Health Risk Assessment Associated with the Reuse of Compost, Urine and Greywater in Agricultural Field in Sahelian Climate

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Dedication

For my Only Lord JESUS CHRIST

For my beloved wife Anne Aurélie N'Goné CISSE

In memory of my dear late father KOUAME Brou Paul and my dear mother KOFFI Affoué who died on August 15, 2012 before I started this course

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Abstract

The aim of this study is to assess the health risks associated with the reuse of human excreta (compost and urine) and greywater in the agricultural field. It will firstly have to assess the health risk from farmers associated with the reuse of human excreta and greywater in agriculture field; and secondary to assess the health risk from consumers associated with the reuse of human excreta and greywater in agriculture field. To achieve these objectives, some hypotheses were considered as for the different exposed groups. For farmers, we assume that they handle compost and urine in fields, and they irrigate crops with greywater without adequate equipment protection (gloves, clothing and shoes). For consumers, we assume that they eat lettuce without washing it thoroughly. Different onsite experimentations have been carried out. It is about combining Compost and Top Water (C+TW), Urine and Top Water (U+TW), Compost, Urine and Greywater (C+U+GW) and Non fertilizer (NoF) which is used as a like controlling tool. An initial number of indicators and pathogens cited above were determined in irrigation greywater, compost and urine before application in the field. Microbiological quality of soil in different combinations was monitored weekly from *E.coli*, Faecal coliform, *Faecal Enterococci, and Salmonella,* and helminthes eggs over two months. Quantitative microbial risk assessment was subsequently evaluated for *Salmonella* and *Ascaris* on these combinations. Results vary from different treatments: For C+TW treatment, there are annual risks of *Salmonella* infection in scenarios where it is assumed that farmers may ingest accidentally 10 to 100 mg of soil which is $3.87x10^{-3}$ pppy. Concerning *Ascaris* infection, annual risk is $4.67x10^{-2}$. From lettuce consumption, *Salmonella* annual risk infection is $1.54x10^{-1}$. For U+TW treatment, *Salmonella* annual risk infection in scenario where it is assumed that farmers can ingest accidentally soil spread with urine is $9.55x10^{-1}$. For lettuce consumption, annual risk is $1.30x10^{-7}$. From GW treatment, *Salmonella* annual risk infection in scenario which assumes that farmers ingest accidentally 10 to 100 mg of soil irrigated with greywater is $8.89x10^{-6}$. From ingestion of irrigation greywater, annual risk infection is $1.02x10^{-4}$. Concerning lettuce consumption, *Salmonella* risk infection is $9.42x10^{-4}$. From C+U+GW treatment, in case of soil ingestion, *Salmonell*a annual risk infection is $1.44x10^{-4}$. For Ascaris infection, risk is $4.67x10^{-2}$. From ingestion of irrigation greywater, *Salmonell*a annual risk infection is $1.53x10^{-3}$. For *Ascaris* infection, risk is $3.97x10^{-1}$. From lettuce consumption, *Salmonell*a annual risk infection is $5.00x10^{-7}$. For *Ascaris* infection, risk is $2.41x10^{-2}$.

Keywords: Health Risk Assessment, Compost, Urine, Greywater, Quantitative Microbial Risk Analysis, *Ascaris, Salmonella,* Reuse and Agriculture.

Résumé

L'objectif de cette étude était d'évaluer les risques sanitaires liés à l'utilisation combinée du compost, de l'urine et des eaux grises en agriculture. Il s'agissait de façon spécifique d'évaluer dans un premier temps les risques sanitaires au niveau des agriculteurs et dans un second temps d'évaluer les risques sanitaires au niveau des consommateurs. Pour atteindre ces objectifs, des scénarios ont été considérés au niveau des différents groupes d'exposition. Ainsi, au niveau des agriculteurs, avons-nous supposé qu'ils manipulent le compost et l'urine dans leurs champs, et qu'ils irriguent les cultures avec les eaux grises sans aucun équipement de protection approprié (gants, habillement et chaussures). Concernant les consommateurs, nous avons supposé qu'ils mangent de la laitue sans la laver correctement. Différentes expérimentations sur site ont été effectuées. A savoir la combinaison du compost et de l'eau de robinet (C+TW), de l'urine et de l'eau de robinet (U+TW), du compost, de l'urine et des eaux grises (C+U+GW) et un témoin arrosé seulement avec l'eau de robinet (NoF). La charge initiale d'organismes indicateurs et pathogènes (Coliformes fécaux, *E.coli*, *Entérocoques*, *Salmonelles* et les œufs d'*Ascaris*) a été déterminée dans, le compost, l'urine et les eaux grises avant leur application. La qualité microbiologique du sol au niveau des différentes combinaisons de traitement a été suivie une fois par semaine pour des paramètres tels que les *E.coli*, coliformes fécaux, *entérocoques fécaux*, *Salmonelles*, et œufs d'*Ascaris* pendant deux mois. L'évaluation quantitative microbienne des risques a été effectuée en utilisant la méthode de simulation de Monte Carlo pour *les salmonelles* (10000 itérations) et *l'ascaris* (1000 itérations) pour chaque combinaison. Les résultats ainsi obtenus, varient selon le type de traitements: Au niveau de C+TW, le risque d'infection aux *Salmonelles* est le plus important ($1.54x10^{-1}$), dans le cadre de la consommation de la laitue. Au niveau du traitement U+TW, le risque annuel d'infection le plus important se trouve au niveau des *salmonelles* $9.55x10^{-1}$dans le scénario selon lequel les agriculteurs peuvent ingérer accidentellement 10 à 100 mg de sol fertilisé avec l'urine. Concernant la matrice eaux grises (GW), le risque annuel d'infection aux *salmonelles* ($1.02x10^{-4}$), le plus important se rencontre dans le scénario selon lequel, les fermiers ingèrent accidentellement 1 à 2 mL d'eaux grises lors de l'arrosage de leurs cultures. Pour la matrice compost, urine et eaux grises (C+U+GW), concernant, l'ingestion de sol, les *Ascaris* présentent le risque annuel d'infection le plus important $4.67x10^{-2}$. Quant à l'ingestion des eaux grises lors de l'irrigation, le risque annuel d'infection aux *salmonelles* est de $1.53x10^{-3}$. Pour l'infection à l'*Ascaris*, le risque est de $3.62x10^{-1}$.

Concernant la consommation de laitue, il ressort que les *Ascaris* présentent le risque d'infection le plus important soit $2.41x10^{-2}$.

Mots-clés : Evaluation des risques sanitaires, Analyse Quantitative des Risques Microbiens, Compost, Urine, Eaux grises, Réutilisation, et Agriculture.

Abbreviations

Ameli-EAUR	Amélioration de l'accès à l'Eau potable et à l'Assainissement en milieu Urbain et Rural
C+TW	Compost and Top Water
C+U+GW	Compost, Urine and Greywater
DALY	Disability Adjusted Life Years
DW	Dry weight
EPA	Environment Protection Agency
FAO	Food and Agriculture Organization of United Nations
FW	Fresh weight
GW	Greywater
JICA	Japanese International Cooperation Agency
NoF	Non Fertilizer
Pinf	Probability of infection
PPPY	Per Person Per Year
Pyr	Annual Probability risk infection
QMRA	Quantitative Microbial Risks Analysis
UNICEF	United Nations Organization for Child and Education Found
U+TW	Urine and Top Water
W/V	Weight/Volume

List of tables

List of figures

Table of contains

I. Introduction

Burkina Faso, like many countries, is confronted with various issues among which food insecurity. In order to address this situation, the level of fertility of the soils has been decreasing; the price of chemical fertilizers is increasing on the market as well as the weakness of the pluviometry. Furthermore, the water resources are insufficient, because 44,15% of rural population have not access of best water quality (DGRE, 2010). In addition, there is the issue of an appropriate sanitation. According to WHO and UNICEF, (2007) Joint Monitoring Program, access to improved sanitation in Burkina Faso was about 17% on national scale (47% urban and 4% rural) in 2007. The lower sanitation distribution is increasing the diseases from population which constitutes a public health issue in Burkina Faso. Therefore, improvement of the agriculture and sanitation is urgent task in the country. Faeces and urine, as well as mixed sewage products, need to be seen as resources rather than waste the resource oriented sanitation for sanitation or composting toilet is an advantage for agriculture. In addition human excreta have traditionally been used for crop fertilization in many countries. In Japan recycling of urine and faeces was introduced in the 12th Century and in China human and animal excreta have been composted for thousands of years (Höglund, 2001). In human excreta, urine contains the major part of essential plant nutrients (nitrogen, phosphorus and potassium). Concerning Faeces, apart from nutrients, can contribute humus-like substances, thus improving soil fertilizer (Schönning et al., 2007). In this case, the reuse of human excreta without previous relevant treatment in agriculture triggers a problem of public health and remains health risk for farmers and consumers. Greywater reuse can alleviate stress on depleted water resources while reducing water cost for residents (Maimon et al., 2010). The reuse of greywater, however also can compromise human and environmental health. Pathogens in greywater may cause diseases through direct contact as well as through the consumption of contaminated plants (Shuval et al., 1997 and Mara et al., 2007a).

However, hazards associated with the recycling of these products include pathogens and pharmaceuticals as well as other micropollutants and heavy metals (Höglund et al., 1998 and Schönning et al., 2007). Thus, consumers can be exposed to diseases, when consuming the contaminated products related to greywater and human excreta reuse in agriculture especially if these products are not appropriately treated before being used in agriculture (FAO and WHO, 2008). Therefore, in order to minimize contamination of farmers and consumers due

to the reuse of human excreta and greywater in agriculture field, several studies were conducted on health risk assessment related to urine, compost or greywater in agriculture field in the world (Höglund et al., 2002; Al-Hamaiedeh, 2010; Fidjeland, 2010 ; Gemmell and Schmidt, 2011; and Nana O.B. Ackerson and Esi Awuah, 2012).

However, in Burkina Faso these kinds of study have not been conducted yet according to our investigations, when we know that the majority of urban farming populations use wastewater to irrigate their crops which is not necessarily treated before use to irrigate crops. In this context, the Japanese International Corporation Agency (JICA) through the Ameli-EAUR project which promotes the valorization of human excreta and greywater in family farming in order to improve sustainable sanitation for rural populations tried to study the health risk assessment related to the reuse of human excreta and greywater in agriculture. It is in this context that a topic was suggested to us within the framework of our master's thesis. The topic is entitled "health risk assessment associated with the reuse of compost, urine and greywater in agricultural field in sahelian climate". The aim of this study is to assess the health risks associated with the reuse of human excreta and greywater in the agricultural field. It will, in a specific way, firstly, assess the health risk for farmers that reuse human excreta and greywater in agriculture field; and secondly assess the health risk for consumers of goods relating to the reuse of human excreta and greywater in agriculture field. To meet these objectives, this present dissertation includes the following parts: the state of the art on the generality on health risk assessment which include the risk for farmers and consumers, the material and methods which are used to do this study, then the results and discussion issue following the different activities and experimentations, and finally the conclusion and perspectives of this study.

II. LITERATURE REVIEW

1. Generalities on health risk assessment

1.1. Health risk assessment

According to WHO, 2006a, the risk is the probability that something with a negative impact may occur. The agent that causes the adverse effect is a hazard. Risk incorporates the probability that an event will occur with the effect that it will have on a population or the environment, considering the sociopolitical context where it takes place (WHO, 2006a). The WHO guideline for the safe use of wastewater, excreta and greywater (WHO, 2006a) gives recommendations on treatment and management in order to avoid unacceptable health risk. It is based on the Stockholm framework, which is a harmonized approach to control water-related diseases (Fidjeland, 2010). Different exposures and diseases are compared through the Disability Adjusted Life Years (DALY) unit, which is a measure of the years lost due to premature death, diseases and chronic effects. The DALY unit enables cross-sectional cost-efficiency comparison of health initiatives (WHO, 2006a); (Fidjeland, 2010). The tolerable risk which is recommended by World Health Organization is 10^{-6} DALY (WHO, 2006a).

Many authors have characterized the risk analysis in three principal steps: risk assessment, risk management and risk communication (WHO, 1999); (Westrell, 2004); (Metcalf & Eddy, 2007);(Fidjeland, 2010).

According to the National Research Council of USA, risk assessment can be defined broadly as the process of the probability of occurrence of an event and the probable magnitude of adverse effects on safety, health, ecology, finances over a specified time period (Metcalf & Eddy, 2007). In other words, the risk assessment is defined as the qualitative or quantitative characterization and estimation of potential adverse health effects associated with exposure of individuals or populations to hazards (here microbial agents) (Westrell, 2004); (Fidjeland, 2010). Risk assessment also includes characterization of the uncertainties inherent in the process of inferring risk.

Risk management is the process of evaluating and, if necessary, controlling sources of exposure and risk. Sound environmental risk management means weighing many different attributes of a decision and developing alternatives (Metcalf & Eddy, 2007). Risk management is an activity much broader than technical risk analysis alone (McDowell and Lemer, 1991).

It is the interactive exchange of information and opinions concerning risk and risk management among risk assessors, risk managers, consumers, and other interested parties about the nature, magnitude, significance, or control of a risk (Metcalf & Eddy, 2007). It concerns the health risk assessment component, is the quantitative or qualitative characterization and estimation of potential adverse health effects associated with exposure of individuals or populations to hazardous materials and situations (Metcalf & Eddy, 2007). Therefore, health risk assessment can be divided into four major steps including: hazard identification, dose-response assessment, exposure assessment, and risk characterization (WHO, 1999). Health risk assessment includes chemical and microbial risk assessment (Metcalf & Eddy, 2007), and in our case of study we will focus on microbial risk assessment.

1.2. Steps of health risk assessment

Many authors have localized the health risk assessment in four steps which are mentioned below (Haas et al., 1999); (WHO, 1999);(Metcalf & Eddy, 2007):

Hazard identification, defined as the process of determining whether exposure to an agent can cause an increase in the incidence of a health condition, is the most easily recognized in the actions of regulatory agencies (Metcalf & Eddy, 2007). Also the identification of microbiology agent capable of causing adverse health effects and which may be present in a food or group of foods(WHO, 1999).

Dose-response may be defined as the determination of the relationship between the magnitude of exposure (dose) to a chemical, biological or physical agent and the severity and/or frequency of associated adverse health effects (response)(WHO, 1999). The dose-response assessment is the process of characterizing the relationship between the dose of an agent administered or received and the incidence of an adverse health effect in exposed populations and then estimating the incidence of the effect as a function of human exposure to the agent (Metcalf & Eddy, 2007).

Exposure assessment is the process of measuring or estimating the intensity, frequency, and duration of human exposures to an agent currently present in the environment. For microbial risk assessment, exposure assessment describes the magnitude and/or probability of actual or anticipated human exposure to pathogenic microorganisms or microbiological toxins (Haas et al., 1999); (Metcalf & Eddy, 2007); (Fidjeland, 2010).

Risk characterization is the process of estimating the incidence of a health effect under various conditions of the human exposure described in exposure assessment. In addition, risk characterization may require compiling all the data necessary for a given model and running simulations (Haas et al., 1999); (WHO, 1999) and (Metcalf & Eddy, 2007).

1.3. Microbial risk assessment

Haas et al., (1999) were defined microbial risk assessment (MRA) as the process that is used to evaluate the likelihood of adverse human health effects that can occur following exposure to pathogenic microorganisms or to a medium in which pathogens occur. Other authors as WHO, (1999),Metcalf & Eddy, (2007) and Fidjeland, (2010) explained the microbial or microbiological risk assessment process includes evaluation and consideration of quantitative information; however, qualitative information is also employed as appropriate. In other words, the microbial risk assessment should explicitly consider the dynamics of microbiological growth, survival, and death in foods and the complexity of the interaction between human and agent following consumption as well as the potential for further spread (WHO, 1999).

Quantitative Microbial Risk Assessment (QMRA) is a tool used to predict the consequences of potential or actual exposure to infectious microorganisms (Haas et al., 1999). The methodology is based on the chemical risk assessment concept for which the National Academy of Sciences published recommended definitions and main principles (Höglund, 2001). QMRA thus starts by a problem formulation where all the transmission routes and pathogens of interest are identified. It then assesses the dose of a certain pathogen to which an individual may be exposed and uses this dose in a dose-response model to calculate the probability of infection. Risks are finally characterized by taking into consideration the frequency of the exposure events for the range of pathogens studied, to estimate a total risk (Haas et al., 1999); (Höglund, 2001).

2. Health risk assessment for farmers

Health risk can be localized in the different activities in the field when, the farm workers use compost, urine and greywater to amend the soil and the crops.

2.1. Spreading compost

When using fertilizer products containing human or animal excreta, the reduction of excreted pathogens is a critical step in minimizing the risk of further spreading of pathogens.

Transmission of disease may occur if humans or animals come in contact with the excreta and accidentally ingest the pathogen-containing material before the pathogens have been inactivated (Schönning et al., 2007). According to WHO, (2006a) the variations in the risk for infection depend on the organism in question. Some Salmonella are able to regrow in stored but unstabilized materials, especially if the materials are partly moist. Viruses and parasites generally have longer survival in the environment as well as lower infectious doses, which resulted in high risks for rotavirus, the protozoa and *Ascaris*. For WHO, (2006a), in considering two mean scenarios which are unconditional (applying the incidence in the population) and conditional (assuming that one member of the family actually had an infection during period of collection). Thus in this situation, the difference in risk between the conditional and unconditional scenario was 1-4 orders of magnitude, and the difference between typical (50%) and worst case (95%) varied from none to five orders of magnitude, depending on the organism. For the unconditional scenario, the risk was never higher than $4x10^{-2}$ (rotavirus). Only after 12 months of storage and taking incidence into consideration were the risks $<10^{-4}$ for all organisms, excluding *Ascaris* ($P_{inf} = 8 \times 10^{-4}$), when emptying the container and applying the material (WHO, 2006a); (Schönning et al., 2007). For Carr, (2005), agricultural field workers are at high risk of parasitic infections because of the long survival of the protozoa and *Ascaris* in the compost because WHO guidelines recommend to reduce the helminth eggs in compost to ≤ 1 egg/L (WHO, 2006a). But exposure to hookworm infection can be reduced, even eliminated, by the use of less contaminating irrigation methods and by the use of appropriate protective clothing (i.e. shoes for field workers and gloves for crop handlers).

2.2. Spreading urine

For the hygienic risks related to the handling and reuse of urine, temperature, dilution, pH ammonia and time are the mean determinants affecting the persistence of organisms in collected urine (WHO, 2006a). Urine contains the majority of plant macronutrients that originate from household wastewater (Swedish EPA, 2007). Furthermore, the potential pathogen content is low, especially compared to faeces. Therefore, separate collection of urine for later use as a fertilizer in agriculture has been promoted through the use of urine separating toilets and latrines (Höglund et al., 2002). The short survival of *E. coli* in urine makes it unsuitable as a general indicator for faecal contamination by, for example, viruses and protozoa (WHO, 2006a). According to WHO (2006a), the Gram-positive faecal streptococci has a longer survival process (normally a T_{90} value of 4-7 days at 20°C, but up to

30 days at 4°C), and spore-forming clostridia are not reduced at all during a period of 80 days. In general, lower temperature and higher dilution result in longer survival of most bacteria (Höglund et al., 1998; WHO, 2006). However, the urine is generally contaminated at the time of the micturition by germs coming from faeces, which increases the load of pathogenic and constitutes a health risk (Tagro, 2012). According to WHO (2006a), the pathogenic germs of bacterial, viral or parasitic origin are responsible for several diseases such as diarrhea, cholera, typhoid fever, salmonellosis, shigelloses, amoebiasis, bacterial dysentery, amoebic dysentery, and parasitism. But, urinary excretion of pathogens that can be transmitted through the environment are uncommon (Höglund et al., 2002). The use of non-treated urines as fertilizer in agriculture can contribute to the transmission of these diseases to the directly exposed field workers (Tagro, 2012). However if the farm workers are used the protective equipment before spreading of urine in the field, the risk of infection can be reduced (WHO, 2006a). Furthermore, Höglund et al., 1998 suggest that estimate the risk of pathogen transmission for handling, transportation and reuse of source separated urine that follow it is necessary to determine the exact amount of faecal material introduced in the urine fraction.

Therefore, the estimated risks of pathogens for different pathways were calculated by Höglund et al., 2002 for three indicator pathogens (*C. jejuni, C. parvum* and rotavirus). It arises that in the case of an epidemic, where no inactivation and accidental ingestion of 1 mL of unstored urine was assumed to occur in the collection tank and spreading in the field, viruses may pose an unacceptably high risk, and bacteria pose a greater risk than protozoa. The annual risk of viral infection at 4°C is 0.81, since very low inactivation of rotavirus occurs at this temperature and slightly lower at 20°C (P_{inf} = 0.55) (WHO, 2006a). The risk from exposure to aerosols when farm workers spread urine in the field depend, according to Höglund et al., 2002 and WHO, 2006 of the technique of spreading of the urine.

2.3. Watering greywater

Greywater is wastewater generated from domestic activities such as laundry, dishwashing and bathing that can be recycled on-site for reuse in landscape irrigation and constructed wetlands (Zuma and Tandlich, 2010). Greywater is thus domestic wastewater, without any input from toilet, which carries finite concentrations microorganisms such as faecal coliforms, *E.coli* and opportunistic pathogens (WHO, 2006a) and (Zuma and Tandlich, 2010). In greywater system, microbial hazards emanate mainly from faecal cross-contamination (e.g. from anal cleansing,

hygienic practices, contaminated laundry and other sources) (WHO, 2006a). Thus, farm workers and their families are at the highest risk when flood or furrow irrigation techniques are used, particularly when protective clothing is not worn and earth is moved by hand (Carr, 2005). Farmers can be exposed by different pathways when they irrigate the field with greywater according to Maimon et al., 2010 as shown in the exposure scenario in the table 1.

Table 1: Different routes of exposure of farmers by irrigation with greywater

Exposure type	Exposure scenario
Direct	Accidental ingestion of greywater Ingestion of greywater from the irrigation system Ingestion of soil contaminated with greywater Inhalation of aerosols from spray irrigation system

Therefore, greywater is comprised of very diverse components, making the drafting creation of comprehension risk assessment, guidelines, and regulations a hard task (Maimon et al., 2010). Furthermore, according to same author, determining an acceptable risk for water reuse schemes will vary from place to place according to the severity of local water stress and the level of background risks as well as the existing ‘’governance’’ in the water sphere and regulatory capacity (Maimon et al., 2010). Greywater used for irrigation may, depending on distribution practices, expose people via inhalation of aerosols as well as through consumption of irrigated contaminated crops, in a similar pathway as for wastewater (WHO, 2006b).

The faecal load in the greywater in the system was assessed on the basis of a range of microbial indicators (E.coli, enterococci, sulfite-reducing clostridia, coliphage) and chemical markers (faecal sterols) (WHO, 2006a). Furthermore the pathogen-related risks of greywater depend on the faecal load or faecal misplacement. According to WHO, 2006a, in all exposure scenarios, rotavirus posed the highest risk, partly due to its excretion in higher numbers, at least during the acute phase, compared to the other pathogens included in the study. Thus, different studies have tried to correlate the rotavirus load with faecal indicators such as *E.coli* (Maimon et al., 2010). The WHO guidelines suggest that there are between 0.1 to 1 rotavirus for every 10^5 *E.coli* in 100 mL of domestic wastewater (WHO, 2006a) and (Mara et al., 2007a). Thus, the tolerable disease risks for these organisms (rotavirus, *Campylobacter* and *Cryptosporidium*)are in the range 10^{-3}- 10^{-4} per person per year (pppy) according to WHO, 2006a.

Reliable epidemiological data relating to the safe use of greywater in agriculture are scarce. As an alternative, the range of tolerable disease risk can be deduced on the QMRA, for which the risks resulting from exposure to greywater, for both its final use and handling (WHO, 2006a). Furthermore, Ottosson & Strenström in 2003, suggested that guidelines for the safe use of greywater in agriculture should not be based on thermotolerant coliforms as a hygienic parameter, because of the large input of non-faecal coliforms and/or growth of coliforms, unless their concentrations are adjusted for false-positive levels (Ottosson & Strenström, 2003a in (WHO, 2006a)). Thus, the overestimation of the faecal load, and risk, resulting from these indicator bacteria is to some degree compensated for by the higher susceptibility to treatment and environmental die-off (WHO, 2006a and Mara et al., 2007). In greywater, a regrowth of *E. coli* sometimes occurs, which may lead to an overestimation of the risks if verification monitoring is based on this parameter. It is suggested that *E. coli* guideline values, which are applicable for wastewater use, be applied cautiously for greywater. If applied, they will give a level of additional safety in this application, since the faecal load is usually 100-1000 times less than wastewater (WHO, 2006a). Thus, a guideline value of $<10^3$ *E. coli* per 100 mL is suggested for unrestricted irrigation with greywater by (WHO, 2006a).

3. Health risk assessment for consumers

In developing countries, foodborne illnesses caused by contaminated fruits and vegetables are frequent and in some areas they cause a large proportion of illness. However, due to lack of foodborne disease investigation and surveillance in most of these countries, most outbreaks go undetected and the scientific literature reports only on very few outbreaks (WHO, 1998). Thus, reuse of human excreta and greywater in agriculture can cause diseases for consumers especially when they eat those crops without cooking. In addition, human waste may be a source of direct contamination if deposited in farms. Alternatively, environmental contamination with pathogens from these sources may be transferred indirectly to products via contaminated water, insects, agents such as dust, tools and equipment (FAO and WHO, 2008). According to FAO and WHO, 2008 fruits and vegetables can become contaminated with microorganisms capable of causing human diseases while still on plant in fields or orchards, or during harvesting, transport, processing distribution and marketing, or in the house. Also, Bacteria such as *Clostridium botulinum*, *Bacillus cereus* and *Listeria monocytogenes*, all capable of causing illness, are normal inhabitants of many soils, whereas *Salmonella*, *Shigella*, *Escherichia coli* and *Campylobacter* reside in the intestinal tracts of

animals, including humans, and are more likely to contaminate fruits and vegetables through contact with faeces, sewage, untreated irrigation water or surface water (WHO, 2006a);(FAO and WHO, 2008); and (Mara and Sleigh, 2010a). Generally, people irrigating with wastewater have higher rates of helminth infections than those using freshwater. In addition, skin and nail problems may occur among farmers using wastewater (Al-Hamaiedeh, 2010). There is substantial evidence that human enteric pathogens which are frequently present in greywater are responsible for low-level incidence of chronic gastroenteritis (upset stomach, vomiting, and diarrhea) as well as other "mild illness in people" (Al-Hamaiedeh, 2010).

To assess potential risks associated with the use of reclaimed wastewater, the following exposure scenario is developed by Asano et al., 1992 for spray irrigation of food crops. The following scenario is used to estimate the risk of infection to an individual for a single or an annual or a lifetime exposure. In this case, Asano et al., 1992 are assumed to 10 mL reclaimed wastewater can be left on the crops eaten raw. However, irrigation with reclaimed wastewater is assumed to stop two weeks before harvesting. Thus, virus die-off due to desiccation and sunlight for 14 days is included in the calculation. Shuval et al., 1997 are corroborated the developed approach by Asano et al., 1992 where they were collected for 100g of long leaf lettuce, 10.8 mL for 12 days before harvesting. Based on these measurements it is possible to estimate the amount of indicator organisms that might remain on the vegetables if irrigated with raw wastewater and with wastewater meeting the WHO guidelines.

In 1989, to mitigate the risks of contamination, in terms of epidemiological and technological data available, the WHO "Health Guidelines for the Use of Wastewater in Agriculture and Aquaculture", recommended the microbial guidelines for wastewater irrigation of vegetables eaten raw of a mean of 1000 faecal coliforms (FC)/100 mL and <1 helminth egg/L in effluent (Shuval et al., 1997). Thus, a study was carried out in Ghana by Nana O.B. Ackerson and Esi Awuah, (2012), and which showed that, the annual probabilities of *Ascaris* and *E. coli* infection associated with the consumption of lettuce where farmers used the shallow well and stream to irrigate lettuce are higher ($7.51x10^{-2}$ for *Ascaris* and $3.63x10^{-1}$ for *E. coli*) than the tolerable risk (10^{-6} pppy) recommended by WHO, (2006a). However, cessation of irrigation before harvest can be adopted to minimize the risk of infection in lettuce consumption (Nana O.B. Ackerson and Esi Awuah, 2012).

III. MATERIAL AND METHODS

1. Experimental site

The experimental site of our study is localized on Kamboinsé campus of the International Institute for Water and Environmental Engineering (2iE) whose geographic details are 12°27'39.74"N and 1°32'54.78"W. This experiment is carried out in the vicinity of the water purification plant on campus (Figure 1).

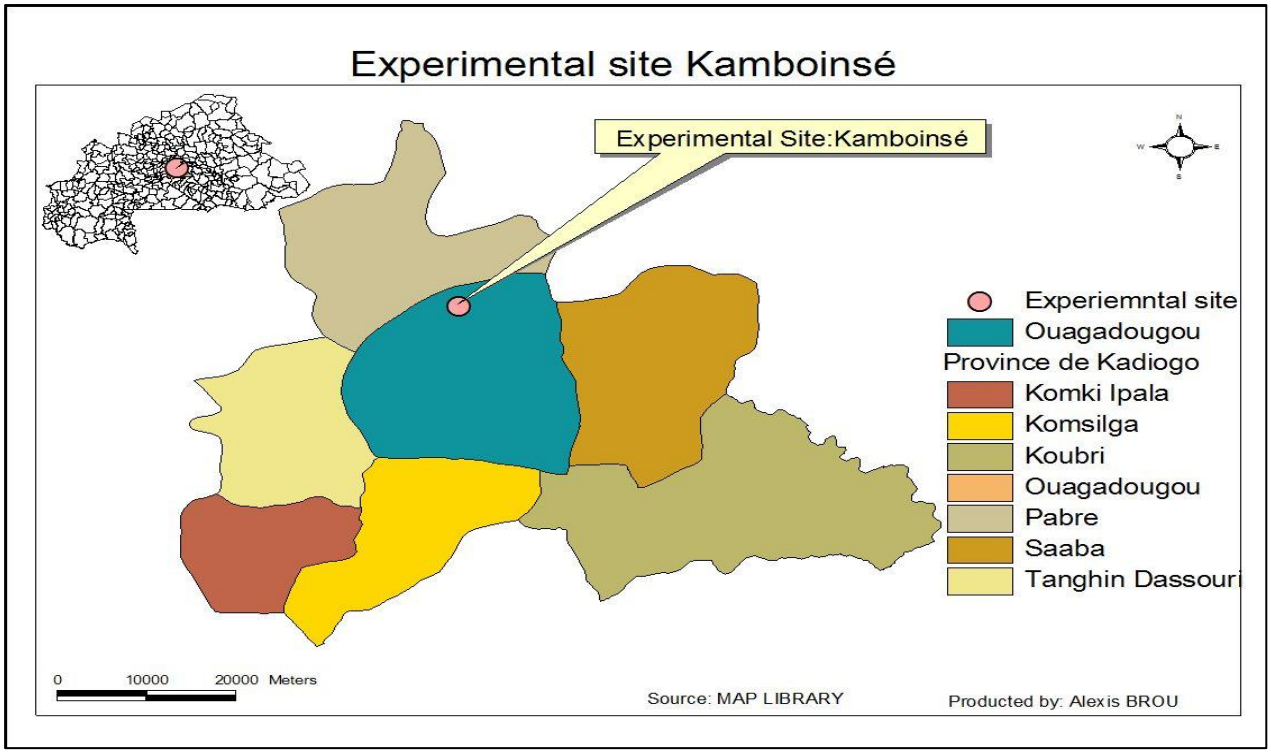

Figure 1: Experimental site of Kamboinsé (source Google earth)

Kamboinsé village is located at approximately 9 kms in the North of Ouagadougou on the road to Kongoussi. The population practice Christianity mainly and has activities such as agriculture, breeding and marketing of traditional drink "dolo". This locality is submitted to the soudano-sahelian climate with a long dry season and a short rain season. The grounds, with the image of the sahelian grounds, are relatively low in organic matter and in total elements (N, P, K), they are generally attached to the classes of average fertility to weak (SOU, 2009). The study is carried out in the experimental site of Ameli-EAUR project. The experimental design is carried out on the lettuce crop which uses the combination of compost and top water (C+TW), urine and top water (U+TW), compost, urine and greywater (C+U+GW), greywater only (GW), and control with which we use only top water to irrigate (NoF). There are 3 replications for each combination (Figure 2 below). The area where the lettuce crop is grown is 1.56 m^2 per plank.

The source of compost, urine and greywater which is used to irrigate the lettuce crop is from the families' pilot of Ziniaré especially from Barkuundouba and Kolongodjessé villages. Ziniaré is located in the eastern section with about thirty kilometers far from Ouagadougou, in the Oubritenga district. Barkuundouba is located at 17 kms of Ziniaré. The populations include in the majority Peulh (Fulani people) and practice Islam as the first religion and then Christianity. Breeding is the principal economic activity of the populations. The second activity is agriculture with rudimentary farming techniques. This activity is dominated by cereal cultures like millet, sorghum and corn. This sector is also confronted with the insufficiency of cultivable grounds, the irregularity of the rains and decreasing soil fertility (Tagro, 2012).

Kolongodjessé, as for it, is located at 7 kms of the city of Ziniaré on the axis Ouagadougou-Kaya. Its population has respectively as first and second activities breeding and agriculture. They also sell traditional drink called "dolo" and mainly include Mossi ethnical group. Contrary to Barkuundouba, the dominant religion with Kolongodjessé is Christianity (NIKIEMA, 2012).

The gap between the lettuce plants on each plank varies from 10 to 15 centimeters. The choice of lettuce is justified by the roughness of surface of the edible sheets and the foliated density of the culture. These characteristics ensure for the micro-organisms a certain disinfecting ability to through solar radiations. Hence this type of consumed vegetables is believed to be a vector of pathogenic micro-organisms particularly dangerous for the consumer (SOU, 2009).

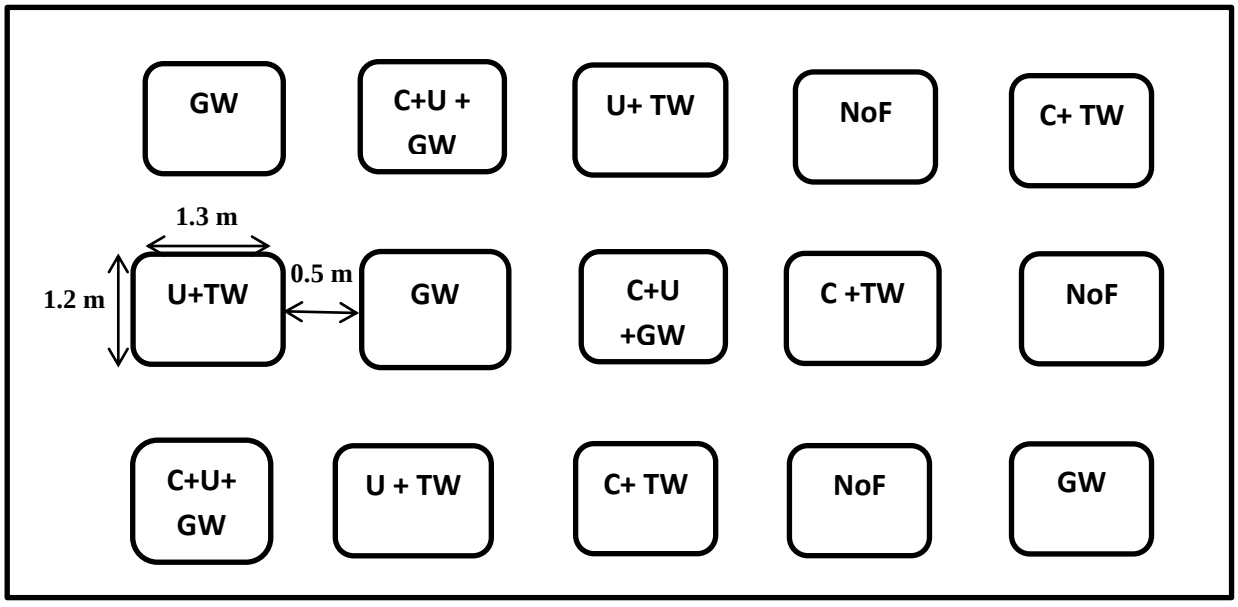

Figure 2: Experimental design in the site

2. Sampling and data collection

2.1. Initial statement of the experimental site

Before planting out the lettuce plants in the soil, the samples of soil, compost, urine and greywater have been made to known the initial concentration of the microbiological parameters. The parameters or indicators which are analyzed in the different matrix are contained in the table 1. And then, samples of soils are taken for each treatment per week to analyze these parameters in table 1 below. Therefore, samplings were carried out from 10 April to 26 May 2014.

Table 2: Different parameters which are analyzed in the matrix

Matrix	Indicators/pathogens
Soil	*E. coli/Faecal coliform, Salmonella,* Helminthes eggs, *Faecal enterococci*
Compost	Helminthes eggs, *E.coli/Faecal coliform, Salmonella*
Urine	*Faecal coliform, Faecal enterococci, Salmonella*
Greywater	*E. coli/ Faecal coliform, Salmonella, Faecal enterococci*

For all these parameters the microbiological analysis will be used.

2.2. Microbiological analysis of matrix (soil, compost, urine, and greywater)

2.2.1. Enumeration of bacteria in soil and compost

Compost or soil samples 25 g (w/v) were homogenized in 225 mL of buffer phosphate water and a 10-fold dilution series was performed in maximum recovery diluents (ringer solution). Fecal coliforms and *E. coli* and *Enterococci* were cultured following a method 9215 A in Standard Methods for the Examination of Water and Wastewater (APHA, 1998). Relevant dilutions were spread on plates in duplicate on the following selective media; chromo cult coliform agar ES (Difco, France) incubated at 44,5°C and for 24 h for Fecal Coliforms, *E. coli*, and *Salmonella*, Slanetz Bartley agar at 37°C for 48 h for *Enterococci*. The bacteria load is expressed in (log_{10} UFC/g-DW soil or compost) through the equation 1:

$$\boldsymbol{N = (log_{10}\left(\frac{n}{\frac{P}{Vl}\times V\times d}\right)\times DW)} \quad \textbf{(Equation 1)}$$

Where:

N = Bacteria load in compost or soil (Log_{10} UFC/g- DW- soil or compost);

n = Number of colonies in box of Petri;

P = Weight of compost or soil samples (25g);

V_l = Volume of Buffer phosphate used to homogenization of compost or soil samples;

V = Volume of test (1 mL);

d = factor of dilution.

DW= Dry weight is expressed by this equation below:

$$\frac{M1-M0}{M2-M0} \qquad \textbf{(Equation 2)}$$

Where:

M_1= 10g fresh weight + empty weight of tube,

M_2= 10g-dry weigth+ empty weight of tube,

M_0= empty weight of tube.

2.2.2. Enumeration of bacteria in urine

The description of *E. coli and Faecal Coliform* (FC) or *Enterococci* was done by the method of culture of spreading out in depth. The samples were diluted with sterile ringer. After dilution, 1 mL of the diluted sample was spread out over media (Chromocult Agar for *E. coli/Faecal coliform* and Slanetz Bartley for Enterococci), contained in box of Petri which were then carried to the drying oven for incubation with 44 °C during 24h for *E. coli*/Faecal coliform and with 37 °C during 48 h for Faecal Enterococci. *E coli* were identified by blue colorant purple and Faecal Enterococci by whitish. The colonies obtained were counted thereafter and numbers obtained was allotted to the number of E coli or enterococci present in the sample. This is why the concentration is expressed in unit forming colony (UFC) reported to 100 mL of sample. Bacteria load is expressed by equation (2):

$$N = \frac{n}{V \times d} \times Vs \qquad \textbf{(Equation 3)}$$

Where:

N = Concentration of bacteria in urine (UFC/100 mL);

n = Number of colonies in box of Petri;

Vs = Reference volume (100 mL);

V = Volume of test (1 mL);

d = dilution factor.

2.2.3. Enumeration of bacteria in greywater

The description of *E. coli and Faecal coliform* (FC) or *Enterococci* was done by the method of culture of spreading out in surface. The samples were diluted with sterile ringer. After dilution, 0.1 mL of the diluted sample was spread out over media (Chromo cult Agar for *E. coli/Faecal coliform* and Slanetz Bartley for Enterococci), contained in box of Petri which were then carried to the drying oven for incubation with 44 °C during 24h for *E. coli*/Faecal coliform and with 37 °C during 48 h for Faecal Enterococci. *E coli* were identified by blue color and purple and Faecal Enterococci by whitish. The colonies obtained were counted thereafter and numbers obtained was allotted to the number of *E coli* or enterococci present in the sample. This is why the concentration is expressed in unit forming colony (UFC) reported to 100 mL of sample. Bacteria load is determined by equation 2 above in similar conditions.

2.2.4. Enumeration of *Salmonella*

- Compost and soil

Compost or soil samples 25 g (w/v) were homogenized in 225 mL of buffer phosphate water and a 10-fold dilution series was performed in maximum recovery diluents (ringer solution). 10 mL of Rappaport Vassiliadis media were added in test tubes of different dilutions (10^0 to 10^{-6}) where three to five repetitions are made per dilution and 1 mL of sample is added in the test tubes. It is illustrated by figure 3 below. Then, test tubes are introduced in incubator during 24h at 37°C for testing process before sowing in ChromAgar media on Petri box and then incubating at 37°C during 24h to confirm the result of first observation. Final result is obtained by the tables of Mac Grady (annex i) where it is expressed in Most Numbers Probable per gramme (MNP/g).

- Urine and greywater

Process is similar as compost and soil analysis (figure 3). However, dilution is made directly without homogenization with buffer phosphate water. Final result is expressed in Most Numbers Probable per liter (MNP/L).

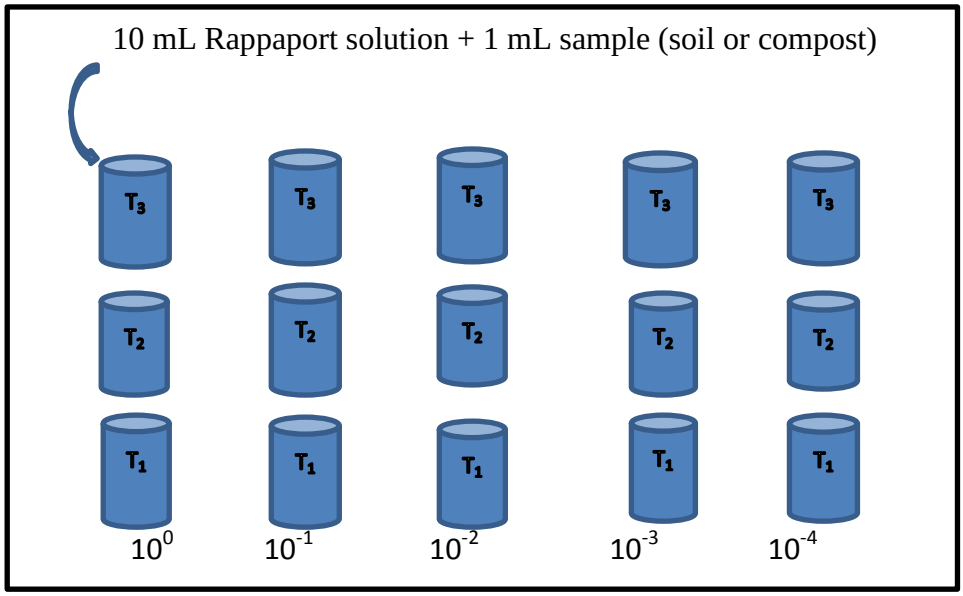

Figure 3: Illustration of step of *Salmonella* analysis

2.2.5. Enumeration of helminth eggs in soil and compost

Briefly, analysis was performed on compost or soil and was based on the recognition of forms and structure of helminth eggs in microscope. Sludge was prepared by adding 225 mL of 0.1% Tween 80 to 25g compost sample. The mixture was homogenized for 1 min using a blender and screened through 4 layers of wet gauze folded. The filtrate was collected in round bottom flasks and allowed to settle for 3 hours and submitted to analysis. Helminth eggs were determined by the US EPA protocol (1999) modified by Schwartzbrod (2003) with a modified density of zinc sulfate ($ZnSO_4$) saline solution. Quantification of Helminth eggs is made through the equation 3:

$$N = (\sum \frac{helminth\ eggs\ present}{V}) \times k \quad \textbf{(Equation 4)}$$

Where:

N = Number of helminth eggs/L

V= Volume of initial sample compost or soil (225 mL);

k = Constant related to the performance of the method (k = 1.42).

Then, result of equation 3 is reduction of the weight of dry compost or soil diluted (25g) where the final result is expressed in eggs/g.

2.3. Following up indicators of pathogen on lettuce leave

Sampling consisted in taking 100 g of vegetable matter at the stage of maturity on each of the 3 repetitions, which are representing 4 samples of each treatment. The collection was carried out in the respect of the conditions of hygiene and of sterility necessary and the samples are preserved at 4 °C until the moment of the analyses which take place within the next 24 h. The analyses relate to the surface of the sheets of lettuce. A quantity of 10 g of lettuce leaves of each treatment was introduced into sterile bottles with broad collar provided with a lid. Each bottle was completed with 90 ml of a solution of NaCl with 1N, then closed and agitated during 15 minutes in horizontal position on a plate agitator. For each flushing water representing a suspension mother of 10^{-1}, two decimal dilutions at 10^{-2} and 10^{-3} were carried out with the NaCl solution with 1N. The suspension mother and dilutions were sown by spreading out of 0.1 mL on the culture media (Chromocult Agar or Slanetz Bartley according to the required type of indicators) cast in boxes of sterile Petri 90 mm in diameter. Each dilution was the two object repetitions.

3. Quantitative Microbial Risk Analysis (QMRA) methods

3.1. Hazard identification

All pathogens that are excreted in human excreta and greywater from insanitary and unhygienic surrounding environment could potentially be found in irrigation waters and vegetables (Nana O.B. Ackerson and Esi Awuah, 2012). A selection of pathogens was made for the risk assessment, representing bacteria (*Salmonella* and *E.coli*) and helminthes (*A. lumbricoides*). From epidemiological reviews, helminthes and bacteria pose the greatest health risks in human excreta and greywater reuse in agriculture (WHO, 2006a); (Mara and Sleigh, 2010b). The choice of *Ascaris* was due to its persistence for months to years in soil under harsh conditions (Amoah et al., 2005) thus making it an ideal reference organism for QMRAs in developing country (Nana O.B. Ackerson and Esi Awuah, 2012) such as Burkina Faso.

3.2. Exposure assessment

Exposure scenarios were identified from 2 target groups of population: farmers and urban consumers.

3.2.1. For farmers

We assume that during spreading compost, urine and irrigation with greywater, farmers did not wear protective clothing and were in direct contact with the different matrix (compost urine and greywater). Furthermore compost which is used to spread in our experimental site is not totally hygienic and mature. It was spread 2 days after it was taken away from family pilot to Ziniaré. Compost is used like basic manure before plant out lettuce. It carried out 1 time per cycle of lettuce crop. Variety of lettuce crop on our site has 50 days as total cycle. Farmers can ingest 100 mg of compost accidentally when they spread it in the field (Schönning et al., 2007). In rainy season, farmers do not grow lettuce crop now in Burkina Faso, rainy season can take 3 months per year. Thus, farmers can be exposed 5 times per year. Concerning urine, we used urine which is stored during 1 week before spreading in our experimental site. For doing so, we used a small bucket for spreading. Urine is applied 3 times per cycle for lettuce crop. Farmers can ingest accidentally 0.43 mL of urine when they spread it in the field after making experimentation (Annex ii). Also farmers spread urine without wear protective clothing. Exposition frequency is 15 times per year. Greywater was used to irrigate lettuce crop with watering cans. Farmers can ingest accidentally 1 to 2 mL of greywater (Nana O.B. Ackerson and Esi Awuah, 2012) during irrigation of the lettuce crop. The exposure days per year to irrigation greywater are 275 days.

Concerning soil ingestion, farmers can ingest accidentally 10 to 100 mg of soil (Haas et al., 1999) contaminated with compost, urine and greywater when they work in fields. We assume that field workers are directly in contact with soil when they are spreading compost, planting out lettuce crops, and weeding the field. Those activities can occur 4 times per cycle. Therefore, the exposure days per year for those activities are 20 days.

3.2.2. From consumers

According to Shuval et al., (1997) 10.8 mL of irrigation water will be left on a 100 g lettuce after harvest. There are two days between lettuce harvest and consumption (WHO, 2006a). The amount of lettuce consumed per person per day was taken as 100 g at a rate of one lettuce per week per consumer in developing country (Shuval et al., 1997); (Nana O.B. Ackerson and Esi Awuah, 2012) such as Burkina Faso. Thus, a consumer can be exposed 52 times per year. The exposure scenarios of different matrix for farmers and consumers are summarized in table 3 below.

Table 3: Different exposure scenarios and pathways which farmers and consumers can be exposed in different cases

Target population	Matrix of manipulation	Exposure scenario	Quantity ingested	Frequency exposed (events/year)
Farmers	Compost	Handle without protection individual (glove, mask,…) before to spread compost	10-100mg[a]	5
	Urine	Handle urine in the field with a small bucket and use this hand to eat without washing it	0.43 mL*	15
	Soil	Ingestion of soil contaminated with greywater, compost or urine.	10-100mg[b]	20
	Greywater	Ingestion of greywater from the irrigation system (watering cans or bucket)	1-2mL[c]	275
Consumers	Lettuce harvest	Accidental ingestion Consumers can eat lettuce without washing it	10.8mL/100g[d]	52

***a*=(Schönning et al., 2007) *; b*=(Haas et al., 1999) *; c*=(Nana O.B. Ackerson and Esi Awuah, 2012)*; d*=(Shuval et al., 1997). *= Protocol of determination of amount of urine ingested (annex 2).**

3.3. Dose-response assessment

For dose-response relationships, the beta-Poisson dose-response model described by Haas et al., (1999) was used for *Salmonella*, *Ascaris*. However, single-hit exponential dose-response can be applied for *Salmonella* and *Ascaris.* Dose-response parameters for exponential and beta-Poisson models from various enteric pathogen ingestion studied by different authors were summarized in table 4 below. To calculate microbial risk, uncertain values (minimum and maximum values) of pathogen amounts will use to evaluate risk for each treatment.

Single-hit exponential model:

$$\boldsymbol{P_{inf}(r, d) = 1 - \exp(-rd)} \quad \textbf{(Equation 5)}$$

Beta-Poisson model:

$$\boldsymbol{P_{inf}(d,\ \alpha,\ N_{50}) \approx 1 - [1 - \frac{d}{N_{50}}\left(2^{1/\alpha} - 1\right)]^{-\alpha}} \quad \textbf{(Equation 6)}$$

Where $\boldsymbol{P_{inf}} =$ the probability of infection which is a function of r and d

$\boldsymbol{r}$ = empirical parameter assumed to be constant for any given host and given pathogen picked to fit the data

$\boldsymbol{d} =$ Mean ingested dose, $\mathbf{N_{50}}$= the median dose, $\boldsymbol{\alpha}$ **and** $\boldsymbol{\beta}$**= slope** parameters, which hold when $\beta \geq 1$ and $\alpha \leq \beta$.

The annual probability of infection is given by:

$$\boldsymbol{P_{yr} = 1 - (1 - P_{inf})^n \approx nP_{inf}} \quad \textbf{(Equation 7)}$$

Where P_{yr} = acceptable annual risk of infection caused by a pathogenic organism

n = number of exposure events per year (events/yr).

A QMRA model for broccoli, cucumber, lettuce, and three cultivars of cabbage constructed by Hamilton et *al*. (2006) was used to calculate the daily dose of pathogenic organism on the lettuce. The beta -Poisson and exponential dose -response models were subsequently used to calculate the probability of infection (Nana O.B. Ackerson and Esi Awuah, 2012).

The daily dose of pathogens, λ=d, taken as a result of consuming the lettuce was calculated as:

$$\boldsymbol{\lambda = M_i M_{body} c_{iw} V_{prod} e^{(-kt)}} \quad \textbf{(Equation 8)}$$

Where,

M_{body} = human body mass (kg)

M_i = daily consumption per capita per kg of body mass [g (kg.ca.day)$^{-1}$]

c_{iw}= concentration of pathogens in irrigation water

V_{prod}= volume of irrigation water caught by product (mL.g^{-1})

k = pathogen kinetic decay constant (day^{-1})

t = time between last reclaimed - water irrigation event and harvest/consumption/storage (day).

M_{body} = 71.8 kg

From survey, M_i = 1.6713 g. (kg.ca.day)$^{-1}$

V_{prod} = 0.125 mL g^{-1} ; t = 2 d.

Table 4: Summary of dose-response parameters for exponential and beta-Poisson models from various enteric pathogen ingestion studies

	Exponential		beta-Poisson	
Constituent	**r**	**α**	**β**	**N_{50}**
Escherichia coli		0.1705[a]	1.61 x 10^{6}[a]	
Salmonella	0,00752[a]	0,313[b]		23600[b]
Ascaris	1[b]	0,104[c]		859[c]

***a*= (Metcalf & Eddy, 2007); *b*= (Schönning et al., 2007); *c*= (Mara and Sleigh, 2010b)**

3.4. Risk characterization

Hazard identification, exposure assessment and dose-response components were integrated to obtain a risk estimate and then comparing this risk estimated with the acceptable annual risk of infection according to WHO guidelines which recommend 10^{-6} DALY. The framework of steps of Monte Carlo method is shown in figure 4.

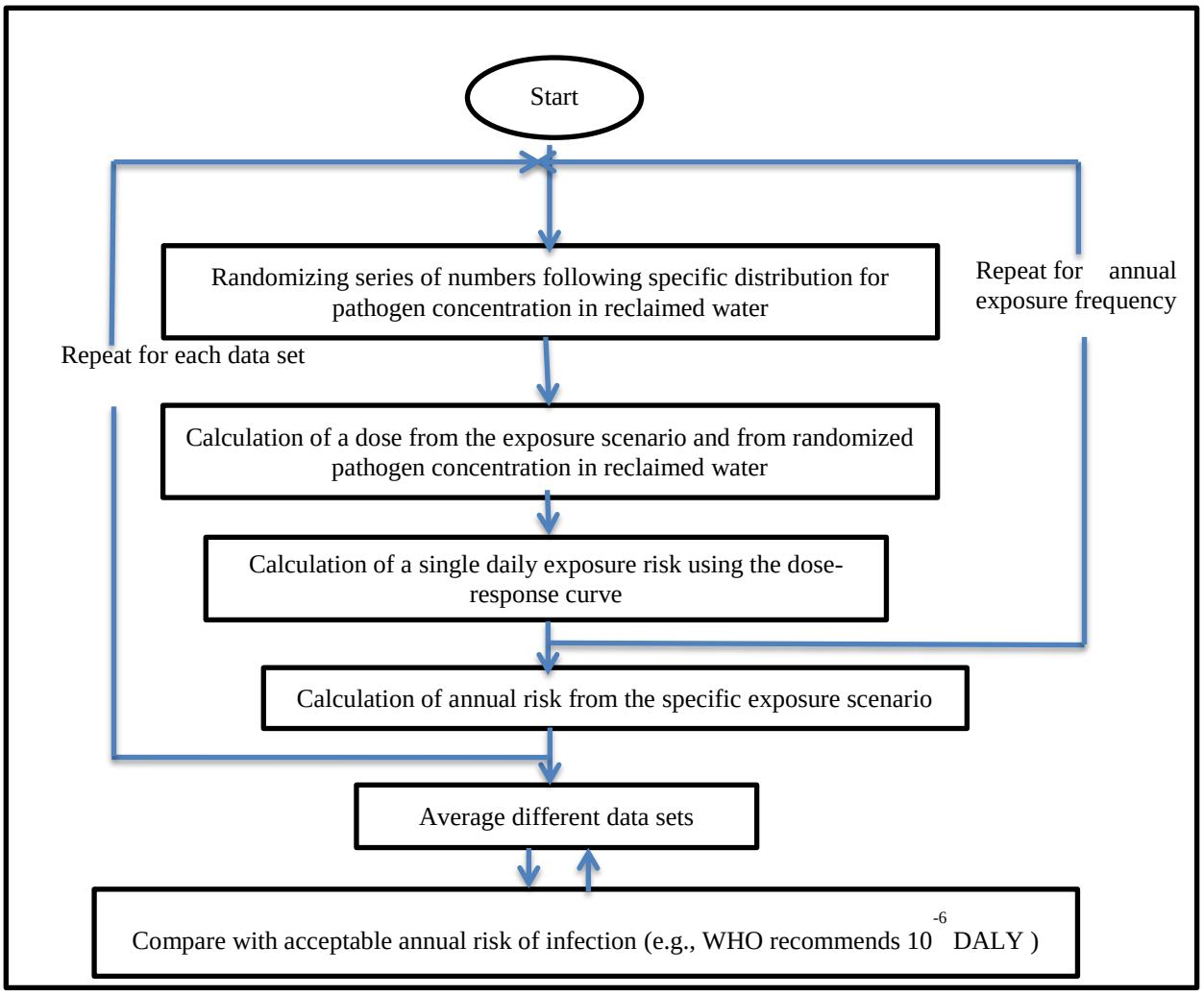

Figure 4: Steps of calculation of Monte Carlo

IV. RESULTS AND DISCUSSION

1. Results

1.1. Quantitative Microbial Risk Assessment from different treatments

1.1.1. From compost and top water (C+TW) treatment

Annual probabilities of *Salmonella* and *Ascaris* infection related to soil ingestion when farmers use only compost to spread in the field and when lettuce harvest is eaten by consumers are showed by table 5 below. Annual risks of *Salmonella* infection in scenarios where it is assumed that farmers can ingest accidentally soil, is $3.87x10^{-3}$ pppy (Annex iii). That is where a risk is possible for one infection of *Salmonella* per 1000 farmers per year. Concerning Ascaris infection, annual risks is $4.67x10^{-2}$ (soil ingestion accidentally) (Annex v). That is where there may be a risk of one infection of *Ascaris* per 100 farmers when they use compost in field.

For lettuce consumption, *Salmonella* annual risk infection is $1.54x10^{-1}$ (i.e. one infection of *Salmonella* per 10 consumers per year) (Annex iv). And Ascaris infection risk is $2.41x10^{-2}$ (Annex vi) i.e. one infection of *Ascaris* per 100 consumers of lettuce per year.

Table 5: Annual probabilities of *Salmonella* and *Ascaris* infection associated with the ingestion of soil combined with compost and consumption of lettuce

Pathogens	Soil Ingestion		Lettuce consumption	
	P_{inf}	$P_{yr}(n=20)$	P_{inf}	$P_{yr}(n=52)$
Salmonella	$1.94x10^{-4}$	$3.87x10^{-3}$	$2.96x10^{-3}$	$1.54x10^{-1}$
Ascaris	$2.33x10^{-3}$	$4.67x10^{-2}$	$4.63x10^{-4}$	$2.41x10^{-2}$

Annual infection risks of *Salmonella* and *Ascaris* which are compared with WHO guideline values in red line according to both scenarios are showed by figure 5 below.

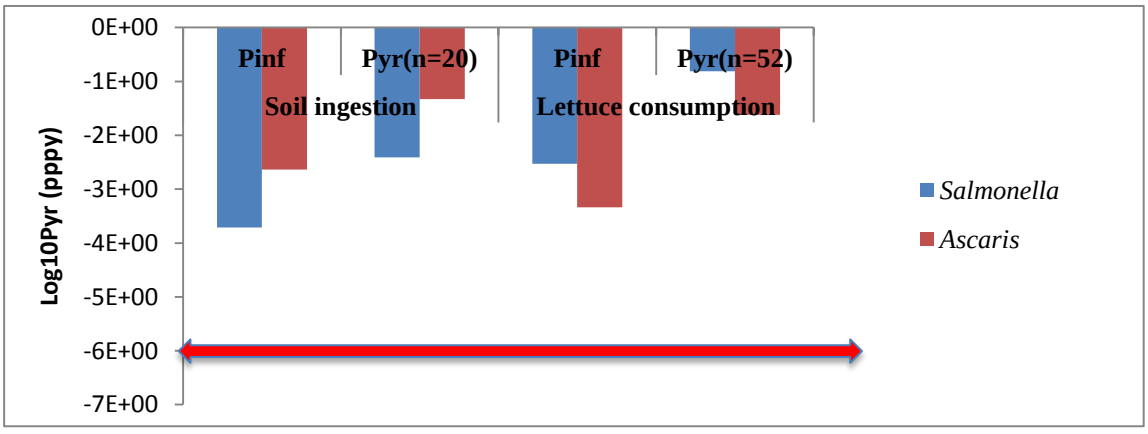

Figure 5: Annual infection risks of *Salmonella* and *Ascaris* in function of scenarios compared with WHO guideline value.

1.1.2. For urine and top water (U+TW) treatment

Annual probabilities infection of *Salmonella* associated with the soil ingestion combined with urine and lettuce consumption are summarized in table 6 below.

Salmonella annual risk infection in a scenario which assumes that farmers can ingest accidentally soil spread with urine is $9.55x10^{-1}$ (Annex vii). It means one infection of *Salmonella* per 10 farmers per year when they use urine to spread in field.

Table 6: Annual probabilities of *Salmonella* infection associated with the ingestion of soil combined with urine and consumption of lettuce

Pathogen	Soil ingestion		Lettuce consumption	
	P_{inf}	$P_{yr}(n=20)$	P_{inf}	$P_{yr}(n=52)$
Salmonella	$4.78x10^{-2}$	$9.55x10^{-1}$	$2.50x10^{-9}$	$1.30x10^{-7}$

For lettuce consumption, annual risk is $1.30x10^{-7}$ (Annex viii) i.e. one infection of *Salmonella* per 10000000 consumers of lettuce per year.

Annual infection risks of *Salmonella* which are compared with WHO guideline values in red line according to both scenarios are showed by figure 6 below.

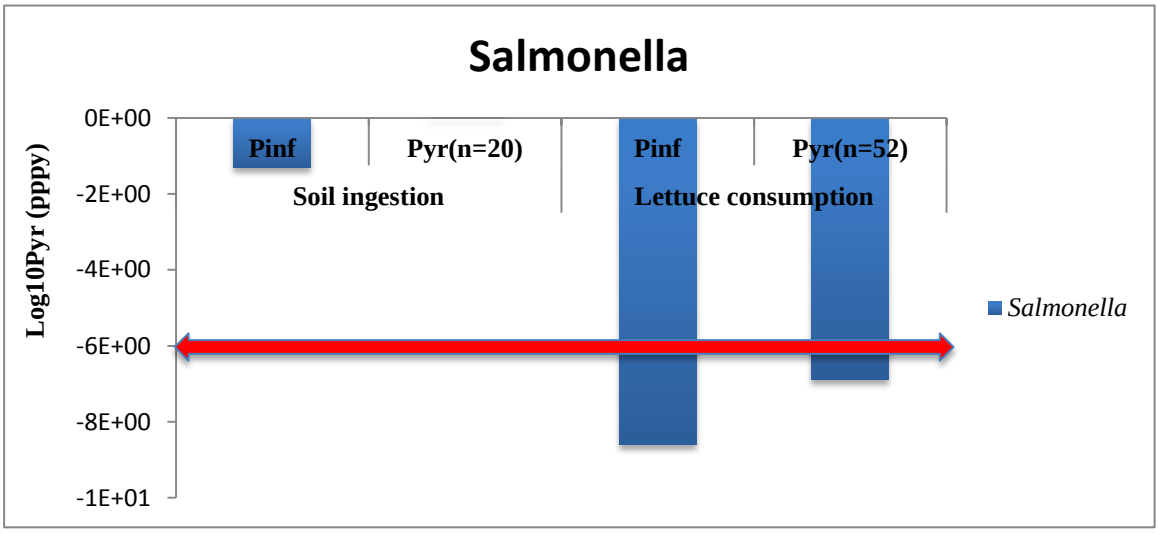

Figure 6 : Annual infection risks of *Salmonella* in function of scenarios compared with WHO guideline value.

1.1.3. From greywater only (GW) treatment

Annual probabilities of infection from *Salmonella* associated with the soil irrigated with greywater and lettuce consumption are summarized in table 7 below. *Salmonella* annual risks

infection in scenario which assumes that farmers ingest accidentally 10 to 100 mg of soil irrigated with greywater is $8.89x10^{-6}$ (Annex ix). It means that one infection of *Salmonella* per 1000000 farmers per year when they are exposure 20 days per year.

From ingestion of irrigation greywater, annual risk infection is $1.02x10^{-4}$ (Annex x) i.e. one infection of *Salmonella* per 10000 farmers per year for 275 days of exposure in worst case.

Table 7: Annual probabilities of *Salmonella* infection associated with the soil and greywater ingestion combined with greywater and lettuce consumption

	Soil ingestion		Irrigation greywater		Lettuce consumption	
Pathogen	P_{inf}	$P_{yr}(n=20)$	P_{inf}	$P_{yr}(n=275)$	P_{inf}	$P_{yr}(n=52)$
Salmonella	$4.45x10^{-7}$	$8.89x10^{-6}$	$3.69x10^{-7}$	$1.02x10^{-4}$	$1.81x10^{-5}$	$9.42x10^{-4}$

Concerning lettuce consumption, *Salmonella* risk infection is $9.42x10^{-4}$ (Annex xi).i.e. one infection of *Salmonella* per 10000 consumers of lettuce leaves per year when they eat it during 52 days per year.

Annual infection risks of *Salmonella* which are compared with WHO guideline values in red line according to three scenarios are showed by figure 7 below.

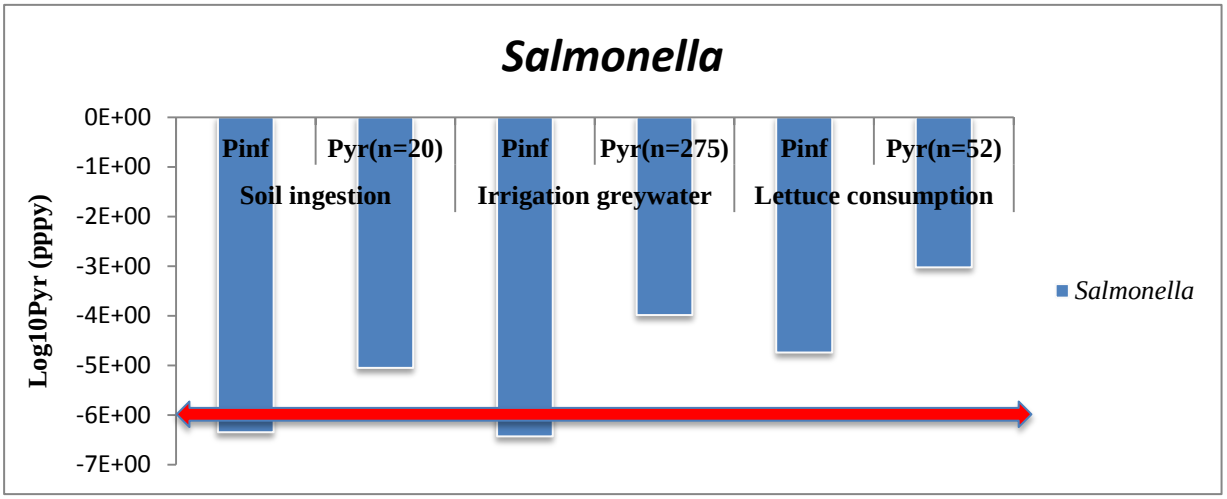

Figure 7 : Annual infection risks of Salmonella in function of scenarios compared with WHO guideline value.

1.1.4. For compost, urine, ant greywater (C+U+GW) treatment

Annual risks infection of *Salmonella* and *Ascaris* are showed by table 8 according to 3 scenarios (soil ingestion, ingestion irrigation greywater and lettuce consumption).

From soil ingestion, *Salmonell*a annual risk infection is $1.44x10^{-4}$ (Annex xii). That is when there will be a risk of one infection of *Salmonella* per 10000 farmers when farmers are exposure during 20 days per year. For *Ascaris* infection, risk is $4.67x10^{-2}$ (Annex xiii). That means one infection of Ascaris per 100 farmers during 20 days exposure per year.

Table 8: Annual probabilities of *Salmonella* and *Ascaris* infection associated with the soil and greywater ingestion combined with compost, urine and greywater and lettuce consumption

Pathogens	Soil ingestion		Irrigation greywater		Lettuce consumption	
	P_{inf}	$P_{yr}(n=20)$	P_{inf}	$P_{yr}(n=275)$	P_{inf}	$P_{yr}(n=52)$
Salmonella	$7.21x10^{-6}$	$1.44x10^{-4}$	$5.58x10^{-6}$	$1.53x10^{-3}$	$2.50x10^{-8}$	$5.00x10^{-7}$
Ascaris	$2.33x10^{-3}$	$4.67x10^{-2}$	$1,44x10^{-3}$	$3.97x10^{-1}$	$4.63x10^{-4}$	$2.41x10^{-2}$

From ingestion of irrigation greywater, *Salmonell*a annual risk infection is $1.53x10^{-3}$ (Annex xiv). That means there will be a risk of one infection of *Salmonella* per 1000 farmers per year during 275 days of exposure. For *Ascaris* infection, risk is $3.97x10^{-1}$ (Annex xv). It means one infection of *Ascaris* per 10 farmers during 275 days of exposure per year.

From lettuce consumption, *Salmonell*a annual risk infection is $5.00x10^{-7}$ (Annex xvi). That means there will be a risk of one infection of *Salmonella* per 1000000 consumers of lettuce leaves per year during 52 days of exposure. For *Ascaris* infection, risk is $2.41x10^{-2}$ (Annex xvii). It means that one infection of *Ascaris* per 100 farmers during 52 days of exposure per year.

Annual infection risks of *Salmonella and Ascaris* which are compared with WHO guideline in red line values according to three scenarios are showed by figure 8 below.

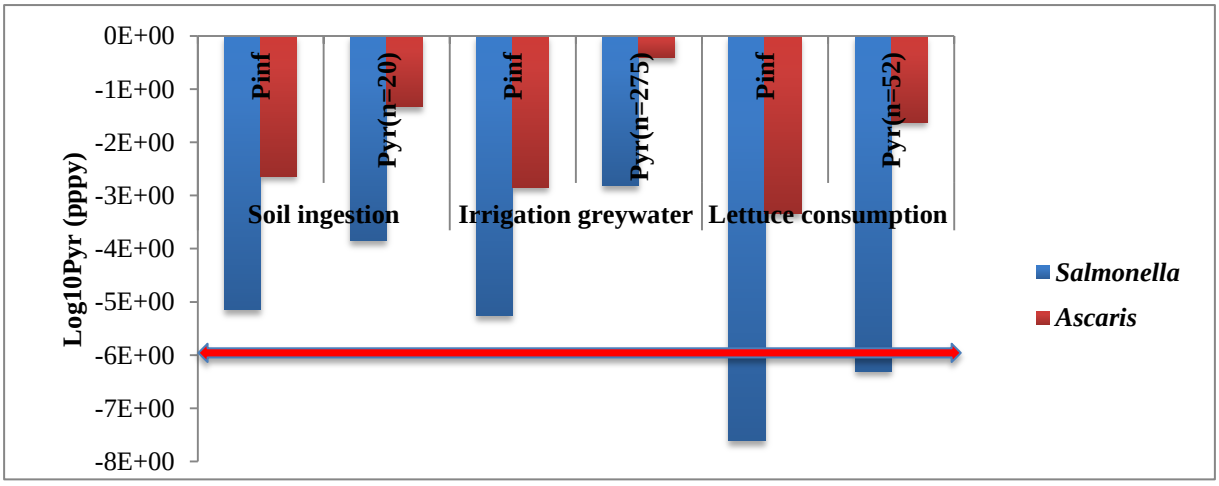

Figure 8: Annual infection risks of *Salmonella* and *Ascaris* in function of scenarios compared with WHO guideline value.

1.2. Comparison of the probabilistic values of different treatments related with the scenarios

1.2.1. For soil ingestion scenario

The probabilistic values of all treatments compared with the WHO guideline values of risk for soil ingestion scenario are showed in the table 9 below. *Salmonella* annual risk of infection in worst case from U+TW ($9.55x10^{-1}$) is higher than C+TW ($3.87x10^{-3}$), C+U+GW ($1.44x10^{-4}$) and GW ($8.89x10^{-6}$) for soil ingestion. The annual risk of infection in all treatment exceeded the tolerable risk of $\leq 10^{-6}$ per person per year (WHO, 2006a). *Ascaris* annual risks of infection in worst case from C+TW and C+U+GW are equal ($4.67x10^{-2}$), however, this probabilistic values are higher than WHO guideline values (2006).

Table 9 : Probabilistic values of different treatments compared with the WHO guideline values of the risk.

	Soil ingestion		WHO guidelines values
	Pathogens		
Treatment	***Salmonella***	***Ascaris***	
C+TW	$3.87x10^{-3}$	$4.67x10^{-2}$	
U+TW	$9.55x10^{-1}$	NA	$\mathbf{10^{-6}}$
GW	$8.89x10^{-6}$	NA	
C+U+GW	$1.44x10^{-4}$	$4.67x10^{-2}$	

C+TW=Compost +Top water; U+TW=Urine + Top water; GW=Greywater; C+U+GW=Compost + Urine + Greywater.

The probabilistic values of all treatments compared with the WHO guideline values in red line of risk for soil ingestion scenario are showed by figure 9 below.

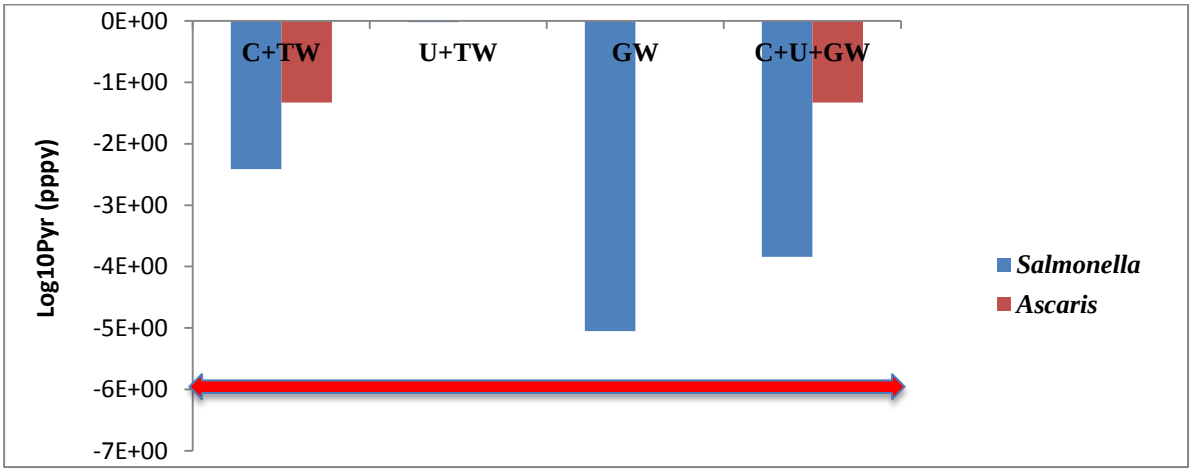

Figure 9 : Probabilistic values of all treatments compared with the WHO guideline values of risk for soil ingestion scenario

1.2.2. Ingestion of irrigated greywater

The probabilistic values of all treatments compared with the WHO guideline values of risk for ingestion of irrigated greywater scenario are showed in the table 10 below. *Salmonella* annual risk of infection in worst case from C+U+GW ($1.53x10^{-3}$) is higher than GW ($1.02x10^{-4}$). The annual risk of infection in all treatment exceeded the tolerable risk of $\leq 10^{-6}$ per person per year (WHO, 2006a).

Table 10: Probabilistic values of Greywater and Compost, Urine and Greywater treatments compared with the WHO guideline values of the risk.

Pathogen	Treatment		WHO guideline values
	GW	C+U+GW	
Salmonella	$1.02x10^{-4}$	$1.53x10^{-3}$	10^{-6}

Probabilistic values of Greywater and Compost, Urine and Greywater treatments compared with the WHO guideline values of the risk are showed by figure 10 below.

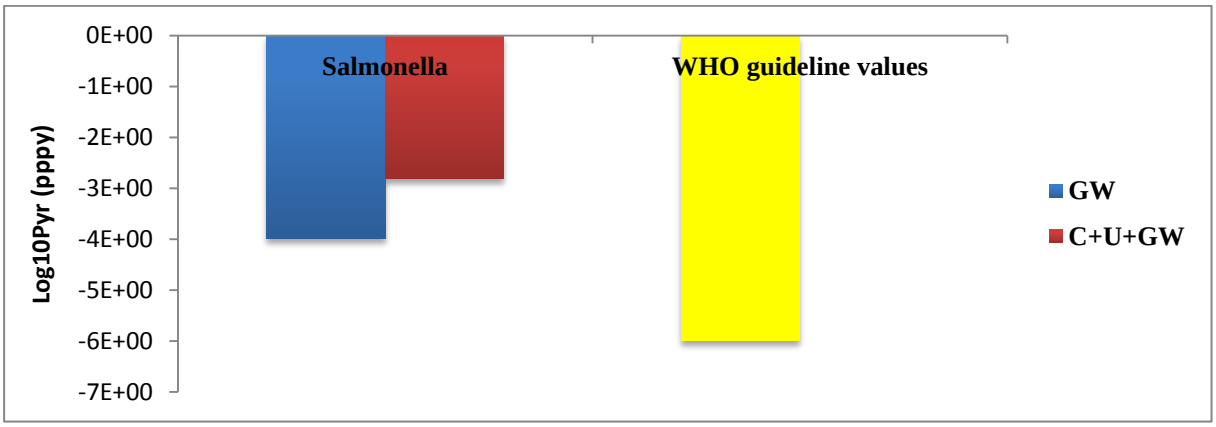

Figure 10 : Probabilistic values of Greywater and Compost, Urine and Greywater treatments compared with the WHO guideline values

1.2.3. Lettuce consumption

The probabilistic values of all treatments compared with the WHO guideline values of risk for lettuce consumption scenario are showed in the table 11 below. *Salmonella* annual risk of infection in worst case from C+TW ($1.54x10^{-1}$) is higher than U+TW ($1.30x10^{-7}$), C+U+GW ($5.00x10^{-7}$) and GW ($9.42x10^{-4}$).

The annual risk of infection in all treatment exceeded the tolerable risk of $\leq 10^{-6}$ per person per year (WHO, 2006a). *Ascaris* annual risks of infection C+TW and C+U+GW are equal ($4.67x10^{-2}$), however, this probabilistic values are higher than WHO guideline values (2006).

Table 11: Probabilistic values of different treatments compared with the WHO guideline values of the risk.

Pathogens	Treatment				WHO guideline values
	C+TW	U+TW	GW	C+U+GW	
Salmonella	$1.54x10^{-1}$	$1.30x10^{-7}$	$9.42x10^{-4}$	$5.00x10^{-7}$	10^{-6} pppy
Ascaris	$2.41x10^{-2}$	NA	NA	$2.41x10^{-2}$	

The probabilistic values of all treatments compared with the WHO guideline values of risk for lettuce consumption scenario are showed by figure 11 below.

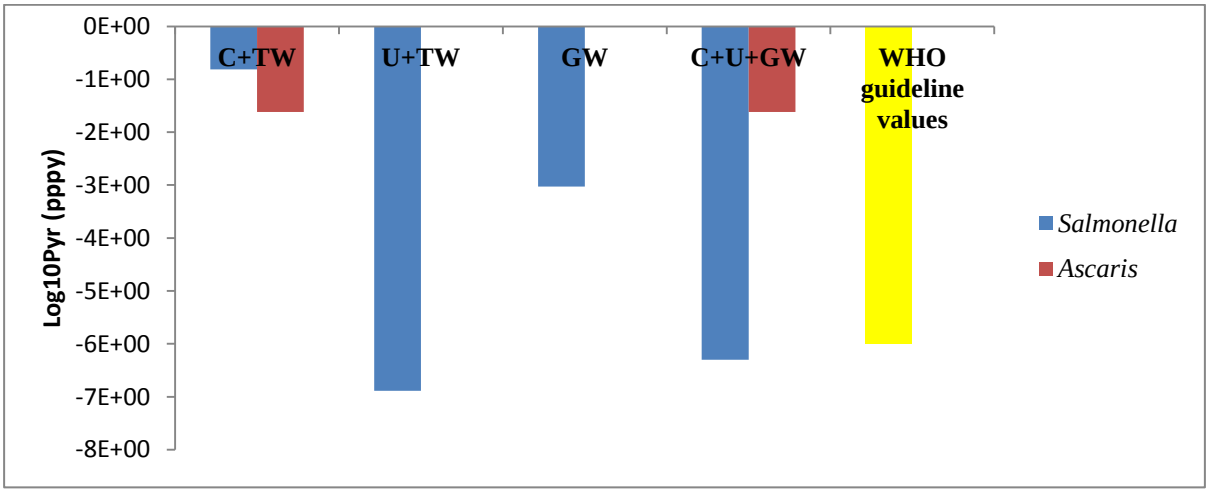

Figure 11: Probabilistic values of all treatments compared with the WHO guideline values of risk for lettuce consumption scenario

2. Discussion

2.1. Quantitative Microbial Risk Assessment from each treatment.

2.1.1. For compost and top water treatment (C+TW)

Annual probabilities of *Salmonella* and *Ascaris* infection related to soil ingestion when farmers use only compost to spread in the field and when lettuce harvest is eaten by consumers are showed by figure 5 above.

Annual risks of *Salmonella* infection in scenarios where it is assumed that farmers can ingest accidentally 10 to 100 mg of soil is $3.87x10^{-3}$ pppy. That means there will be a risk of one infection of *Salmonella* per 1000 farmers per year. *Salmonella* risk infection ($3.87x10^{-3}$) for accidental soil ingestion is relatively high and exceeds the benchmark in this scenario by a 3 order magnitude (10^{-3}). Thus farmers may be at risk of contracting typhoid fever (Westrell, 2004) and (Nana O.B. Ackerson and Esi Awuah, 2012). Concerning *Ascaris* infection, annual risk is $4.67x10^{-2}$ (soil ingestion accidentally). That means there will be a risk of one infection of *Ascaris* per 100 farmers when they use compost in field. The annual infection risk is relatively high and exceeds the benchmark by a 4 order of magnitude (10^{-4}). According to Amoah et al., (2011) farmers may be at risk of contracting ascariasis.

From lettuce consumption, *Salmonella* annual risks infection is $1.54x10^{-1}$ (i.e. one infection of *Salmonella* per 10 consumers per year). *Salmonella* annual risk infection is relatively high and exceeds the benchmark by a 5 order of magnitude (10^{-5}). Consumers may be at risk of contracting typhoid fever when they eat lettuce leaves (Mara et al., 2010). *Ascaris* infection risk for lettuce consumption is $2.41x10^{-2}$ i.e. one infection of *Ascaris* per 100 consumers of lettuce per year. The annual infection risk is relatively high and exceeds the benchmark by a 4 order of magnitude (10^{-4}). According to Amoah et al., (2011) consumers may be at risk of contracting ascariasis.

Any single pathogen that is ingested can multiply and form a clone which is capable of causing infection (Haas et al., 1993). The annual risk of infection for all pathogens in both scenarios exceeded the tolerable risk of $\leq 10^{-6}$ per person per year (WHO, 2006a).

2.1.2. For urine and top water (U+TW) treatment

Annual probabilities infection of Salmonella associated with the soil ingestion combined with urine and lettuce consumption are summarized in figure 6 above.

Salmonella annual risk infection in scenario which assumes that farmers can ingest accidentally soil spread with urine is $9.55x10^{-1}$. It means one infection of *Salmonella* per 10 farmers per year when they use urine to spread in field. This is high and exceeds the benchmark by 5 orders of magnitude. Thus, farmers may be at risk of contracting diseases (Höglund, 2001).

For lettuce consumption, annual risk is respectively $1.20x10^{-7}$ and $1.30x10^{-7}$ for 50 and 95 percentile i.e. one infection of *Salmonella* per 10000000 consumers of lettuce per year. Risk is relatively low and respects the tolerable risk recommended (10^{-6} pppy) by WHO guidelines (WHO, 2006b). In this scenario consumers may eat lettuce leaves without any high risk (Carr, 2005).

2.1.3. For greywater only (GW) treatment

Annual probabilities of infection by *Salmonella* associated with the soil irrigated with greywater and lettuce consumption are summarized in figure 7 above.

Salmonella annual risks infection in a scenario which assumes that farmers ingest accidentally 10 to 100 mg of soil irrigated with greywater is $8.89x10^{-6}$. It means that one infection of *Salmonella* per 1000000 farmers per year when they is exposure of 20 days per year. This result complies with the tolerable annual risk (10^{-6} per person per year) recommended by WHO, (2006a). This exposure do not constitute a public health from farmers (Zuma and Tandlich, 2010).

For irrigation with greywater (farmers can ingest 1 to 2 mL), annual risk infection is $1.02x10^{-4}$ i.e. one infection of *Salmonella* per 10000 farmers per year for 275 days of exposure in worst case. The order of magnitude is 2. In this situation, farmers may be at risk of contracting diseases according to WHO, (2006a) guidelines.

It is concerning lettuce consumption, *Salmonella* risk infection is $9.42x10^{-4}$ .i.e. one infection of *Salmonella* per 10000 consumers of lettuce leaves per year when they eat it during 52 days per year. The magnitude is 2 orders compared to WHO recommendations (10^{-6} pppy). These

lettuce leaves cannot eat because of high load remain on leaves when they irrigate it with greywater. However last irrigation before harvest must be considered (2 days) in evaluation of risk according to Shuval et al., (1997) and WHO, (2006a).

2.1.4. For compost, urine, and greywater (C+U+GW) treatment

Annual risks infection of *Salmonella* and *Ascaris* are showed by figure 8 above according to 3 scenarios (soil ingestion, ingestion irrigation greywater and lettuce consumption).

For soil ingestion, *Salmonell*a annual risk infection is $1.44x10^{-4}$. That means there will be a one infection of *Salmonella* per 10000 farmers for the worst case when farmers are exposed during 20 days per year. This is relatively high and exceeded the benchmark 2 order of magnitude compared to WHO guidelines (10^{-6} pppy). Farmers may be at risk of contracting salmonellosis or typhoid fever (Nana O.B. Ackerson and Esi Awuah, 2012). For Ascaris infection, risk is $4.67x10^{-2}$. It means one infection of Ascaris per 100 farmers during 20 days exposure per year. This is relatively high and exceeds the benchmark by 4 order of magnitude compared to WHO guidelines (10^{-6} pppy). Farmers can be infected by ascariasis.

From ingestion of irrigation greywater, *Salmonell*a annual risk infection is $1.53x10^{-3}$. That means there will be a risk of one infection of *Salmonella* per 1000 farmers per year during 275 days of exposure. This is high and exceeds the benchmark in both cases by 3 orders of magnitude (10^{-3}). Farmers may be at risk of contracting salmonellosis or typhoid fever (Höglund et al., 1998). For *Ascaris* infection, risk is $3.97x10^{-1}$. It means one infection of Ascaris per 10 farmers during 275 days of exposure per year. This is relatively high and exceeds the benchmark by 5 order of magnitude compared to WHO guidelines (10^{-6} pppy). Farmers can be infected by ascariasis (Mara and Sleigh, 2010b).

From lettuce consumption, *Salmonell*a annual risk infection is $5.00x10^{-7}$. That means there will be a risk of one infection of *Salmonella* per 10000000 consumers of lettuce leaves per year during 52 days of exposure. This result complies with the tolerable annual risk (10^{-6} per person per year) recommended by WHO, (2006a). This exposure does not constitute a public health risk from farmers. Concerning *Ascaris* infection, risk is $2.41x10^{-2}$. It means one infection of Ascaris per 100 farmers during 52 days of exposure per year. This is relatively high and exceeds the benchmark by 4 order of magnitude compared to WHO guidelines (10^{-6} pppy). Farmers can be infected by ascariasis (Mara and Sleigh, 2010b).

2.2. Comparison of the probabilistic values of different treatments related with the scenarios

The probabilistic values of all treatments compared with the WHO guideline values of risk for soil ingestion scenario are showed in the figure 9 above. *Salmonella* annual risk of infection from U+TW ($9.55x10^{-1}$) is higher than C+TW ($3.87x10^{-3}$), C+U+GW ($1.44x10^{-4}$) and GW ($8.89x10^{-6}$) for soil ingestion. The annual risk of infection in all treatment exceeded the tolerable risk of $\leq 10^{-6}$ per person per year (WHO, 2006a). The recorded values were above the recommended annual risk of infection by a 5 order of magnitude (U+TW ($9.55x10^{-1}$)).

Ascaris annual risks of infection in worst case from C+TW and C+U+GW are equal to ($4.67x10^{-2}$) however; these probabilistic values are higher than WHO guideline values (2006). The recorded value was above the recommended annual risk of infection by a 4 order of magnitude. This is more than the range of annual risk of *Ascaris* infection of 10^{-3} to 10^{-4} reported by Seidu et *al*. (2008) who used data from studies in Ghana to assess the annual risk of infection associated with the reuse of diluted wastewater for irrigation (Nana O.B. Ackerson and Esi Awuah, 2012).

The probabilistic values of all treatments compared with the WHO guideline values of risk for ingestion of irrigated greywater scenario are showed in the figure 10 above.

Salmonella annual risk of infection in worst case from C+U+GW ($1.53x10^{-3}$) is higher than GW ($1.02x10^{-4}$). The annual risk of infection in all treatment exceeded the tolerable risk of $\leq 10^{-6}$ per person per year (WHO, 2006a). The recorded value was above the recommended annual risk of infection by a 3 order of magnitude.

However, USEPA considers an annual risk of 10^{-4} to be acceptable for microbial contamination of drinking water, therefore the annual risk of infection for C+U+GW is above this recommended annual risk of infection by a 1 order of magnitude (Shuval et al., 1997).

The probabilistic values of all treatments compared with the WHO guideline values of risk for lettuce consumption scenario are showed in the figure 11 above. *Salmonella* annual risk of infection in worst case from C+TW ($1.54x10^{-1}$) is higher than U+TW ($1.30x10^{-7}$), C+U+GW ($5.00x10^{-7}$) and GW ($9.42x10^{-4}$).

The annual risk of infection in all treatment exceeded the tolerable risk of $\leq 10^{-6}$ per person per year (WHO, 2006a). *Ascaris* annual risk of infection from C+TW and C+U+GW is equal ($4.67x10^{-2}$), however, this probabilistic value is higher than WHO guideline values (2006). The recorded value was above the recommended annual risk of infection by a 4 order of

magnitude. This is attributed to the relative low levels of *Ascaris* counts in lettuce. This is more than the range of annual risk of *Ascaris* infection of 10^{-3} to 10^{-4} reported by Seidu et *al*. (2008) who used data from studies in Ghana to assess the annual risk of infection associated with the reuse of diluted wastewater for irrigation (Nana O.B. Ackerson and Esi Awuah, 2012).

2.2.1. Risk assessment from farmers

The risk more proven meets at the time of the ingestion of the soil on which the urine was spread urine (U+TW) is the *Salmonella* risk infection ($9.55x10^{-1}$). That could be explained by the fact why the urines used for the fertilization go back to less than one week of storage. With a significant load of the pathogens (*Salmonellas*) or indicator of the pathogen such as the enterococci (Annex xix). However, if the time of storage of urine is long, that contributes to the reduction of the risks of infection of the pathogen. That was proven by the works of Höglund et al., (2002) which showed that for a time of storage of 4 weeks, the risk of infection of bacteria is at least of 10^{-15}. In combination of C+TW, C+U+GW and GW (figure 9), the annual risk of infection of *Salmonella* is higher than benchmark (10^{-6} DALY) proposed by WHO, (2006a). *Ascaris* annual risk infection is high in C+TW and C+U+GW ($4.67x10^{-2}$) combination. However, compost which is used to spread in field, go back to less than 3 days (unstored) where 27 eggs/g dry weight of compost were fund. Risk could be reduced, if faeces were stored at least 12 months before its use for the fertilization of the cultures such as confirm by Schönning et al., (2007) who were determined values above 10^{-4} recommended by Swedish EPA, (2007).

For ingestion of irrigated greywater, the risk of infection of *Salmonella* in both combinations (figure 10) is higher than the WHO guideline value. But value of C+U+GW ($1.53x10^{-3}$) is higher than GW ($1.02x10^{-4}$). Risk could be mitigated, if farmers use adequate equipment of protection before to irrigate the crops. In addition, if irrigated greywater amount of fecal indicators is below of 10^3CFU/100 mL recommended by WHO, (2006a).

2.2.2. Risk assessment from consumers

The risk more proven meets at the time of the consumption of lettuce is the C+TW treatment where *Salmonella* risk infection is $1.54x10^{-1}$, then GW treatment ($9.42x10^{-4}$). Infection of risk from C+TW is higher than WHO recommendations (10^{-6}). This high value could be explained by the fact that, compost was not mature before use to spread in the field. Therefore, when

they irrigate the crop microorganisms may be regrow and then to settle on the lettuce leaves thanks to the water drops (FAO and WHO, 2008). From GW, risk could be reduced if last irrigation before harvest must be considered (2 days) according to Shuval et al., (1997) and WHO, 2006a. *Ascaris* annual risks of infection C+TW and C+U+GW are equal ($4.67x10^{-2}$), however, this probabilistic values are higher than WHO guideline values (2006). Risk could be reduced thanks to recommendation above given by WHO, (1998) concerning conservation of foods. Furthermore, according to Shuval et al., (1997), pathogens attached to plants will be inactivated with time due to natural attrition and the effects of desiccation, UV irradiation, heat and biological competition.

IV. Conclusion and perspectives

The risks related to the reuse of compost, urine and greywater in agriculture were varied according to the different treatments and scenarios which were assumed from farmers and consumers.

Therefore, the risk more proven meets at the time of the ingestion of the soil on which the urine was spread urine (U+TW) is the *Salmonella* risk infection > 10^{-6}. That could be explained by the fact why the urines used for the fertilization go back to less than one week of storage. With a significant load of the pathogens (*Salmonellas*) or indicator of the pathogen such as the enterococci. However, if the time of storage of urine is long, that contributes to the reduction of the risks of infection of the pathogen. *Ascaris* annual risk infection is high in C+TW and C+U+GW combination. However Risk could be reduced, if faeces were stored at least 6-12 months before its use for the fertilization of the cultures.

For ingestion of irrigated greywater, the risk of infection of *Salmonella* in both combinations (GW and C+U+GW) is higher than the WHO guideline value. Risk could be mitigated, if farmers use adequate equipment of protection before to irrigate the crops. In addition, if irrigated greywater amount of fecal indicators is below of 10^3CFU/100mL.

For lettuce consumption, risk infection of pathogens is high than benchmark (10^{-6}), however it could be reduced by observing the WHO recommendations.

Through this study, compost, urine may be used to fertilize the soil and greywater may be used to irrigate the crop which can eat freshly.

These results may be contributed for managing the public health by reducing diseases from populations. But if farmers and consumers observe the recommendations of protection by wearing protection equipment for farmers and washing the lettuce leaves with clean water before eating for the consumers.

In sahelian climate, risk infection of pathogens could be reduced thank to sun because the sunbeam play a significant role in the inactivation of pathogen in the soil.

This study has given us an insight into many other research possibilities. For example, risk assessment can study in handle faeces and urine from families' pilot. Also, health risk could be assessing from urban farmers in Ouagadougou city where they use wastewater and dam water to irrigate crops.

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Annex

Tables de Mac Grady

2 tubes par dilution		*3 tubes par dilution*					
Nombre caractéristique	Nombre de cellules	Nombre caractéristique	Nombre de cellules	Nombre caractéristique	Nombre de cellules	Nombre caractéristique	Nombre de cellules
000	0.0	000	0.0	201	1.4	302	6.5
001	0.5	001	0.3	202	2.0	310	4.5
010	0.5	010	0.3	210	1.5	311	7.5
011	0.9	011	0.6	211	2.0	312	11.5
020	0.9	020	0.6	212	3.0	313	16.0
100	0.6	100	0.4	220	2.0	320	9.5
101	1.2	101	0.7	221	3.0	321	15.0
110	1.3	102	1.1	222	3.5	322	20.0
111	2.0	110	0.7	223	4.0	323	30.0
120	2.0	111	1.1	230	3.0	330	25.0
121	3.0	120	1.1	231	3.5	331	45.0
200	2.5	121	1.5	232	4.0	332	110.0
201	5.0	130	1.6	300	2.5	333	140.0
210	6.0	200	0.9	301	4.0		
211	13.0						
212	20.0						
220	25.0						
221	70.0						
222	110.0						

5 tubes par dilution							
Nombre caractéristique	Nombre de cellules	Nombre caractéristique	Nombre de cellules	Nombre caractéristique	Nombre de cellules	Nombre caractéristique	Nombre de cellules
000	0.0	203	1.2	400	1.3	513	8.5
001	0.2	210	0.7	401	1.7	520	5.0
002	0.4	211	0.9	402	2.0	521	7.0
010	0.2	212	1.2	403	2.5	522	9.5
011	0.4	220	0.9	410	1.7	523	12.0
012	0.6	221	1.2	411	2.0	524	15.0
020	0.4	222	1.4	412	2.5	525	17.5
021	0.6	230	1.2	420	2.0	530	8.0
030	0.6	231	1.4	421	2.5	531	11.0
100	0.2	240	1.4	422	3.0	532	14.0
101	0.4	300	0.8	430	2.5	533	17.5
102	0.6	301	1.1	431	3.0	534	20.0
103	0.8	302	1.4	432	4.0	535	25.0
110	0.4	310	1.1	440	3.5	540	13.0
111	0.6	311	1.4	441	4.0	541	17.0
112	0.8	312	1.7	450	4.0	542	25.0
120	0.6	313	2.0	451	5.0	543	30.0
121	0.8	320	1.4	500	2.5	544	35.0
122	1.0	321	1.7	501	3.0	545	45.0
130	0.8	322	2.0	502	4.0	550	25.0
131	1.0	330	1.7	503	6.0	551	35.0
140	1.1	331	2.0	504	7.5	552	60.0
200	0.5	340	2.0	510	3.5	553	90.0
201	0.7	341	2.5	511	4.5	554	160.0
202	0.9	350	2.5	512	6.0	555	180.0

Annex i: Table of Mac Grady

Annex ii: Determination of amount of urine ingested from farmers

Protocol

The simulation of this experimentation is done through spreading the urine in the field in the same way a farmer would do. However, we used gloves before handling urine. After we finished handling urine, we withdrew the gloves and put them in sterilized bags to be analyzed. Then, the gloves were rinsed in a fixed volume (100 mL) of ultra-pure water. The amount of nitrogen was determined in this rinsed water. However, we must soak the gloves before analysis to know if nitrogen is not on these gloves where the result may be of use for control.

Assuming that all nitrogen proceeds from urine, we use the equation of conservation of concentration to determine the amount of urine ingested.

$$V_i = \frac{C_f \times V_f}{C_i}$$

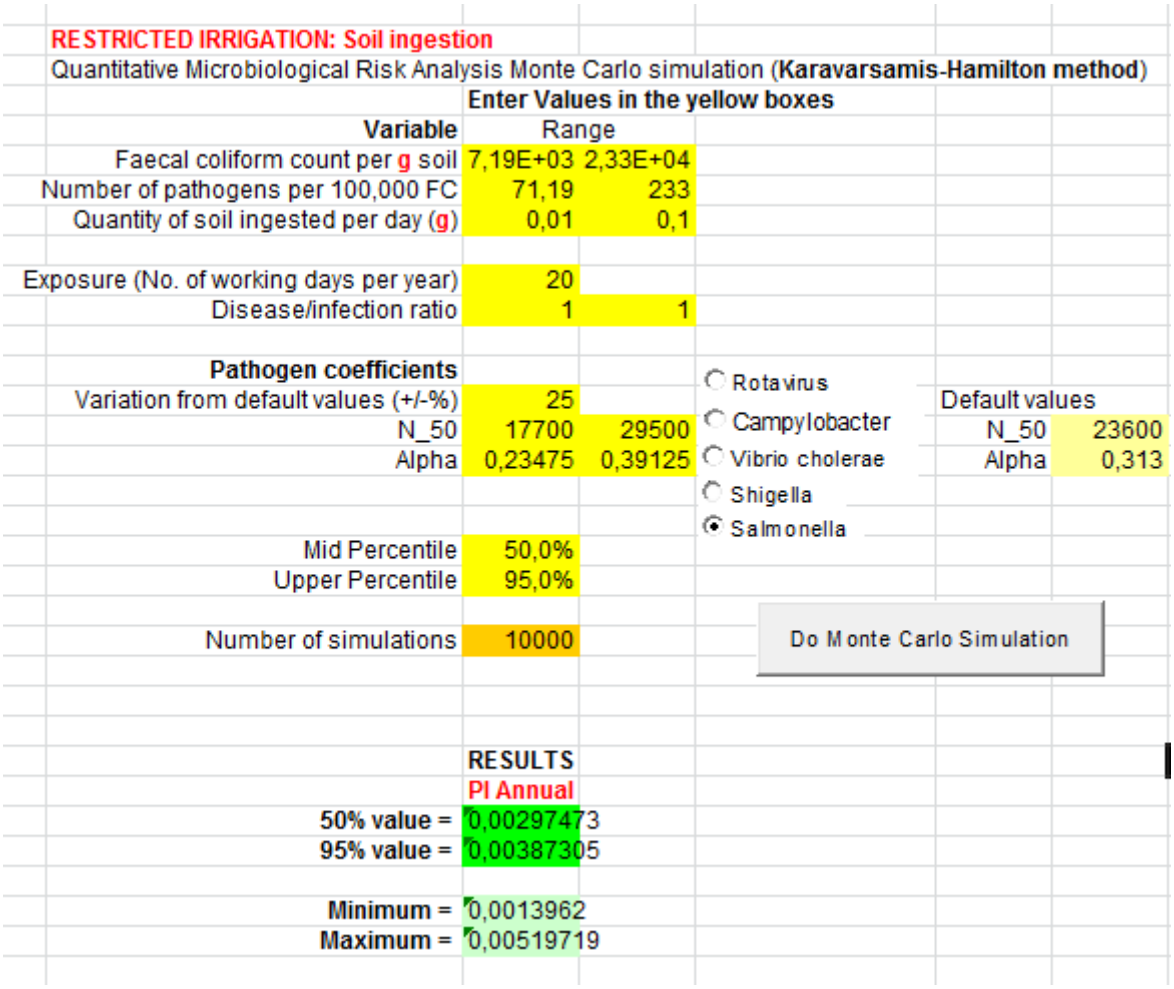
Where:

V_i= Volume of urine ingested (L),

C_i= Initial concentration of nitrogen contained in stored urine (mg/L),

V_f= Volume of water where gloves soak (L),

C_f= Concentration of nitrogen of urine in rinsed water (mg/L).



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	7,19E+03	2,33E+04
Number of pathogens per 100,000 FC	71,19	233
Quantity of soil ingested per day (g)	0,01	0,1
Exposure (No. of working days per year)	20	
Disease/infection ratio	1	1
Pathogen coefficients		
Variation from default values (+/-%)	25	
N_50	17700	29500
Alpha	0,23475	0,39125
Mid Percentile	50,0%	
Upper Percentile	95,0%	
Number of simulations	10000	

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

Default values: N_50 23600; Alpha 0,313

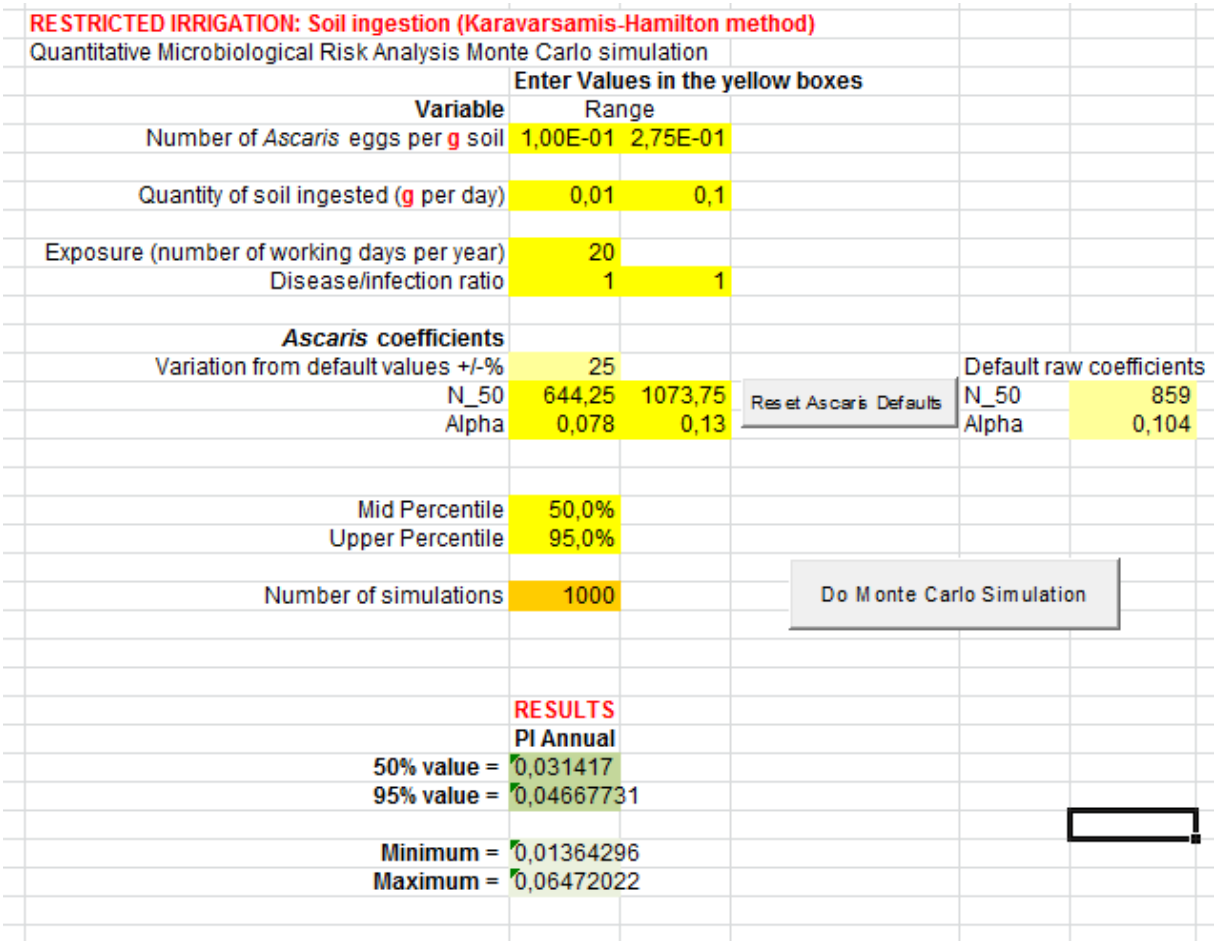
Do Monte Carlo Simulation

RESULTS	
	PI Annual
50% value =	0,00297473
95% value =	0,00387305
Minimum =	0,0013962
Maximum =	0,00519719

Annex iii: Calculation of *Salmonella* infection risk from soil ingestion of C+TW treatment

Variable	Range				
Faecal coliform count per 100 mL	7,19E+03	2,33E+04			
No.of pathogens per 100,000 FC	7,19	100			
Wastewater on 100 g lettuce (mL)	10	15			
Quantity of lettuce consumed (g/day)	100	100			
Reduction factor (n log)	0	0	Factor	1	1
Exposure (every n days)	7	7	Exposure (days/year)	52,142857	52,14286
Disease/infection ratio	1	1			
Pathogen coefficients					
Variation from default value (+/-%)	25		○ Rotavirus	Default values:	
N_50	17700	29500	◉ Salmonella	N_50	23600
Alpha	0,23475	0,39125	○ Shigella	Alpha	0,313
			○ Campylobacter		
Mid Percentile	50,0%		○ Vibrio cholerae		
Upper Percentile	95,0%				
			Do Monte Carlo Simulation		
Number of simulations	10000				
	RESULTS				
	PI Annual				
50% value =	0,00620572				
95% value =	0,0072455				
Minimum =	0,00413519				
Maximum =	0,00852683				

Annex iv : Calculation of *Salmonella* infection risk from lettuce consumption of C+TW treatment

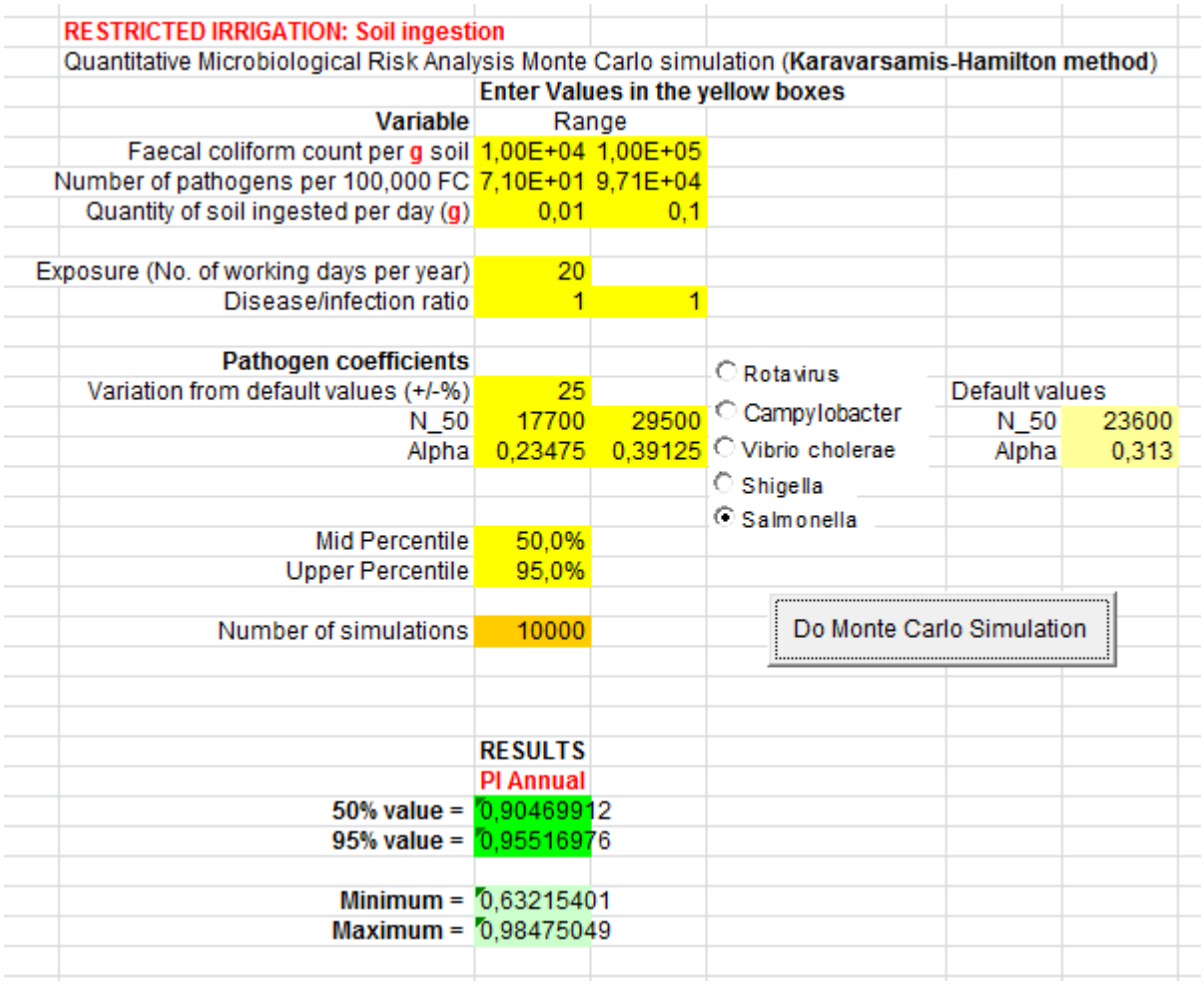


RESTRICTED IRRIGATION: Soil ingestion (Karavarsamis-Hamilton method)					
Quantitative Microbiological Risk Analysis Monte Carlo simulation					
	Enter Values in the yellow boxes				
Variable	Range				
Number of *Ascaris* eggs per g soil	1,00E-01	2,75E-01			
Quantity of soil ingested (g per day)	0,01	0,1			
Exposure (number of working days per year)	20				
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	1000		Do Monte Carlo Simulation		
	RESULTS				
	PI Annual				
50% value =	0,031417				
95% value =	0,04667731				
Minimum =	0,01364296				
Maximum =	0,06472022				

Annex v Calculation of Ascaris infection risk from soil ingestion of C+TW treatment

UNRESTRICTED IRRIGATION: Ascaris					
Quantitative Microbiological Risk Analysis Monte Carlo simulatio	**(Karavarsamis-Hamilton method)**				
	Enter Values in the yellow boxes				
Variable	Range				
Number of *Ascaris* eggs per litre of treated wastewater	0,1	0,275			
Irrigation water remaining on 100 g food (mL)	10	15			
Quantity of food consumed (g/day)	100	100			
[No *Ascaris* die-off]			Factor	1	1
Exposure (every n days)	7	7	Exposure (days/year)	52,14286	52,1429
Disease/infection ratio	1	1			
***Ascaris* coefficients**					
Variation from default values (+/-%)	25			Default values	
N_50	644,25	1073,75	Reset As caris Defaults	N_50	859
Alpha	0,078	0,13		Alpha	0,104
Mid Percentile	50,0%				
Upper Percentile	95,0%				
Number of simulations	1000		Do Monte Carlo Simulation		
	RESULTS				
	PI Annual				
50% value =	0,01941851				
95% value =	0,0240759				
Minimum =	0,01177122				
Maximum =	0,02877247				
Mean P_I_d =	0,00037964				
(mean infection risk per person per exposure event)					

Annex vi: Calculation of Ascaris infection risk from lettuce consumption of C+TW treatment



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	1,00E+04	1,00E+05
Number of pathogens per 100,000 FC	7,10E+01	9,71E+04
Quantity of soil ingested per day (g)	0,01	0,1
Exposure (No. of working days per year)	20	
Disease/infection ratio	1	1

Pathogen coefficients		
Variation from default values (+/-%)	25	
N_50	17700	29500
Alpha	0,23475	0,39125
Mid Percentile	50,0%	
Upper Percentile	95,0%	
Number of simulations	10000	

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

Default values: N_50 23600; Alpha 0,313

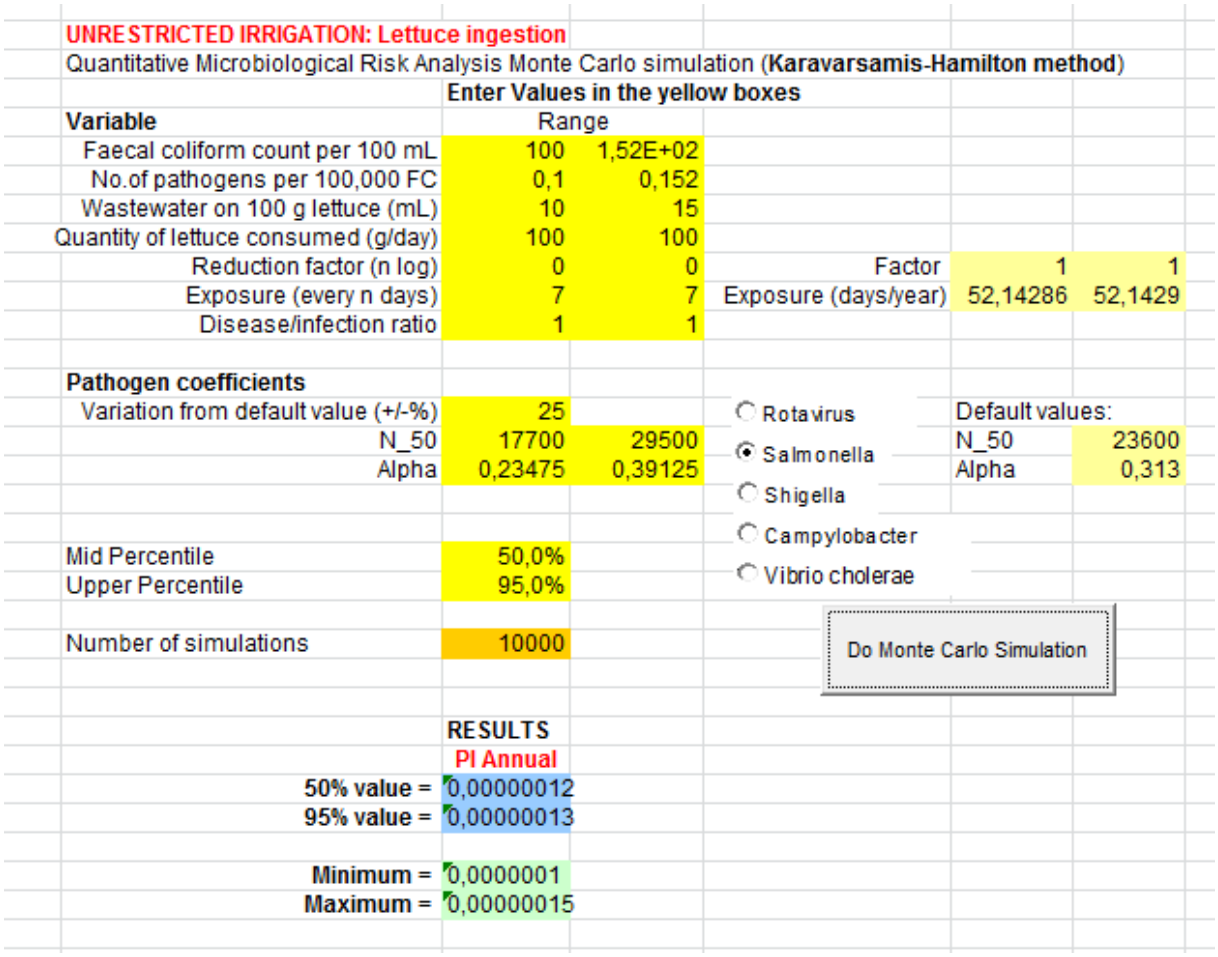
Do Monte Carlo Simulation

RESULTS

PI Annual

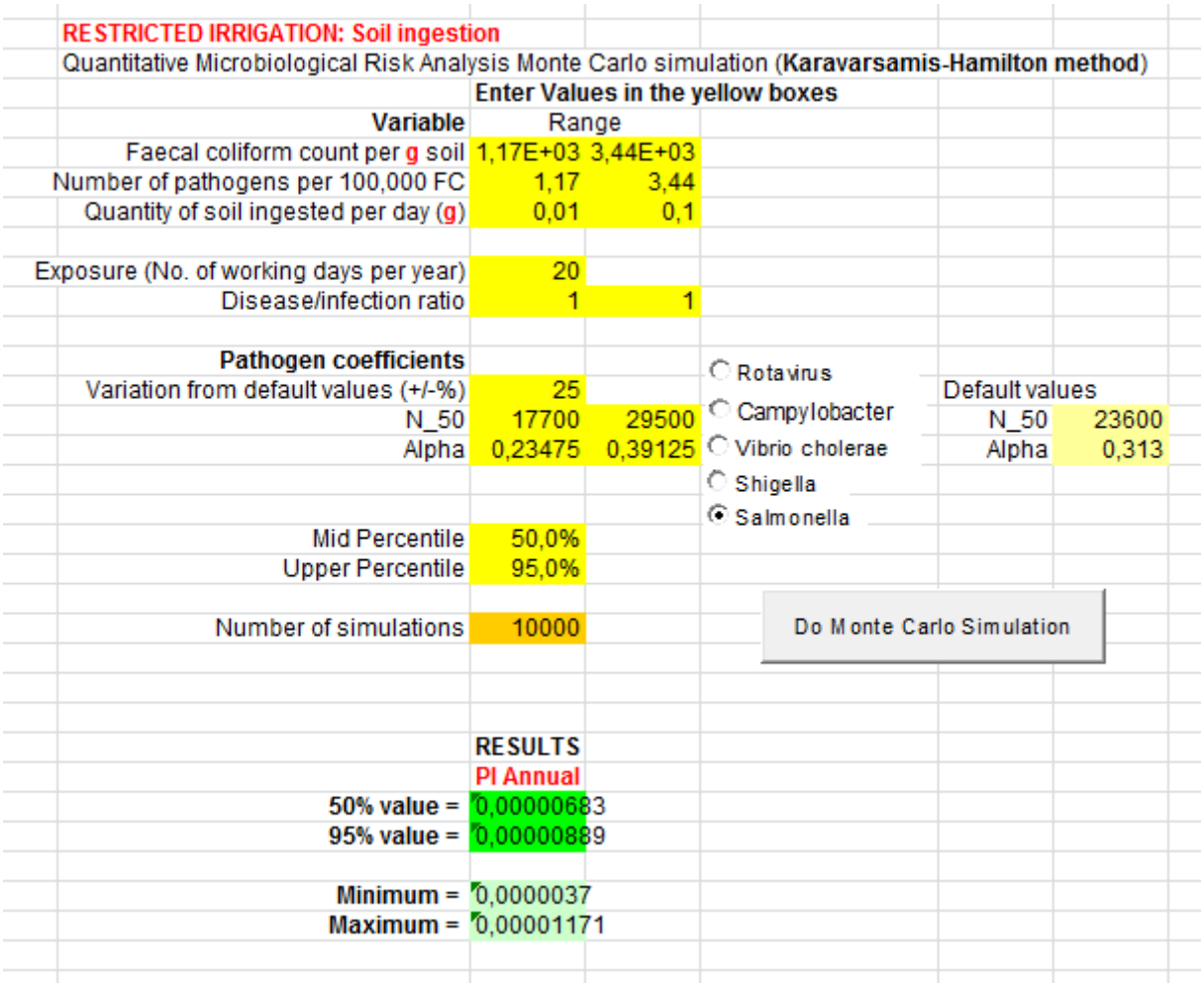
50% value =	0,90469912
95% value =	0,95516976
Minimum =	0,63215401
Maximum =	0,98475049

Annex vii : Calculation of *Salmonella* infection risk from soil ingestion of U+TW treatment



UNRESTRICTED IRRIGATION: Lettuce ingestion					
Quantitative Microbiological Risk Analysis Monte Carlo simulation (**Karavarsamis-Hamilton method**)					
	Enter Values in the yellow boxes				
Variable	Range				
Faecal coliform count per 100 mL	100	1,52E+02			
No.of pathogens per 100,000 FC	0,1	0,152			
Wastewater on 100 g lettuce (mL)	10	15			
Quantity of lettuce consumed (g/day)	100	100			
Reduction factor (n log)	0	0	Factor	1	1
Exposure (every n days)	7	7	Exposure (days/year)	52,14286	52,1429
Disease/infection ratio	1	1			
Pathogen coefficients					
Variation from default value (+/-%)	25		○ Rotavirus	Default values:	
N_50	17700	29500	◉ Salmonella	N_50	23600
Alpha	0,23475	0,39125	○ Shigella	Alpha	0,313
			○ Campylobacter		
Mid Percentile	50,0%		○ Vibrio cholerae		
Upper Percentile	95,0%				
			Do Monte Carlo Simulation		
Number of simulations	10000				
	RESULTS				
	PI Annual				
50% value =	0,00000012				
95% value =	0,00000013				
Minimum =	0,0000001				
Maximum =	0,00000015				

Annex viii : Calculation of *Salmonella* infection risk from lettuce consumption of U+TW treatment



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	1,17E+03	3,44E+03
Number of pathogens per 100,000 FC	1,17	3,44
Quantity of soil ingested per day (g)	0,01	0,1
Exposure (No. of working days per year)	20	
Disease/infection ratio	1	1
Pathogen coefficients		
Variation from default values (+/-%)	25	
N_50	17700	29500
Alpha	0,23475	0,39125
Mid Percentile	50,0%	
Upper Percentile	95,0%	
Number of simulations	10000	

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

Default values: N_50 23600; Alpha 0,313

Do Monte Carlo Simulation

RESULTS

PI Annual

50% value =	0,00000683
95% value =	0,00000889
Minimum =	0,0000037
Maximum =	0,00001171

Annex ix : Calculation of *Salmonella* infection risk from soil ingestion of GW treatment for 20 days exposure

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	1,17E+03	3,44E+03
Number of pathogens per 100,000 FC	1,17	3,44
Quantity of soil ingested per day (g)	0,01	0,1
Exposure (No. of working days per year)	275	
Disease/infection ratio	1	1

Pathogen coefficients			Default values	
Variation from default values (+/-%)	25			
N_50	17700	29500	N_50	23600
Alpha	0,23475	0,39125	Alpha	0,313

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

Mid Percentile	50,0%	
Upper Percentile	95,0%	
Number of simulations	10000	

Do Monte Carlo Simulation

RESULTS

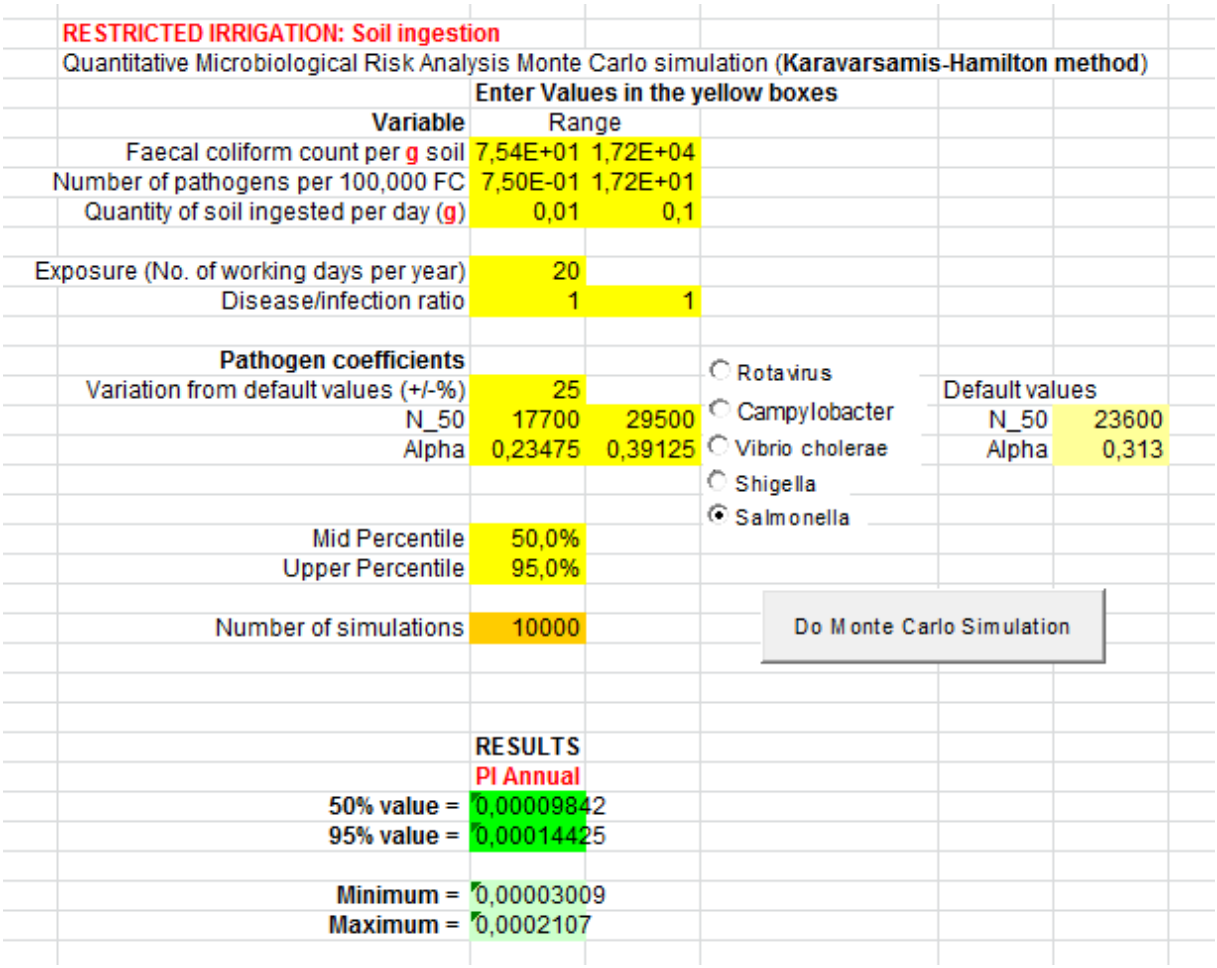
PI Annual

50% value =	0,00009451
95% value =	0,00010153
Minimum =	0,00007906
Maximum =	0,00011056

Annex x : Calculation of *Salmonella* infection risk from soil ingestion of GW treatment for 275 days exposure

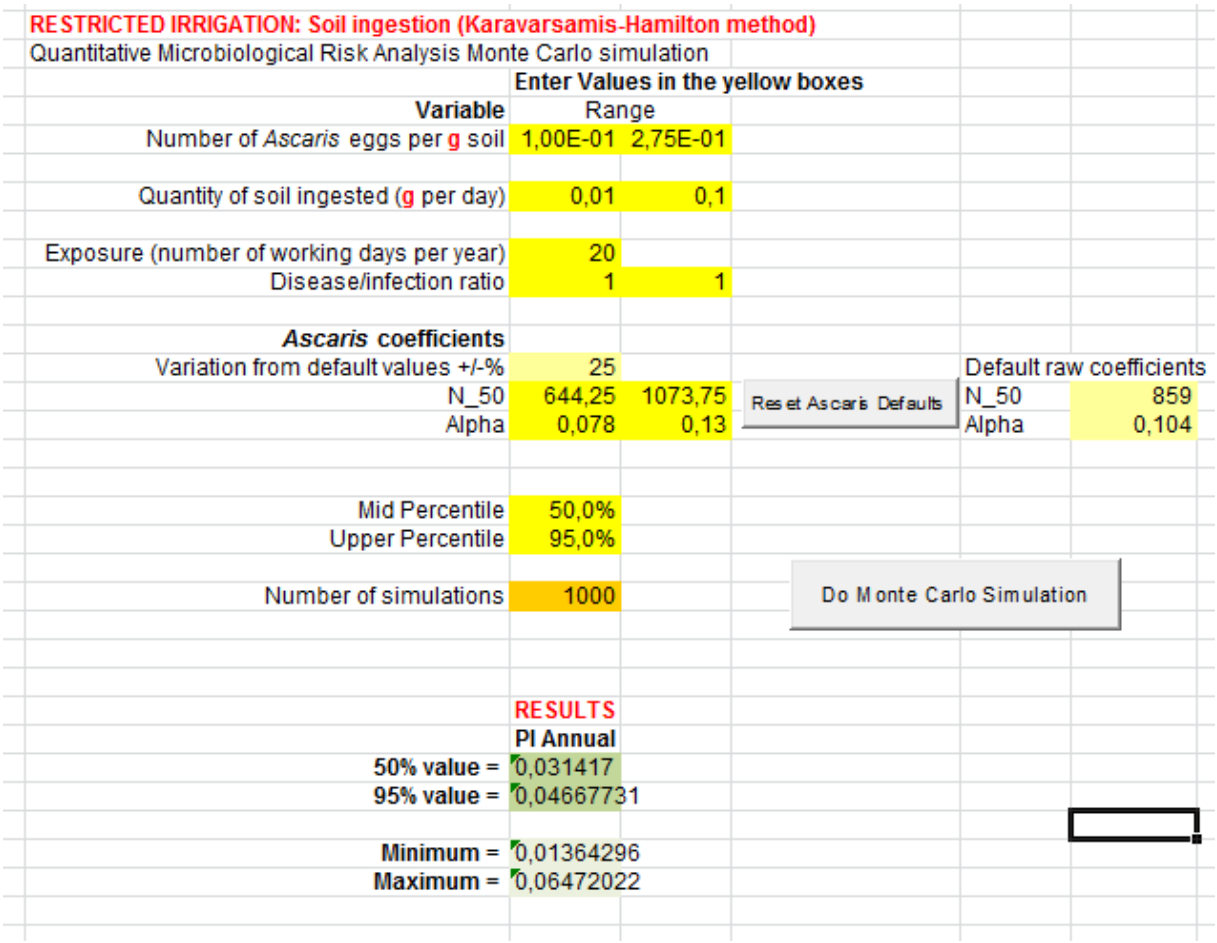
UNRESTRICTED IRRIGATION: Lettuce ingestion					
Quantitative Microbiological Risk Analysis Monte Carlo simulation (**Karavarsamis-Hamilton method**)					
	Enter Values in the yellow boxes				
Variable	Range				
Faecal coliform count per 100 mL	1000	1,92E+04			
No.of pathogens per 100,000 FC	1	19,2			
Wastewater on 100 g lettuce (mL)	10	15			
Quantity of lettuce consumed (g/day)	100	100			
Reduction factor (n log)	0	0	Factor	1	1
Exposure (every n days)	7	7	Exposure (days/year)	52,14286	52,1429
Disease/infection ratio	1	1			
Pathogen coefficients					
Variation from default value (+/-%)	25		○ Rotavirus	Default values:	
N_50	17700	29500	◉ Salmonella	N_50	23600
Alpha	0,23475	0,39125	○ Shigella	Alpha	0,313
			○ Campylobacter		
Mid Percentile	50,0%		○ Vibrio cholerae		
Upper Percentile	95,0%				
			Do Monte Carlo Simulation		
Number of simulations	10000				
	RESULTS				
	PI Annual				
50% value =	0,00077694				
95% value =	0,00094233				
Minimum =	0,00047578				
Maximum =	0,00122148				

Annex xi : Calculation of *Salmonella* infection risk from lettuce consumption of GW treatment



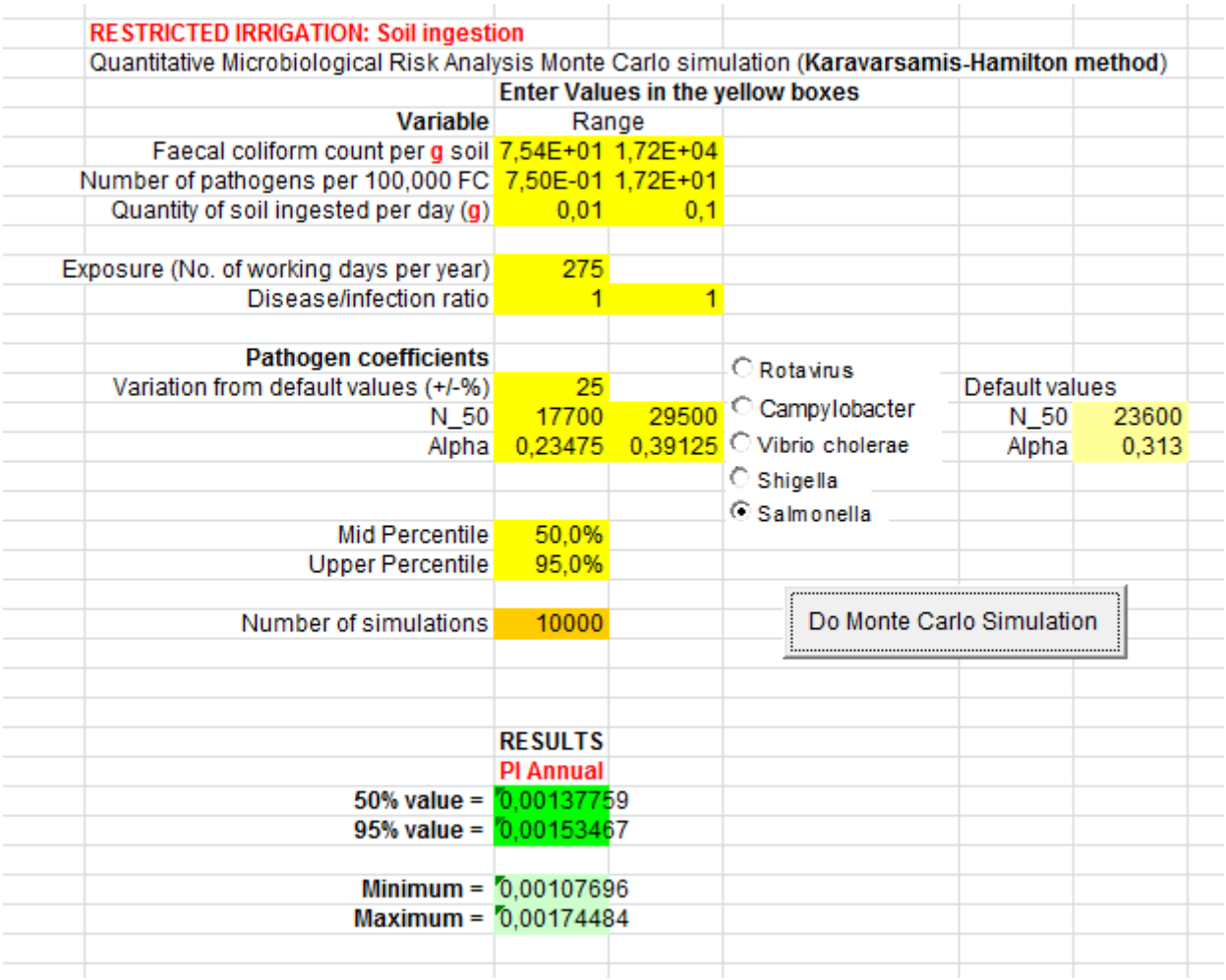
RESTRICTED IRRIGATION: Soil ingestion					
Quantitative Microbiological Risk Analysis Monte Carlo simulation (**Karavarsamis-Hamilton method**)					
	Enter Values in the yellow boxes				
Variable	Range				
Faecal coliform count per g soil	7,54E+01	1,72E+04			
Number of pathogens per 100,000 FC	7,50E-01	1,72E+01			
Quantity of soil ingested per day (g)	0,01	0,1			
Exposure (No. of working days per year)	20				
Disease/infection ratio	1	1			
Pathogen coefficients			○ Rotavirus		
Variation from default values (+/-%)	25		○ Campylobacter	Default values	
N_50	17700	29500	○ Vibrio cholerae	N_50	23600
Alpha	0,23475	0,39125	○ Shigella	Alpha	0,313
			◉ Salmonella		
Mid Percentile	50,0%				
Upper Percentile	95,0%				
Number of simulations	10000		Do Monte Carlo Simulation		
	RESULTS				
	PI Annual				
50% value =	0,00009842				
95% value =	0,00014425				
Minimum =	0,00003009				
Maximum =	0,0002107				

Annex xii : Calculation of *Salmonella* infection risk from soil ingestion of C+U+GW treatment for 20 days exposure



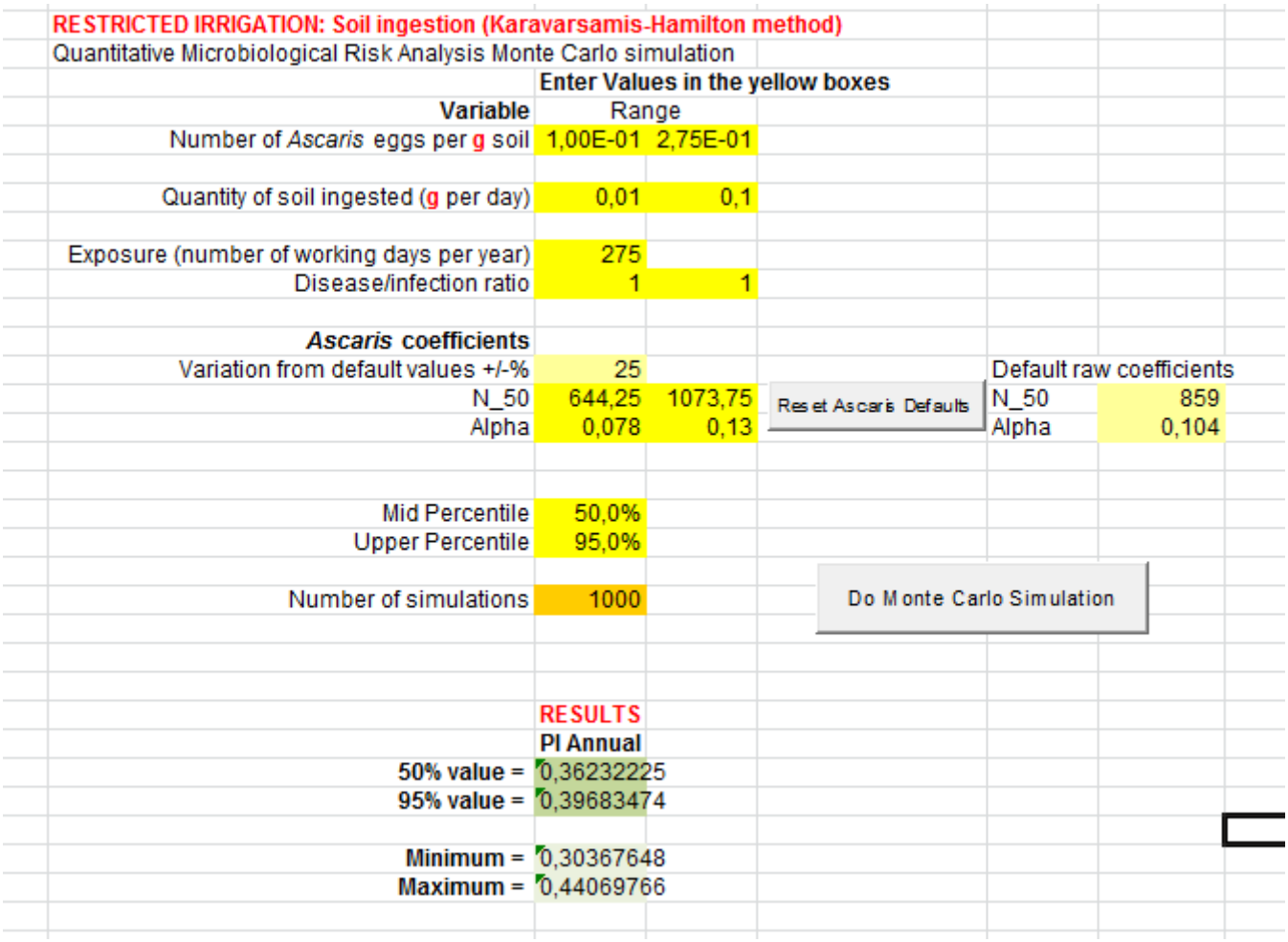
RESTRICTED IRRIGATION: Soil ingestion (Karavarsamis-Hamilton method)					
Quantitative Microbiological Risk Analysis Monte Carlo simulation					
	Enter Values in the yellow boxes				
Variable	Range				
Number of *Ascaris* eggs per g soil	1,00E-01	2,75E-01			
Quantity of soil ingested (g per day)	0,01	0,1			
Exposure (number of working days per year)	20				
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	1000		Do Monte Carlo Simulation		
	RESULTS				
	PI Annual				
50% value =	0,031417				
95% value =	0,04667731				
Minimum =	0,01364296				
Maximum =	0,06472022				

Annex xiii : Calculation of *Ascaris* infection risk from soil ingestion of C+U+GW treatment from 20 days exposure



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	7,54E+01	1,72E+04			
Number of pathogens per 100,000 FC	7,50E-01	1,72E+01			
Quantity of soil ingested per day (g)	0,01	0,1			
Exposure (No. of working days per year)	275				
Disease/infection ratio	1	1			
Pathogen coefficients			○ Rotavirus		
Variation from default values (+/-%)	25		○ Campylobacter	Default values	
N_50	17700	29500	○ Vibrio cholerae	N_50	23600
Alpha	0,23475	0,39125	○ Shigella	Alpha	0,313
			◉ Salmonella		
Mid Percentile	50,0%				
Upper Percentile	95,0%				
Number of simulations	10000		Do Monte Carlo Simulation		
	RESULTS				
	PI Annual				
50% value =	0,00137759				
95% value =	0,00153467				
Minimum =	0,00107696				
Maximum =	0,00174484				

Annex xiv : **Calculation of *Salmonella* infection risk from soil ingestion of C+U+GW treatment for 275 days exposure**



RESTRICTED IRRIGATION: Soil ingestion (Karavarsamis-Hamilton method)					
Quantitative Microbiological Risk Analysis Monte Carlo simulation					
	Enter Values in the yellow boxes				
Variable	Range				
Number of *Ascaris* eggs per g soil	1,00E-01	2,75E-01			
Quantity of soil ingested (g per day)	0,01	0,1			
Exposure (number of working days per year)	275				
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	1000		Do Monte Carlo Simulation		
	RESULTS				
	PI Annual				
50% value =	0,36232225				
95% value =	0,39683474				
Minimum =	0,30367648				
Maximum =	0,44069766				

Annex xv: Calculation of *Ascaris* infection risk from soil ingestion of C+U+GW treatment from 275 days exposure

UNRESTRICTED IRRIGATION: Lettuce ingestion					
Quantitative Microbiological Risk Analysis Monte Carlo simulation (**Karavarsamis-Hamilton method**)					
	Enter Values in the yellow boxes				
Variable	Range				
Faecal coliform count per 100 mL	100	3,79E+02			
No.of pathogens per 100,000 FC	0,1	0,379			
Wastewater on 100 g lettuce (mL)	10	15			
Quantity of lettuce consumed (g/day)	100	100			
Reduction factor (n log)	0	0	Factor	1	1
Exposure (every n days)	7	7	Exposure (days/year)	52,14286	52,1429
Disease/infection ratio	1	1			
Pathogen coefficients					
Variation from default value (+/-%)	25		○ Rotavirus	Default values:	
N_50	17700	29500	◉ Salmonella	N_50	23600
Alpha	0,23475	0,39125	○ Shigella	Alpha	0,313
			○ Campylobacter		
Mid Percentile	50,0%		○ Vibrio cholerae		
Upper Percentile	95,0%				
			Do Monte Carlo Simulation		
Number of simulations	10000				
	RESULTS				
	PI Annual				
50% value =	0,00000044				
95% value =	0,0000005				
Minimum =	0,00000033				
Maximum =	0,0000006				

Annex xvi : Calculation of *Salmonella* infection risk from lettuce consumption of C+U+GW treatment

UNRESTRICTED IRRIGATION: Ascaris					
Quantitative Microbiological Risk Analysis Monte Carlo simulatio	**(Karavarsamis-Hamilton method)**				
	Enter Values in the yellow boxes				
Variable	Range				
Number of *Ascaris* eggs per litre of treated wastewater	0,1	0,275			
Irrigation water remaining on 100 g food (mL)	10	15			
Quantity of food consumed (g/day)	100	100			
[No *Ascaris* die-off]			Factor	1	1
Exposure (every n days)	7	7	Exposure (days/year)	52,14286	52,1429
Disease/infection ratio	1	1			
***Ascaris* coefficients**					
Variation from default values (+/-%)	25			Default values	
N_50	644,25	1073,75	Reset As caris Defaults	N_50	859
Alpha	0,078	0,13		Alpha	0,104
Mid Percentile	50,0%				
Upper Percentile	95,0%				
Number of simulations	1000		Do Monte Carlo Simulation		
	RESULTS				
	PI Annual				
50% value =	0,01941851				
95% value =	0,0240759				
Minimum =	0,01177122				
Maximum =	0,02877247				
Mean P_I_d =	0,00037964				
(mean infection risk per person per exposure event)					

Annex xvii : Calculation of *Ascaris* infection risk from lettuce consumption of C+U+GW treatment

BESOINS EN EAU DES CULTURES

Station ETo: OUAGADOUGOU
Station Pluie: OUAGADOUGOU

Culture: Small Vegetables
Date de plantation: 20/03

Mois	Décade	Phase	Kc coeff	ETc mm/jour	ETc mm/dec	Pluie eff. mm/dec	Bes. Irr. mm/dec
Mar	2	Init	0.70	5.33	5.3	0.0	5.3
Mar	3	Init	0.70	5.28	58.1	0.0	58.1
Avr	1	Crois	0.70	5.24	52.4	0.0	52.4
Avr	2	Crois	0.79	5.83	58.3	0.0	58.3
Avr	3	Crois	0.90	6.60	66.0	0.0	66.0
Mai	1	Mi-sais	1.02	7.31	73.1	0.0	73.1
Mai	2	Mi-sais	1.05	7.42	74.2	0.0	74.2
Mai	3	Mi-sais	1.05	6.99	76.9	0.0	76.9
Jui	1	Arr-sais	1.05	6.53	65.3	0.0	65.3
Jui	2	Arr-sais	0.99	5.76	57.6	0.0	57.6
Jui	3	Arr-sais	0.95	5.25	10.5	0.0	10.5
					597.7	0.0	597.7

Annex xviii : Besoins en eau de la laitue

Annex xix : Initial amount of indicators and pathogens in urine and greywater

Indicators/Pathogens	Matrix	
	Urine	Greywater
E.coli **(log_{10}CFU/100mL)**	0	4.73±1.73
Fecal coliforms (log_{10}CFU/100mL)	5.12±0.71	5.35±1.4
Enterococci **(log_{10}CFU/100mL)**	4.00±0.41	4.42±1.63
Salmonella **(log_{10} MPN/100mL)**	3.95 ± 2.27	156.63±199.80
Ascaris **(eggs/L)**	0	0

Annex xx: Initial amount of indicators and pathogens in compost and soil

Indicators/Pathogens	Matrix	
	Compost	Soil
E.coli (log_{10}CFU/gDW)	4.21 ± 3.53	2.10 ± 2.20
Fecal coliforms (log_{10}CFU/gDW)	4.40 ± 3.55	0
Enterococci (log_{10}CFU/gDW)	5.82 ± 4.32	0
Salmonella (log_{10}MPN/g DW)	3.31	0
Ascaris (eggs/gDW)	27	0