00	15.2	10.2	0.000424744	-3.720097104	0.024231615	250.0258651
8.5	8:30	14.9	9.9	0.000425119	-3.743803619	0.023663923	247.014877
9.0	9:00	14.5	9.5	0.000425745	-3.783407669	0.022745052	244.1094289
9.5	9:30	14.0	9.0	0.0004265	-3.831086932	0.021686032	241.3490144
10.0	10:00	14.0	9.0	0.0004265	-3.831086932	0.021686032	238.7461999
10.5	10:30	13.7	8.7	0.000427004	-3.862967098	0.021005581	236.1714553
11.0	11:00	13.4	8.4	0.000427383	-3.886926759	0.020508276	233.7039763
11.5	11:30	13.0	8.0	0.000428017	-3.926954216	0.019703594	231.3197882
12.0	12:00	12.9	7.9	0.000428144	-3.934973948	0.019546209	229.0520616
12.5	12:30	12.5	7.5	0.000428779	-3.97514405	0.018776597	226.8244151
13.0	13:00	12.5	7.5	0.000428779	-3.97514405	0.018776597	224.7048848
13.5	13:30	13.3	8.3	0.000427637	-3.902923517	0.02018282	222.6051602
14.0	14:00	15.8	10.8	0.000423746	-3.657083788	0.025807664	220.3700569
14.5	14:30	20.8	15.8	0.000416415	-3.19375112	0.04101772	217.5447068
15.0	15:00	26.5	21.5	0.000408406	-2.687650357	0.068040623	213.1285529
15.5	15:30	33.5	28.5	0.000398929	-2.088719303	0.123845643	205.9998018
16.0	16:00	39.7	34.7	0.000390937	-1.583658599	0.205222895	193.630632
16.5	16:30	43.3	38.3	0.000386438	-1.299366338	0.272704541	174.7473013
17.0	17:00	46.4	41.4	0.000382548	-1.053522341	0.348707317	152.4731733

17.5	17:30	48.2	43.2	0.000380432	-0.919772942	0.398609538	128.0774308
18.0	18:00	48.6	43.6	0.000379931	-0.888145689	0.411417943	104.9338592
18.5	18:30	48.5	43.5	0.000380031	-0.89446448	0.408826475	85.42350253
19.0	19:00	49.4	44.4	0.000378934	-0.825140163	0.438173572	69.63087191
19.5	19:30	48.3	43.3	0.000380332	-0.913440827	0.401141588	55.9311374
20.0	20:00	47.3	42.3	0.000381437	-0.983278074	0.374082816	45.7664116
20.5	20:30	46.9	41.9	0.000381941	-1.015156616	0.362345676	37.9590916
21.0	21:00	46.8	41.8	0.000382042	-1.021542445	0.360039171	31.66893414
21.5	21:30	46.9	41.9	0.000381941	-1.015156616	0.362345676	26.45159924
22.0	22:00	46.2	41.2	0.000382853	-1.072750976	0.342066206	22.06833512
22.5	22:30	46.3	41.3	0.00038265	-1.059928489	0.346480587	18.59905324
23.0	23:00	44.6	39.6	0.000384791	-1.195246252	0.302629422	15.6406045
23.5	23:30	43.3	38.3	0.000386335	-1.292832718	0.274492122	13.44430604
24.0	0:00	39.1	34.1	0.000391679	-1.630566381	0.195818635	11.72015035
24.5	0:30	33.4	28.4	0.000399039	-2.095685899	0.122985859	10.62702511
25.0	1:00	29.3	24.3	0.000404517	-2.441830392	0.087001459	9.993225027
25.5	1:30	26.3	21.3	0.000408638	-2.702257664	0.067053956	9.567831983
26.0	2:00	24.2	19.2	0.000411666	-2.893668434	0.055372708	9.252369293
26.5	2:30	22.6	17.6	0.000413908	-3.035349729	0.048057853	8.999718547

27.0	3:00	21.6	16.6	0.000415337	-3.125630503	0.04390924	8.786042457
27.5	3:30	20.8	15.8	0.000416535	-3.20134191	0.040707541	8.595250282
28.0	4:00	20.1	15.1	0.000417498	-3.262226244	0.038303031	8.422072913
28.5	4:30	19.6	14.6	0.000418224	-3.308074645	0.036586548	8.262312166
29.0	5:00	18.8	13.8	0.000419439	-3.38484377	0.033882935	8.1125415
29.5	5:30	18.3	13.3	0.000420171	-3.431119685	0.032350698	7.976260797
30.0	6:00	17.6	12.6	0.000421151	-3.493072834	0.030407292	7.848279854
30.5	6:30	16.8	11.8	0.000422383	-3.570922	0.028129906	7.729859874
31.0	7:00	16.2	11.2	0.00042325	-3.625687946	0.026630771	7.621900758
31.5	7:30	15.8	10.8	0.000423871	-3.664944268	0.025605598	7.521084904
32.0	8:00	15.3	10.3	0.000424494	-3.704315989	0.02461705	7.425407739
32.5	8:30	14.9	9.9	0.000425119	-3.743803619	0.023663923	7.334572096
33.0	9:00	14.6	9.6	0.00042562	-3.775477521	0.02292614	7.248301105
33.5	9:30	14.0	9.0	0.0004265	-3.831086932	0.021686032	7.165687727
34.0	10:00	13.5	8.5	0.000427257	-3.878935479	0.02067282	7.088409781
34.5	10:30	13.0	8.0	0.000428017	-3.926954216	0.019703594	7.015518438
35.0	11:00	12.8	7.8	0.000428398	-3.951027689	0.019234924	6.946742314
35.5	11:30	12.5	7.5	0.000428779	-3.97514405	0.018776597	6.880252528
36.0	12:00	12.0	7.0	0.000429545	-4.023505899	0.017890134	6.815960931

36.5	12:30	12.0	7.0	0.000429545	-4.023505899	0.017890134	6.75526358
37.0	13:00	11.8	6.8	0.000429928	-4.047751617	0.017461591	6.69510675
37.5	13:30	12.6	7.6	0.000428652	-3.967100491	0.018928236	6.636907575
38.0	14:00	15.6	10.6	0.00042412	-3.68067908	0.025205852	6.574391394
38.5	14:30	20.9	15.9	0.000416295	-3.186164706	0.04133008	6.492054757
39.0	15:00	27.0	22.0	0.000407715	-2.643927419	0.071081554	6.359272881
39.5	15:30	33.8	28.8	0.000398489	-2.060891366	0.127340412	6.137228569
40.0	16:00	38.5	33.5	0.000392424	-1.677652626	0.186811979	5.758649935
40.5	16:30	42.4	37.4	0.000387475	-1.364895388	0.255407393	5.245114496
41.0	17:00	45.0	40.0	0.000384279	-1.162890556	0.31258134	4.616299336
41.5	17:30	47.4	42.4	0.000381336	-0.976912468	0.376471675	3.948369484
42.0	18:00	48.0	43.0	0.000380632	-0.932447186	0.393589345	3.270905531
42.5	18:30	48.4	43.4	0.000380131	-0.900786599	0.406249978	2.686588559
43.0	19:00	47.8	42.8	0.000380833	-0.9451348	0.388627181	2.1927297
43.5	19:30	47.8	42.8	0.000380833	-0.9451348	0.388627181	1.805492875
44.0	20:00	47.5	42.5	0.000381235	-0.970550223	0.378874515	1.486642208
44.5	20:30	47.3	42.3	0.000381437	-0.983278074	0.374082816	1.230084379
45.0	21:00	47.3	42.3	0.000381538	-0.989647044	0.371707864	1.020243536
45.5	21:30	47.3	42.3	0.000381437	-0.983278074	0.374082816	0.847205009

46.0	22:00	47.3	42.3	0.000381538	-0.989647044	0.371707864	0.702679791
46.5	22:30	45.0	40.0	0.000384279	-1.162890556	0.31258134	0.583501701
47.0	23:00	43.5	38.5	0.000386128	-1.279775959	0.278099599	0.499075156
47.5	23:30	40.6	35.6	0.000389776	-1.510304506	0.22084272	0.434287561
48.0	0:00	35.7	30.7	0.000396084	-1.908929215	0.148239034	0.388885726
48.5	0:30	31.5	26.5	0.000401592	-2.256987077	0.104665359	0.361104005
49.0	1:00	28.3	23.3	0.000405995	-2.535265444	0.079240682	0.342692431
49.5	1:30	26.1	21.1	0.000408985	-2.724199652	0.065598683	0.329380297
50.0	2:00	24.6	19.6	0.00041108	-2.856638617	0.057461586	0.318752092
50.5	2:30	23.6	18.6	0.00041249	-2.945687881	0.052565888	0.309724399
51.0	3:00	22.8	17.8	0.000413671	-3.020363279	0.048783493	0.301689976
51.5	3:30	22.0	17.0	0.00041474	-3.087937886	0.045595881	0.294420252
52.0	4:00	21.3	16.3	0.000415696	-3.148298156	0.042925117	0.28778401
52.5	4:30	20.7	15.7	0.000416655	-3.20893708	0.040399532	0.28167324
53.0	5:00	20.5	15.5	0.000416896	-3.224140578	0.039789963	0.276040587
53.5	5:30	20.5	15.5	0.000416896	-3.224140578	0.039789963	0.270603034
54.0	6:00	20.5	15.5	0.000416896	-3.224140578	0.039789963	0.265272592
54.5	6:30	19.8	14.8	0.000417982	-3.29277415	0.037150645	0.260047151
55.0	7:00	19.2	14.2	0.00041883	-3.346403542	0.035210761	0.255261278

55.5	7:30	18.3	13.3	0.000420049	-3.423395815	0.032601538	0.250806634
56.0	8:00	17.4	12.4	0.000421397	-3.508606319	0.02993861	0.246751435
56.5	8:30	16.7	11.7	0.000422507	-3.578731972	0.027911068	0.243085246
57.0	9:00	16.2	11.2	0.00042325	-3.625687946	0.026630771	0.239716423
57.5	9:30	16.5	11.5	0.000422754	-3.594365634	0.027478109	0.236545663
58.0	10:00	16.5	11.5	0.000422754	-3.594365634	0.027478109	0.233317973
58.5	10:30	17.2	12.2	0.000421766	-3.531940573	0.029248103	0.230134325
59.0	11:00	17.9	12.9	0.00042066	-3.462060165	0.031365078	0.226793318
59.5	11:30	18.4	13.4	0.000419927	-3.415676437	0.032854175	0.223264366
60.0	12:00	18.0	13.0	0.000420538	-3.454318287	0.031608845	0.219626743
60.5	12:30	17.6	12.6	0.000421151	-3.493072834	0.030407292	0.216182954
61.0	13:00	17.3	12.3	0.00042152	-3.516379862	0.029706783	0.212921044
61.5	13:30	18.1	13.1	0.000420416	-3.446580917	0.031854363	0.209781816
62.0	14:00	20.2	15.2	0.000417378	-3.254600304	0.038596244	0.206467051
62.5	14:30	22.8	17.8	0.000413671	-3.020363279	0.048783493	0.202520824
63.0	15:00	26.1	21.1	0.000408985	-2.724199652	0.065598683	0.197640746
63.5	15:30	29.8	24.8	0.000403951	-2.406074783	0.090168532	0.191263418
64.0	16:00	33.4	28.4	0.000399039	-2.095685899	0.122985859	0.182831939
64.5	16:30	37.1	32.1	0.000394246	-1.792754213	0.166500958	0.171927768

65.0	17:00	40.3	35.3	0.000390198	-1.536928268	0.215040635	0.158194289
65.5	17:30	42.5	37.5	0.000387371	-1.358326663	0.257090617	0.14206769
66.0	18:00	44.1	39.1	0.000385407	-1.234187075	0.291071285	0.124930593
66.5	18:30	44.7	39.7	0.000384688	-1.188768217	0.30459623	0.108009878
67.0	19:00	45.5	40.5	0.000383666	-1.124177197	0.324919702	0.092751565
67.5	19:30	45.3	40.3	0.00038387	-1.137067928	0.32075813	0.078843488
68.0	20:00	45.2	40.2	0.000384075	-1.149972374	0.316645517	0.067160525
68.5	20:30	45.4	40.4	0.000383768	-1.13062085	0.322832764	0.057326494
69.0	21:00	45.2	40.2	0.000384075	-1.149972374	0.316645517	0.048781274
69.5	21:30	45.5	40.5	0.000383666	-1.124177197	0.324919702	0.041638439
70.0	22:00	44.2	39.2	0.000385304	-1.227688279	0.292969058	0.035394764
70.5	22:30	43.2	38.2	0.000386542	-1.305903457	0.270927653	0.030571842
71.0	23:00	41.8	36.8	0.000388205	-1.410975357	0.243905272	0.026698718
71.5	23:30	39.0	34.0	0.000391786	-1.637282037	0.19450799	0.023633446
72.0	0:00	34.6	29.6	0.000397501	-1.998502569	0.13553809	0.021443229
72.5	0:30	29.9	24.9	0.000403725	-2.391800529	0.09146485	0.020038188
73.0	1:00	26.3	21.3	0.000408638	-2.702257664	0.067053956	0.019142432
73.5	1:30	24.1	19.1	0.000411784	-2.901087069	0.054963439	0.018511284
74.0	2:00	22.4	17.4	0.000414146	-3.050353371	0.047342192	0.018009488

74.5	2:30	20.9	15.9	0.000416295	-3.186164706	0.04133008	0.01758819
75.0	3:00	19.9	14.9	0.00041774	-3.27749136	0.037722771	0.017228459
75.5	3:30	18.8	13.8	0.000419317	-3.377146795	0.034144738	0.016906552
76.0	4:00	18.0	13.0	0.000420538	-3.454318287	0.031608845	0.016620367
76.5	4:30	17.4	12.4	0.000421397	-3.508606319	0.02993861	0.016359756
77.0	5:00	16.8	11.8	0.00042226	-3.563116595	0.02835033	0.016116686
77.5	5:30	16.5	11.5	0.000422754	-3.594365634	0.027478109	0.015889841
78.0	6:00	16.1	11.1	0.000423374	-3.633530003	0.026422747	0.015673022
78.5	6:30	15.7	10.7	0.000423995	-3.672809364	0.025404998	0.015467322
79.0	7:00	15.2	10.2	0.000424744	-3.720097104	0.024231615	0.015272091
79.5	7:30	14.8	9.8	0.000425369	-3.75963124	0.023292328	0.015088174
80.0	8:00	14.2	9.2	0.000426248	-3.815175079	0.022033856	0.014913474
80.5	8:30	13.7	8.7	0.000427004	-3.862967098	0.021005581	0.014750075
81.0	9:00	13.2	8.2	0.000427763	-3.910929004	0.020021892	0.014595968
81.5	9:30	13.0	8.0	0.000428017	-3.926954216	0.019703594	0.014450578
82.0	10:00	12.7	7.7	0.000428525	-3.959061705	0.01908101	0.014308913
82.5	10:30	12.5	7.5	0.000428779	-3.97514405	0.018776597	0.014173048
83.0	11:00	12.0	7.0	0.000429545	-4.023505899	0.017890134	0.014040609
83.5	11:30	11.8	6.8	0.000429928	-4.047751617	0.017461591	0.013915575

84.0	12:00	11.5	6.5	0.000430313	-4.072040686	0.017042574	0.01379461
84.5	12:30	11.3	6.3	0.000430698	-4.09637322	0.01663289	0.013677562
85.0	13:00	11.3	6.3	0.000430569	-4.088257539	0.016768426	0.013564285
85.5	13:30	12.8	7.8	0.000428271	-3.942998438	0.019389988	0.013451034
86.0	14:00	16.2	11.2	0.00042325	-3.625687946	0.026630771	0.013321257
86.5	14:30	21.1	16.1	0.000416055	-3.171004994	0.041961406	0.013145055
87.0	15:00	27.0	22.0	0.000407715	-2.643927419	0.071081554	0.012872135
87.5	15:30	32.2	27.2	0.0004007	-2.200648797	0.110731293	0.012422684
88.0	16:00	36.7	31.7	0.000394785	-1.826811234	0.160925905	0.011753587
88.5	16:30	40.0	35.0	0.000390514	-1.556933888	0.210781361	0.010844907
89.0	17:00	43.0	38.0	0.000386749	-1.318988198	0.267405727	0.009760122
89.5	17:30	44.3	39.3	0.000385099	-1.214701086	0.296798718	0.008538642
90.0	18:00	45.0	40.0	0.000384279	-1.162890556	0.31258134	0.00736105
90.5	18:30	45.3	40.3	0.00038387	-1.137067928	0.32075813	0.006295984
91.0	19:00	46.4	41.4	0.000382548	-1.053522341	0.348707317	0.00536305
91.5	19:30	47.4	42.4	0.000381336	-0.976912468	0.376471675	0.004504961
92.0	20:00	46.9	41.9	0.000381941	-1.015156616	0.362345676	0.003731997
92.5	20:30	46.7	41.7	0.000382245	-1.034324245	0.355466508	0.003113572
93.0	21:00	47.0	42.0	0.00038184	-1.008774164	0.364665726	0.002606576

93.5	21:30	46.9	41.9	0.000381941	-1.015156616	0.362345676	0.002172122
94.0	22:00	45.6	40.6	0.000383564	-1.117736967	0.327019013	0.001812182
94.5	22:30	43.8	38.8	0.000385819	-1.260216985	0.283592484	0.00153883
95.0	23:00	41.5	36.5	0.000388622	-1.437384717	0.237548203	0.001335393
95.5	23:30	39.0	34.0	0.000391786	-1.637282037	0.19450799	0.00118584
96.0	0:00	34.7	29.7	0.000397392	-1.991589557	0.136478313	0.001075943
96.5	0:30	30.7	25.7	0.000402712	-2.327763437	0.097513599	0.001004971
97.0	1:00	27.3	22.3	0.000407369	-2.622121455	0.072648579	0.000957147
97.5	1:30	24.8	19.8	0.00041073	-2.834471282	0.05874958	0.000923003
98.0	2:00	23.3	18.3	0.000412843	-2.968045667	0.051403672	0.000896285
98.5	2:30	22.0	17.0	0.00041474	-3.087937886	0.045595881	0.000873542
99.0	3:00	20.7	15.7	0.000416655	-3.20893708	0.040399532	0.000853852
99.5	3:30	20.0	15.0	0.000417619	-3.269856595	0.038011878	0.000836778
100.0	4:00	19.3	14.3	0.000418709	-3.338728872	0.035482031	0.000821024
100.5	4:30	18.8	13.8	0.000419317	-3.377146795	0.034144738	0.000806587
101.0	5:00	18.3	13.3	0.000420171	-3.431119685	0.032350698	0.000792933
101.5	5:30	17.6	12.6	0.000421151	-3.493072834	0.030407292	0.000780211
102.0	6:00	16.8	11.8	0.00042226	-3.563116595	0.02835033	0.000768438
102.5	6:30	16.3	11.3	0.000423126	-3.617850484	0.026840308	0.000757622

103.0	7:00	15.6	10.6	0.00042412	-3.68067908	0.025205852	0.000747523
103.5	7:30	15.0	10.0	0.000424994	-3.735896796	0.023851771	0.000738161
104.0	8:00	14.5	9.5	0.000425745	-3.783407669	0.022745052	0.00072941
104.5	8:30	14.0	9.0	0.0004265	-3.831086932	0.021686032	0.000721162
105.0	9:00	13.6	8.6	0.000427131	-3.870948927	0.020838586	0.000713384
105.5	9:30	13.3	8.3	0.00042751	-3.89492277	0.020344946	0.00070599
106.0	10:00	13.0	8.0	0.000428017	-3.926954216	0.019703594	0.000698845
106.5	10:30	13.2	8.2	0.000427763	-3.910929004	0.020021892	0.000691994
107.0	11:00	14.0	9.0	0.0004265	-3.831086932	0.021686032	0.000685101
107.5	11:30	13.8	8.8	0.000426878	-3.854989988	0.021173815	0.000677712
108.0	12:00	13.3	8.3	0.000427637	-3.902923517	0.02018282	0.000670575
108.5	12:30	13.5	8.5	0.000427257	-3.878935479	0.02067282	0.000663842
109.0	13:00	13.8	8.8	0.000426878	-3.854989988	0.021173815	0.000657016
109.5	13:30	15.4	10.4	0.000424369	-3.696432389	0.024811888	0.000650097
110.0	14:00	18.3	13.3	0.000420171	-3.431119685	0.032350698	0.000642082
110.5	14:30	22.3	17.3	0.000414265	-3.057861648	0.046988065	0.000631779
111.0	15:00	27.8	22.8	0.000406681	-2.578620067	0.075878639	0.000617109
111.5	15:30	32.6	27.6	0.000400145	-2.165564213	0.114685209	0.000594135
112.0	16:00	36.1	31.1	0.000395542	-1.874647785	0.15340899	0.000561024

112.5	16:30	40.0	35.0	0.000390514	-1.556933888	0.210781361	0.0005196
113.0	17:00	42.4	37.4	0.000387475	-1.364895388	0.255407393	0.000467626
113.5	17:30	44.3	39.3	0.000385201	-1.221192951	0.294878182	0.000411564
114.0	18:00	46.0	41.0	0.000383056	-1.085587069	0.337703472	0.000355145
114.5	18:30	46.5	41.5	0.000382447	-1.047119586	0.350947168	0.000299968
115.0	19:00	47.3	42.3	0.000381538	-0.989647044	0.371707864	0.000251691
115.5	19:30	47.4	42.4	0.000381336	-0.976912468	0.376471675	0.000209003
116.0	20:00	46.9	41.9	0.000381941	-1.015156616	0.362345676	0.000173142
116.5	20:30	46.3	41.3	0.00038265	-1.059928489	0.346480587	0.000144451
117.0	21:00	46.4	41.4	0.000382548	-1.053522341	0.348707317	0.000121474
117.5	21:30	46.5	41.5	0.000382447	-1.047119586	0.350947168	0.000102038

Annex 5a: Simulation program for the estimation of Health risk (*Ascaris* eggs)

RESTRICTED IRRIGATION: Soil ingestion (Method of Andrew Hamilton)					I													
Quantitative Microbiological Risk Analysis Monte Carlo simulation																		
	Enter Values in the yellow boxes																	
Variable	Range																	
Number of *Ascaris* eggs per **g** compost	2.14E+00	2.14E+00																
Quantity of compost ingested (**g** per day)	0.5	0.8																
Exposure (number of working days per year)	3																	
Disease/infection ratio	1	1																
***Ascaris* coefficients**																		
Variation from default values +/-%	25				Default raw coefficients													
N_50	644.25	1073.75	Reset Ascaris Defaults		N_50	859												
Alpha	0.078	0.13			Alpha	0.104												
Mid Percentile	50.0%																	
Upper Percentile	95.0%																	
Number of simulations	10000																	
	RESULTS																	
	PI Annual																	
50% value =	0,24844895																	
95% value =	0,35268687																	
Minimum =	0,09456869		State log of reduction at this point															
Maximum =	0,46009929																	
			Undetectable limit		Assumed 1 log reduction in 2 hrs													

Do Automated Monte Carlo Simulation

<Click for starting calculation

No of sheets 7

< Check the number of worksheets to be calculated

Do Monte Carlo Simulation

Sheet No.	Sheet nam	No. of data
1	Data1	288
2	Data2	288
3	Data3	325
4	Data4	127
5	Data5	288
6	Data6	286
7	Data7	288

< Input worksheet name and number of data on the sheet

Use "count" function for counting the data

Annex 5b: Simulation program for the estimation of Health risk (Norovirus)

RESTRICTED IRRIGATION: Soil ingestion (Karavarsamis-Hamilton method)

Quantitative Microbiological Risk Analysis Monte Carlo simulation

Enter Values in the yellow boxes

Variable	Range	
Faecal coliform (FC) numbers per g soil	5.48E-05	5.48E-05
Number of noroviruses per 100,000 FC	100000	100000
Quantity of soil ingested (g per day)	0.5	0.8
Exposure (number of days per year)	3	
Disease/infection ratio	1	1

Pathogen coefficients

Variation from dose/respose data (+/-%)	25
Filename of dose/response data	dose_response_noro_virus.csv

Do Monte Carlo Simulation

Mid Percentile	50.0%
Upper Percentile	95.0%
Number of simulations	10000

RESULTS

50% value =	4.48E-05
95% value =	5.36E-05
Minimum =	2.93E-05
Maximum =	6.24E-05
Mean P_I_d =	1.5E-05

Do Automated Monte Carlo Simulation

<Click for starting calculation. Save before starting.

No of sheets 1 < Check the number of worksheets to be calculated

Sheet No.	Sheet name	No. of data	elapse	calculation time (s)
6	Data6	1586	1586/1586	

< Input worksheet name and number of data on the sheet

Use "count" function for counting the data

Annex 5c: QMRA-MC Simulation program for the estimation of Health risk (*Salmonella*)

RESTRICTED IRRIGATION: Soil ingestion

Quantitative Microbiological Risk Analysis Monte Carlo simulation (**Karavarsamis-Hamilton method**)

Enter Values in the yellow boxes

Variable	Range	
Faecal coliform count per g soil	5.53E+03	5.53E+03
Number of pathogens per 100,000 FC	0.1	1
Quantity of soil ingested per day (g)	0.5	0.8
Exposure (No. of working days per year)	3	
Disease/infection ratio	1	1

Pathogen coefficients

Variation from default values (+/-%): 25

- ○ Rotavirus
- ○ Campylobacter
- ○ Vibrio cholerae
- ○ Shigella
- ◉ Salmonella

			Default values	
N_50	17700	29500	N_50	23600
Alpha	0.23475	0.39125	Alpha	0.313

Mid Percentile: 50.0%

Upper Percentile: 95.0%

Number of simulations: 10000

Do Monte Carlo Simulation

Do Automated Monte Carlo Simulation

<Click for starting calculation. Save before starting.

No of sheets: 1 < Check the number of worksheets to be calculated

Sheet No.	Sheet nam	No. of data	elapse	calculation time (s)
1	Data12	966	966/966	< Input worksheet name and number of data on the sheet
2				Use "count" function for counting the data
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				

RESULTS

PI Annual

50% value =	6.81E-06
95% value =	1.124E-05
Minimum =	1.33E-06
Maximum =	1.802E-05

References

1. Moodley, P., Archer, C., and Hawksworth, D. 2008. Standard Methods for the Recovery and Enumeration of. Helminth Ova in Wastewater, Sludge, Compost and Urine–Diversion Waste in South Africa [online] http://www.researchgate.net/profile/Colleen_Archer2/publication/266469512_Standard_Methods_for_the_Recovery_and_Enumeration_of_Helminth_Ova_in_Wastewater_Sludge_Compost_and_UrineDiversion_Waste_in_South_Africa/links/54d350150cf28e069728045d.pdf (Accessed June 10, 2015)
2. Berger, S. 2015. *Echinococcosis: global status*. GIDEON Informatics Inc. Available at: https://books.google.bf/books?id=c2QBwAAQBAJ&pg=PA49&lpg=PA49&dq=Prevalence+of+salmonella+in+Burkina+Faso&source (Accessed 12 June, 2014).

Websites

3. http://www.phsource.us/PH/PARA/Chapter_5.htm (Life cycles of Helminth)
4. DPDxTaeniasis."<http://www.dpd.cdc.gov/dpdx/HTML/Taeniasis.asp?body=Frames/Z/Taeniasis/body_Taeniasis_page1.htm