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Microbial risk assessment on stakeholders of unrestricted irrigation

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Abstract
Scarcity of conventional water sources coupled with population growth, wastewater has become an alternative source for irrigation, though food-borne diseases are increasing and food safety has now been considered as an important global health issue. This present study was carried out purposely to assess the health risk associated with farm workers exposure to wastewater irrigation practices and consumers of wastewater irrigated lettuce. Kumasi was strategically selected as the study site due to its location and in addition, the second largest city in Ghana with a population size of over two million (2,035,064) and an average household size of 3.8 with 2.7% annual growth rate. Majority of farmers in urban Kumasi engage in unrestricted wastewater irrigation practices on vegetables eaten raw such as lettuce, cabbage and spring onions among few others. In total 107 lettuce heads, manured soil and wastewater used for irrigation samples respectively were aseptically collected and analyzed using standard microbiological methods. A quantitative risk assessment tool was used to analyzed the health risk, the results indicates a high level of risk of infection caused by *E.coli 0157:H7* to both farmers and consumers irrespective of the seasons (dry and wet seasons), these were found to exceed the tolerable level of WHO by 3 order of magnitude, however, *Salmonella* spp risk of illness was found to be 1 order of magnitude lower in the dry season and 3 order of magnitude higher in the wet season.

Key words: *Kumasi, Wastewater, Lettuce, manured Soil, Risk Assessment*

1.0 INTRODUCTION

Wastewater has become an alternative source for irrigation due to scarcity of conventional water sources coupled with population growth. Although this practice enhances the income of farmers and improves their livelihood, it is not without health risk due to the presence of high concentrations of pathogens such as bacteria, viruses, protozoa and helminths (Toze, 2006). Municipal wastewater consists of domestic wastewater, industrial wastewater, storm water and ground water seepage entering the sewage network. Homsi (2000) estimates that only 10% of all the wastewater in developing countries receives treatment which conforms to the report in Accra, Ghana, where also only 10% of wastewater collected in piped sewage systems receives primary or secondary treatment (Drechsel *et al.,* 2002; Scott *et al.,* 2004). There are standard guidelines for the use of wastewater for unrestricted irrigation but farmers use untreated wastewater unlawfully for irrigation practices (Melloul *et al.,* 2001). World Health Organization recommends a threshold of 10^3/100ml of faecal coliforms for unrestricted irrigation (crops that can be eaten uncooked) (WHO, 1989) In Accra, Ghana more than 200,000 people eat vegetables produced with wastewater every day (Amoah *et al.,* 2007). Seidu and Drechsel, 2010, also reported that, each day, about 800,000 people in the cities of Ghana consume wastewater irrigated salad food.

Food-borne diseases are increasing and becoming wide spread, therefore food safety has been considered as an important global health issue for a long time now (Harris *et al.,* 2003) and researchers have also made it an essential case for effective study into areas that enhance it outcome. In Kentucky, *Salmonella* spp resistance to ciprofloxacin was indicated to have increased from 22% in 2003 to 57% in 2009, which resulted in a major health concern because *Salmonella* infection limits the ability to treat invasive diseases (Portnoy and Geopfert, 1996). Outbreaks of food borne diseases caused by *Escherichia coli* are a serious public health concern and a report from CDC indicated that 73,000 cases of infection with *E. coli* O157:H7 and 61 deaths on average occur in the United States annually (Seto *et al.,* 2007). Assessment of microbiological health risk associated with wastewater irrigation practices have been carried out in many countries but little has been done in Ghana, though studies in Ghana have shown that, wastewater used for irrigation practices contain high microbial loads (Amoah *et al.,* 2007). However, there is little documentation on the microbial loads, and also health risk assessment has always been based on extrapolations and assumptions (Seidu *et al.,* 2008).

2.0 Materials and Methods

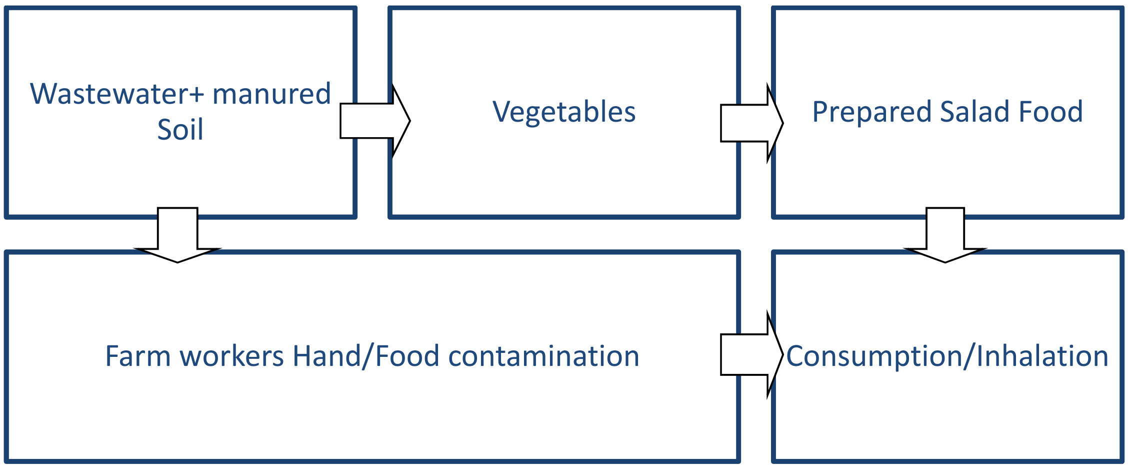
2.1 Description of the study area

This study was carried out in the Kumasi Metropolis which is the second largest city in Ghana with a population size of over two (2) million with a household size of 3.8 and an annual growth rate of 2.7% (Ghana Statistical Service, 2010). Ashanti Region where Kumasi is located has two major seasons, the wet and dry seasons. The wet season starts from March to July which is the major rainy season and minor rains from September to November with an annual rainfall of about 1300mm. The average minimum and maximum temperatures are 21.5^{o} and 30.7^{0}c respectively. The average humidity is about 84.16 per cent at 0900 GMT and 60 per cent at 1500 GMT. Majority of the people in the region are farmers who engage in crop cultivation. Among this group of farmers are those who go into vegetable cultivation, mainly, lettuce, cabbage and spring onions among few others. Due to the high demand for portable water, almost about 97% of the farmers depend on wastewater for irrigating their crops all year round. Kumasi also has quite a number of hotels, restaurants and other food vendors who engage in serving lettuce salad to their consumers.

2.2 Study design

This study was carried out for both the wet and the dry seasons where lettuce heads, manured soil and wastewater irrigation samples respectively, were collected and analyzed aseptically. First survey was carried out mapping out all the areas where wastewater irrigation was practiced within the Kumasi Metropolis. There were eight sites located within the study site namely; Poku Sika, Gyinyase, Ayeduase, Ramseyer, Appeadu, Kentinkrono, Nima and Georgia.

Figure 2: 1 Conceptual frame work of the risk pathway for both farm workers and consumers



2.3 Sampling

Irrigation water, manured soil, Lettuce heads from irrigation farms as well were aseptically sampled into sterile suitable containers and plastic bags, kept in cooling boxes and sent to the laboratory for analysis within the same day.

2.4 Microbial Quality Analysis

2.4.1 *Salmonella* spp

Samples were homogenized and added to peptone water incubated at 37°c for 18 -24hrs. Positive tubes were inoculated into selenite broth, incubated under the same temperature and time. Positive colonies were streaked on Salmonella Shigella (SS) agar plates, and later purified and confirmed with biochemical test and latex agglutination test from Oxoid. The Test Kit is 100% sensitive and between 97.2 % -100 % specific.

2.4.2 *E. coli O157:H7*

Samples were homogenized and mixed with saline, inoculated on Eosine Methylene Blue Agar (EMBA) plates and incubation done at 44°c for 24hrs. Plates showing growth of *E.coli* colonies were subculture onto Sorbitol MacConkey ager (SMA) plates and incubated at 44°C for 24hrs. Positive colonies were purified (Baron *et al.,* 1994) and serologically confirmed with E. coli prolex™ latex agglutination test (Oxoid Limited, 2012) which has a specificity and sensitivity 100% and 99% respectively.

3.0 Quantitative microbial risk assessment (QMRA)

QMRA tool used for this study include Hazard identification, Exposure assessment, Dose response and Risk characterization.

3.1 Hazard Identification

Salmonella spp

Salmonella is a bacterium which causes diarrhea, fever, and abdominal cramps 12 to 72 hours after infection usually lasting 4 to 7 days. In some persons, the diarrhea may be so severe and infection may sometimes become systemic, which could result in death. The elderly, infants, and those with impaired immune systems are more likely to have a severe illness. Every year, approximately 42,000 cases of Salmonellosis are reported in the United States (Seto *et al.,* 2007). Children are the most likely to get Salmonellosis and the rate of diagnosed infections in children less than five years old is higher than the rate in all other persons. Young children, the elderly, and the immune-compromised are most likely to have severe infections. In Africa, over 30,000 children are reported to die every year due to the consumption of contaminated food (Mensah, 2005). Fresh fruits and vegetables are increasingly recognized as potential sources of diseases (Ibenyassine *et al.,* 2006). Vegetables that are eaten uncooked are among the products associated with food borne disease outbreaks in the Unites States (Sivapalasingam *et al.,* 2004). Vegetables can become contaminated by pathogens while growing, during harvest, by post-harvest handling, or distribution. Information from CDC, 2013, states that an estimated 400 persons die each year with acute Salmonellosis but in others infection could only result joint pains, irritation of the eyes, and painful urination which could last for months or years and sometimes leading to chronic arthritis.

3.2 *E. coli* O157:H7

E. coli O157:H7 is a bacterium commonly found in the gut of humans and warm-blooded animals. Most strains of *E. coli* are harmless but *E. coli* O157:H7 which is a pathogenic strain can cause severe food borne disease resulting in abdominal cramps and diarrhoea that may in some cases progress to bloody diarrhoea (haemorrhagic colitis). Fever and vomiting may also occur. It is basically acquired from faecal contamination of vegetables, raw or undercooked ground meat products and raw milk. Illness could be life-threatening disease leading to haemolytic uraemic syndrome (HUS), especially in young children and the elderly. Its significance as a public health problem was recognized in 1982, following an outbreak in the United States of America. *E. coli* O157:H7 is the most important EHEC serotype in relation to public health; however, other serotypes have frequently been involved in sporadic cases and outbreaks.

3.3 Exposure Assessment

The quality of water of which the dose *d* is ingested through the exposures was done through a comprehensive field and laboratory work studies over two seasons (*dry and wet seasons in Ghana covering 6 months each).* Data was collected based on exposure scenarios in the WHO guidelines (WHO, 2006). These were:

(i) Consumption of vegetables irrigated with wastewater.
(ii) Accidental ingestion of water by the farmers
(iii) Accidental ingestion of soil by the farmers

3.4 Consumers Exposure

The study assumed majority of the population within the Kumasi community prepared lettuce salad with the use of lettuce from wastewater irrigation sites which is the main vegetable grown by urban and peri-urban farmers in Ghana (Obuobie *et al.,* 2006). The amount of lettuce consumed was uniformly distributed from 10 g to 12 g accounting for low consumption of lettuce in most diet in the Ghanaian populace (Seidu *et al.,* 2008), whereas the frequency of consumption was uniformly distributed from 3 to 4 times a week also to justify for the low level of lettuce salad consumption in the various homes as compared to the consumption frequency of 4 times a week (Seidu *et al.,* 2008). Socio economic data study on the pre-consumption preparation of lettuce salad in various homes and vendors indicates practices which includes washing with tap water and disinfection before consumption, which was also which was factored into the model as a uniform distribution from 1 log/cfu to 3 logs/cfu (Shuval *et al.,* 1997, WHO, 2006 and Seidu *et al.,* 2008).

3.5 Farmers Exposure

Accidental ingestion of manured soil was uniformly distributed from 10 mg to 100 mg (Seidu *et al.*, 2008) accounting for the non-mechanized nature of wastewater irrigation farming practices in Ghana. The frequency of the working days was also uniformly distributed from 60 days to 70 days accounting for differences in seasonal planting and harvesting which mainly lasted for little over 2 months.

With accidental ingestion of water and aerosol during irrigation, a uniform distribution was assumed from 1 ml to 5 ml accounting for the frequency of the use of improvised equipment for the irrigation of vegetables in urban and peri-urban vegetable fields in Kumasi, again the frequency of the irrigation period was the same as the number of working days, since vegetable fields are irrigated throughout to ensure freshness of the vegetables until harvesting, which was also uniformly distributed from 60 days to 70 days.

3.6 Dose Response Assessment

Salmonella spp *and E. coli* O157:H7 were selected due to their intense involvement in gastro-enteritis. The dose response model for the estimation of the daily infection risk of these two identified pathogens was the β – poison model derived from an outbreak data set for different Salmonella serovars (Anonnymous, 2002). The result is two expected values for the parameters α and β with α = 0.1324 and β = 51.45. Since it is obtained from outbreak data, the resulting Beta Poisson model can be interpreted to describe the probability of illness from a certain dose. Hence the probability of infection (equation 1) was used for the individual exposure scenario for each exposure to *Salmonella* spp and *E. coli* O157:H7.

$$P_{inf} = 1 - (1 + d/\beta)^{-\alpha} \qquad 1$$

$$P_{I(A)}(d) = 1 - (1 - P_{inf}(d))^{n} \qquad 2$$

Where,

P_{inf} is the risk of infection of an exposure following an ingestion of a single dose (*d*) of a pathogen;

$P_{I(A)}(d)$ is the annual probability of infection/illness of an individual from *n* exposures per year.

Annual infection was estimated (equation 2). A 10,000 trial MC simulation using the Hypercube sampling was run with @ RISK (Palisades software, 2010) with a sensitivity analysis been performed which measured the influence of each of the input variables as to the risk.

3.5 Risk Characterization

The median risk of infection of *E. coli O157*:H7 and *Salmonella* spp for accidental ingestion of wastewater and manured soil by farmers and the consumption of lettuce by consumers are then characterized for both the dry and the wet season. The annual probability of illness for lettuce, manured soil and wastewater were then compared against the tolerable risk level by the World Health Organization per person per year (WHO, 2006).

4.0 Results

4.1 Risk of infection

The annual probability risk of infection for *E. coli* O157:H7 during the dry season was 10^{-3} for both consumers and farmers which were also closed to the 95th percentile of the annual risk in all the samples (Table 4.1). This indicated a risk of 3 illness of *E. coli* O157:H7 per 1000 farmers and consumers in the dry season. However the annual probability risk of illness for *Salmonella* spp for consumers was a little below the acceptable level but that of farmers remained as high as the annual probability of risk of illness presented by *E. coli* O157:H7 (Table 4.1).

Table 1: Risk of infection during the dry season

	***E. coli* O157:H7**			***Salmonella* spp**		
	Median	**5th Percentile**	**95th Percentile**	**Median**	**5th Percentile**	**95th Percentile**
Lettuce						
Prob. of infection	1.69×10^{-3}	1.00×10^{-2}	2.48×10^{-3}	1.00×10^{-5}	1.00×10^{-6}	9.80×10^{-5}
Annual prob. of infection	9.9×10^{-3}	1.00×10^{-2}	9.90×10^{-3}	1.00×10^{-5}	1.00×10^{-6}	7.80×10^{-2}
Manured soil						
Prob. of infect.	2.52×10^{-3}	1.89×10^{-3}	2.85×10^{-3}	4.10×10^{-4}	1.19×10^{-2}	2.85×10^{-3}
Annual prob. of infection	9.99×10^{-3}	9.96×10^{-3}	9.99×10^{-3}	9.36×10^{-3}	5.46×10^{-1}	9.99×10^{-3}
wastewater						
Prob. of illness	1.97×10^{-3}	1.38×10^{-3}	2.42×10^{-3}	1.97×10^{-3}	1.49×10^{-4}	6.72×10^{-5}
Annual prob. of infection	9.99×10^{-3}	9.99×10^{-3}	9.99×10^{-3}	9.99×10^{-3}	9.61×10^{-3}	9.90×10^{-3}

The results in table 4.2 indicates both probability infection and annual probability risk of infection of 10^{-3} considering about 50 % of the population during the wet season for *E. coli* O157:H7 and *Salmonella* spp for all the samples (lettuce, wastewater and manured soil), respectively. The results exceeded the tolerable level of 10^{-4} stated by WHO by 3 order of magnitude for all pathogens (table 4.2). This simply means that annual probabilities risk of illness that will be presented by both *E. coli* O157:H7 and *Salmonella* spp infections for example in consumers is 10 out of 1000 persons exposed to contaminated lettuce (table 4.2)

Table 2: Risk of infection of during the wet season

	***E. coli* O157:H7**			***Salmonella* spp**		
	Median	**5th Percentile**	**95th Percentile**	**Median**	**5th Percentile**	**95th Percentile**
Lettuce						
Prob. of infection	3.81×10^{-3}	2.56×10^{-3}	4.85×10^{-3}	1.66×10^{-3}	6.55×10^{-4}	2.90×10^{-3}
Annual prob. of infection	9.99×10^{-3}	9.99×10^{-3}	9.99×10^{-3}	9.99×10^{-3}	9.98×10^{-3}	9.99×10^{-3}
Manured soil						
Prob. of infection	3.34×10^{-3}	2.58×10^{-3}	3.70×10^{-3}	1.70×10^{-4}	3.50×10^{-5}	2.58×10^{-3}
Annual prob. of infection	9.99×10^{-3}	9.99×10^{-3}	9.99×10^{-3}	6.79×10^{-3}	2.23×10^{-4}	9.99×10^{-3}
Wastewater						
Prob. of illness	2.77×10^{-3}	2.05×10^{-3}	3.39×10^{-3}	1.48×10^{-3}	6.43×10^{-5}	2.53×10^{-3}
Annual prob. of infection	9.99×10^{-3}	9.99×10^{-3}	9.99×10^{-3}	9.99×10^{-3}	9.87×10^{-3}	9.99×10^{-3}

4.2 Seasonal variation of annual risk of infection for *E. coli* O157:H7 and *Salmonella* spp

The variation of risk of infection showed a major significant deviation from a highly risk of salmonellosis among consumers during the wet season to a lower risk during the dry season (Figure 4.1), as the incidence of salmonellosis shows a lower risk below the tolerable level for consumers, however, the exposure scenario for the farmers to manured soil and wastewater was high for both seasons, the risk of exposure to wastewater was the same throughout the year irrespective of the season whereas the risk of infection by exposure to manured soil is a little lower in the dry season than the exposure to wastewater though exceeding the tolerable level by 3 order of magnitude. However the risk of infection associated with *E.coli 0157:H7* for both farmers and consumers by exposure to wastewater, manured soil and consumption of lettuce were the same in both seasons (Table 4.1), the seasons show no impact on risk of infection as they all exceed the tolerable level of 10^{-4} by 1 order of magnitude. The high level of the risk gives credence to the infectiousness of *E. coli* O157:H7 irrespective of seasonality changes.

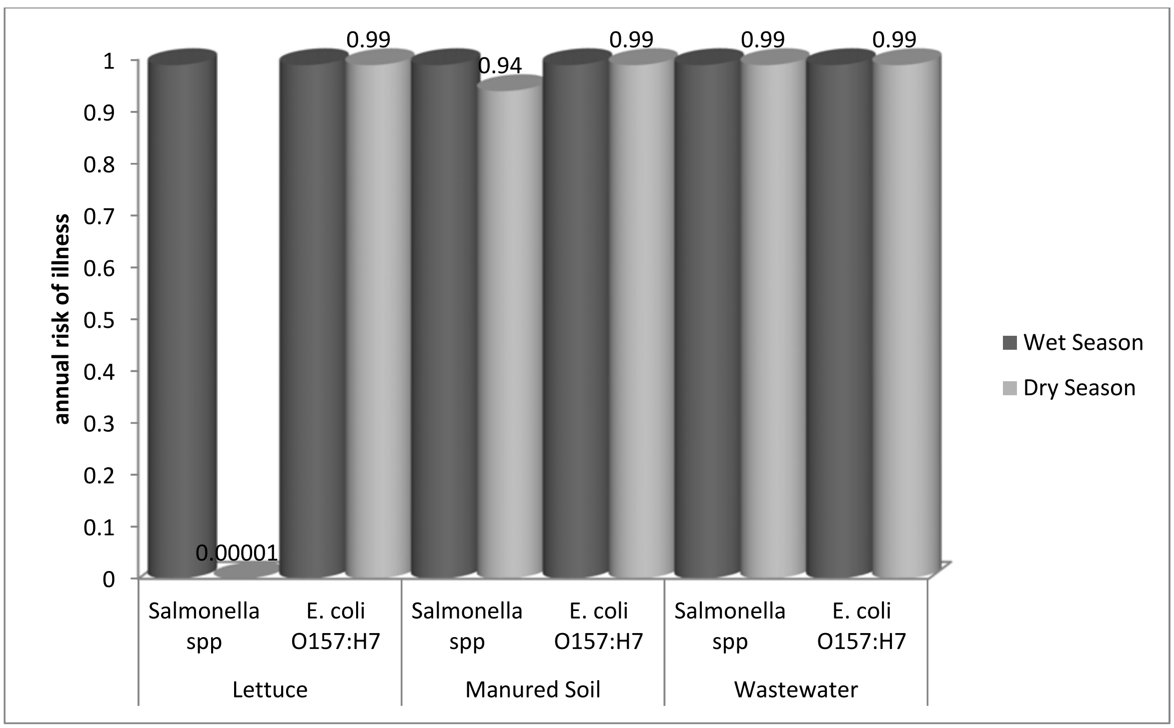


Figure 1: Seasonal variation as against risk of illness for *E. coli* O157:H7 and *Salmonella* spp

5.0 Discussion

Microbial health risk assessment on consumers of unrestricted irrigated vegetables and farmers exposed to wastewater irrigated fields and poorly treated manured soil had attracted the attention of researchers for many years now (Seidu *et al.,* 2015). The World Health Organization (WHO, 1989) has layed down recommendations for standard thresholds of 10^3 FC/100 ml and ≤ 1 egg/L of faecal coliform and helminth eggs, respectively in wastewater used for unrestricted irrigation. This is to minimize any associated health risk problems especially in poor countries due to lack of strict policies on the use of wastewater. Manured soil applied by agricultural farmers provides nutrients to the vegetables but introduces a lot of pathogens which poses threats to human health which needs attention (Yang *et al.,* 2004). A study in Zaria, Nigeria, showed that water sampled from households with livestock contains *Salmonella* spp while water from households without livestock had no *Salmonella* spp (Abakpa *et. al.*, 2011). The high risk of illness associated with this current study is believed to occur due to the introduction of the pathogen through animal husbandry operations such as the application of their faeces as organic manure without adequate treatment (Amoah *et al.,* 2005). Other studies have shown that poultry manure habour a lot of *Salmonella* spp which contribute immensly to the risk of ilness to consumers and farmers observed in this current study (Orji *et al.,* 2005). Food borne disease outbreaks have been widely implicated with consumption of unrestricted wastewater irrigated vegetables (Gould *et al.,* 2013). This current study indicated high risk of illness of *Escherichia coli* O157: H7 among consumers and farmers which can cause life threatening haemolytic uremic syndrome (Banatvala *et al.,* 2001). Many other studies have also associated *E. coli* O157:H7 primarily with animal products and contaminated fresh produce and outbreaks attributed to *E. coli* O157:H7 have been a recurring issue (Erickson and Doyle, 2012). Similarly, this current study has shown presence of *E. coli O157:H7* in wastewater irrigated lettuce and therefore high risk beeb posed to consumers of the product. *Salmonella* spp has also been significantly present in the same lettuce samples in which *E. coli* O157:H7 were detected. It has been noted from other studies that *Salmonella* spp and *E. coli O157:H7* infect more than an estimated 1.6 million humans in the United States at a cost of more than $15 billion per year (Scharff, 2010). This study also detected *Salmonella* spp and *E. coli O157:H7* in wastewater used by farmers for irrigation and lettuce salad from street food which agrees with report from studies that indicated that, pathogenic microorganisms are generally transmitted either directly through contaminated foods or indirectly through contaminated water and crops (LeJeune and Kersting, 2010; Pachepsky *et al*., 2011). The role of free-ranging birds and livestock in pathogen recirculation and local transmission has been noted on and around farms (Gaukler *et al*., 2009; Cernicchiaro *et al.,* 2012).

This present study observed that, annual risk for consumers associated with *Salmonella* spp in the dry season was below the tolerable risk level, the annual risk was found to be10^{-5}, a one (1) order of magnitude lower than the tolerable level (WHO, 2006), and also representing an acceptable level of annual risk of illness of one illness of salmonellosis per 100000 consumers in the dry season. This could be attributed to the low survival rate of *Salmonella* spp on lettuce during the high temperatures of the dry season which may not support pathogen growth. In addition the leaves have large surface area which enhanced exposure of prevailing pathogens to the unfavorable conditions of the season. However manured soil and wastewater modeled annual risk of illness exceeding the tolerable level by a 3 order of magnitude (WHO, 2006).

When the median annual probability of infection was considered for *E. coli O15:H7* and *Salmonella* spp for the accidental ingestion of wastewater and manured soil by farmers, the annual probability of illness for wastewater and manured soil exceeded the tolerable risk of $\leq 10^{-4}$ per person per year for *E. coli O157:H7* as reported by Mara (2010). All exposures indicated high risk of illness to both farmers and consumers during the wet season as a result of low temperature during the wet season which ensures a conducive atmosphere for sustaining pathogen life which includes the provision of suitable substrate for growth and reproduction (Oliveira *et al*., 2012). In addition, the high risk of illness associated with consumers during the wet season could have resulted from run-off from other polluted fields into already polluted waters used for irrigation (Islam *et al*., 2004). This then implies that farmers and consumers are all at a risk of infections/illness associated to these pathogens in both the wet and the dry seasons but more pronounced in the wet and could therefore lead to increased morbidity and mortality as reported (Mead *et al*., 1999).

Conclusion

Both farmers and consumers are at risk of microbial infection from exposures to wastewater, manured soil and unrestricted vegetable consumption and the risk levels indicated are higher than the WHO standard. This therefore invites attention from policy makers to take strict measures and enforce the existing regulations on the use of wastewater for irrigation.

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