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Microbial Risk Assessment of Urban Agricultural Farming: A Case Study on Kwame Nkrumah University of Science and Technology Campus, Kumasi, Ghana

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ABSTRACT

This study was carried out on KNUST campus, Kumasi, Ghana, to assess the microbial quality of irrigation water and on vegetables as well as the risk associated with vegetable irrigated farming. Microbiological contamination of irrigation water and lettuce was monitored weekly for two months from eight farms for faecal and total coliform, and helminth eggs levels. Quantitative microbial risk assessment was subsequently evaluated. Faecal coliform and helminth egg concentrations of irrigation water ranged from $3.2x10^3$ to $3x10^4$ faecal coliform/100 ml and 6 to 15 eggs/l respectively and that of the lettuce ranged from $7x10^2$ to $1.8x10^3$ faecal coliform/10 g and 6 to 19 eggs/100 g respectively. *Ascaris lumbricoides, Schistosoma, Trichuris trichiura,* and *Strongyloides* larvae were isolated from both irrigation water and on lettuce. *Ascaris* and *Escherichia coli* were the reference organisms used in the quantitative microbial risk assessment. The annual risk of infections to the farmers for both pathways was 10^{-2} for *Ascaris* and 10^{-1} for *E. coli*.

Keywords: *Faecal coliform, helminth egg, irrigation water, lettuce, microbial risk assessment*

1. INTRODUCTION

On an increasingly urban planet, one practice that has gained importance in both the developing and developed worlds is urban farming. It was estimated in 1996 that about 800 million people were actively engaged in urban agriculture worldwide and 200 million were considered to be market producers and contribute to feeding urban residents (FAO, 1999). Worldwide, agriculture is being put under a strong pressure to produce more food with less water, on the strength of rapid population growth and consequentially rapidly growing urban centres, especially in developing countries as well as an increased demand for drinking water and agricultural products with an increased water scarcity (Merker, 2004). As a result of the stress on the limited water and other factors farmers in urban agriculture have adopted the use of wastewater for irrigation. Research estimates that at least 20 million ha are irrigated with wastewater, and about 200 million farmers are involved (Raschid-Sally and Jayakody, 2008).

Little wastewater in the developing world undergoes treatment of any kind, and even in affluent countries the cost of treatment is a key criterion determining the likely success or failure of a reuse scheme (Robinson, 2003). Based on average per capita daily consumption rate of 60 L and a wastewater flow of 80% (Cofie and Awuah, 2008), it is estimated that a population of 1.58 million inhabitants of Kumasi will generate 76000 m^3 of wastewater daily.

Wastewater contains pathogenic microorganisms- bacteria, viruses, helminths, and protozoa. In Ghana, high levels of faecal coliforms and helminth eggs were isolated in both grey and black water (Awuah *et al*., 2002). Due to the contamination of food with these pathogens, which poses public health risk, wastewater irrigation has been approached with trepidation (Toze, 2006). Consequently methods are required to determine the microbial risk in wastewater irrigation to safeguard the health of farmers and develop realistic schemes for the practice.

One powerful tool for the estimating order-of-magnitude risk associated with specific scenarios is the quantitative microbial risk assessment (QMRA) (Hamilton *et al*., 2006).
Haas *et al*. (1999) identified four formal procedures of determining QMRA, namely:

- Hazard identification: This step involves weighing the available evidence and determining whether a substance or constituent exhibits a particular adverse health hazard.
- Exposure Assessment: The exposure assessment stage refers to measuring process or the estimate of the intensity, frequency and duration of human exposure to a particular agent, with the purpose of determining the quantity of organisms that correspond to a single exposure or the total quantity of organisms that comprise a set of exposures.
- Dose-Response Assessment: The fundamental goal of a dose-response assessment is to define relationship (typically mathematical) between the quantity (concentration) of a micro-organism to which the

person or population is exposed and the risk of this concentration producing adverse effects.

- Risk Characterisation: It involves the integration of exposure and dose-response assessments to arrive at the quantitative probabilities that effects will occur in humans for a given set of exposure conditions.

Several risk assessment studies have been performed for the contamination of vegetables with viruses by reclaimed irrigation water where simplistic models (Asano *et al.*, 1992; Shuval *et al.*, 1997) and probability distribution function (Petterson *et al.*, 2002; Hamilton *et al.*, 2006) were used to define parameters. The number of human infections was assessed.

The objectives of this paper were to examine the microbiological contamination of irrigation water and vegetables and to assess the quantitative microbial risk of urban vegetable farming.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in the Kwame Nkrumah University of Science and Technology (KNUST) campus, Kumasi, Ghana (Fig. 1).

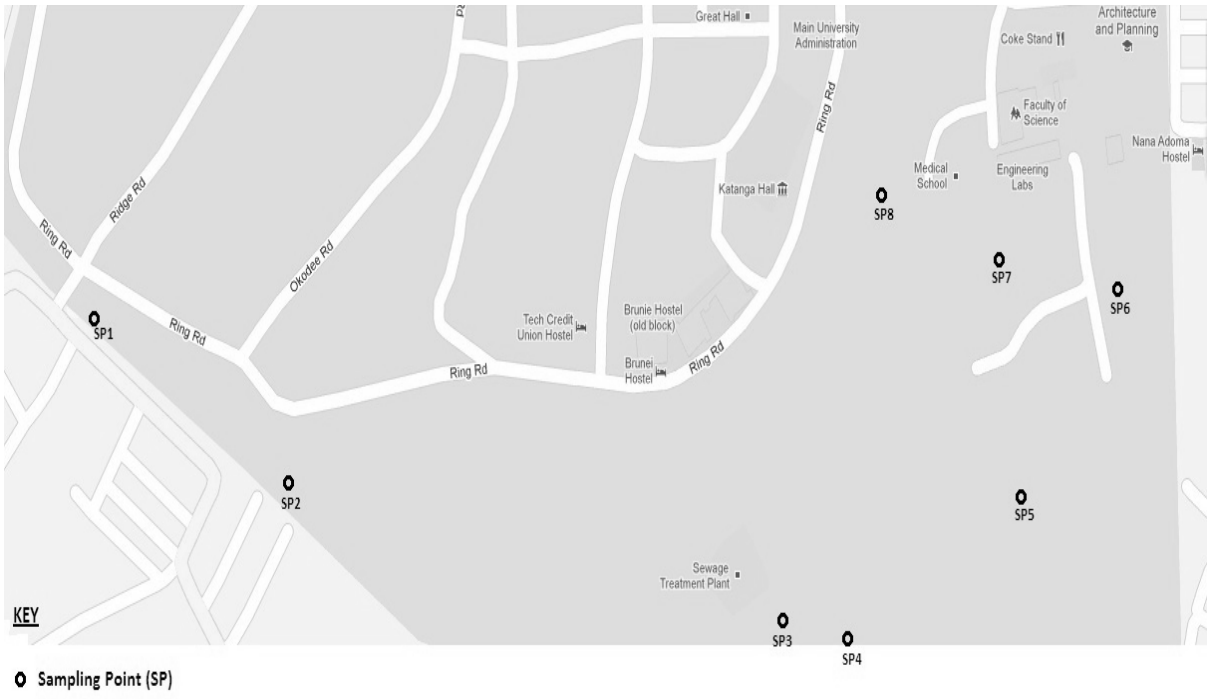

Figure 1. Study area with sampling points

The University is located in the eastern section of the Kumasi metropolis (MLGRDE, 2006). The University campus has portions of undeveloped plots of land which are used by both immigrants from rural areas and the indigenous community for vegetable farming. There are five major urban vegetable farming sites on the campus that were identified. These farming sites were:

- Between the School of Medical Sciences (A);
- Behind the College of Engineering Laboratories (B);
- Behind the KNUST School of Business (C);
- Areas around the University Sewage treatment plant (D); and
- Areas around the entrance of the University at Ahinsan gate (E).

Farmers are involved in irrigated vegetable farming which is a commercial market-oriented activity. It is carried out all year round though, inconsistent in the rainy season. High value and easily perishable exotic vegetables are grown. These include lettuce, carrot, cabbage, spring onions, green pepper, and cauliflower. The vegetables are cultivated on raised beds. The sources of irrigation water are shallow wells, and streams. Irrigation is carried out with buckets and watering cans. Other farmers also use motorised-pumps with hose pipes.

2.2 Sampling and Data Collection

Irrigation water used by farmers was sampled for laboratory analyses. Eight sampling points were identified from the 5 major sites representing the samples (SP1, SP2, SP3, SP4, SP5, SP6, SP7, and SP8) (Fig. 1) for microbiological analysis. Also, vegetables (lettuce) from the same sampling points were randomly collected just before harvesting.

Microbiological analysis of irrigation water and lettuce, which comprised of faecal and total coliform and helminth

eggs, from the different sampling sites were analysed from February to April 2009.

A 500 mL composite sample of irrigation water was collected from each sampling point during the day in keeping with farmer's irrigation practices. A total of 64 composite water samples were analysed. Also a minimum of three samples of whole lettuce were randomly collected just before harvesting from the selected farm sites, put in labelled sterile polythene bag to form a composite sample and transported on ice to the laboratory where they were analysed immediately. A total of 24 composite samples of lettuce were analysed.

2.3 Microbiological Examination

Both irrigation water and lettuce were analysed quantitatively for faecal and total coliform using membrane filtration method (Ayres and Mara, 1996). Helminth eggs were also counted using the concentration method (Schwartzbrod, 1998). The morphological features like shape, size, and colour of the eggs were established using colour charts for the diagnosis of intestinal parasites (WHO, 1994).

2.4 Data Analysis

Total and faecal coliform populations were normalised by log transformation before analysis of variance (ANOVA). Results of analysis are quoted at $p < 0.05$ level of significance.

3. RESULTS AND DISCUSSION

3.1 Microbiological Quality of Irrigation Water

The irrigation water sampled during the study period showed mean faecal coliform levels exceeding the mean count of $1x10^3$/100 mL recommended by WHO (2006) for unrestricted irrigation of crops likely to be eaten raw (fig. 2). There was significant difference in mean faecal coliform levels in irrigation water at different sampling points.

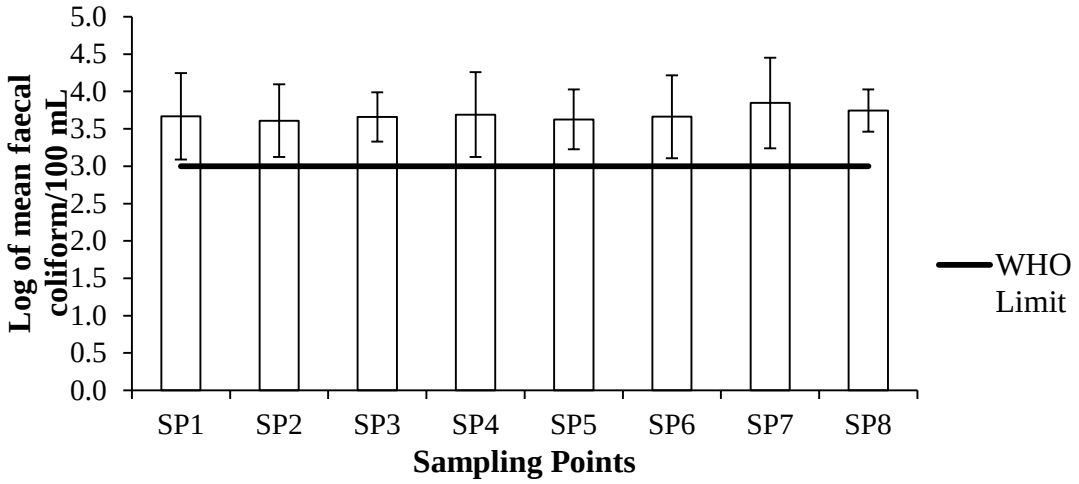

Figure 2. Faecal coliform levels of irrigation water from different sampling points on KNUST campus

The poor quality of irrigation water may be due to the fact that the shallow wells found on the sites were not well constructed and protected exposing them to contaminants from mainly fresh poultry manure on site and runoffs. Heaps of poultry manure in the farm were not well protected exposing them to contaminants mainly fresh poultry manure and faecal matter from the farmland and surrounding farm environment through runoffs. Fresh poultry manure without sufficient drying used for vegetable production in Kumasi recorded high faecal coliform counts (Drechsel *et al*., 2000). Also, the continual use of the land all year-round may render the land contaminated and subsequently contaminating the irrigation water through runoff. The faecal coliform recorded in the research was lower than what was previously assessed in Kumasi by Obuobie *et al*. (2006) who worked on lands located at densely populated areas.

SP7 recorded the highest faecal coliform levels. This may be as a result of grey water from nearby hostels and residences polluting the shallow well.

Total coliform level was relatively high. Total coliform levels ranged from $4x10^3$ to $8x10^4$/100 mL. SP7 recorded the highest total coliform levels.

Helminth eggs were identified in all water samples. The mean helminth eggs population exceeded the recommended level of $\leq$ 1 egg L^{-1} for unrestricted irrigation (WHO, 2006). Significant difference was observed in the mean helminth eggs populations recorded in irrigation water at different sampling points (table 1).

Table 1. Arithmetic mean number of helminth eggs in irrigation water

Sampling points	Arithmetic mean[1] of helminth eggs/L				
	Average of all species of helminth eggs	Species of helminth eggs			
		Ascaris	*Schistosoma*	*Trichuris*	*Strongyloides*
SP1	12 ± 6	6 ± 3	4 ± 4	3 ± 5	0
SP2	6 ± 6	5 ±3	2 ± 5	0	0
SP3	6 ± 3	4 ± 4	0	1 ± 2	1 ± 2
SP4	6 ± 4	4 ± 3	2 ± 3	0	0
SP5	13 ± 8	6 ± 7	9 ± 7	0	0
SP6	8 ± 6	3 ± 4	1 ± 1	4 ± 8	0
SP7	6 ± 4	3 ± 4	3 ± 3	0	0
SP8	7 ± 4	3 ± 3	0	3 ± 5	1 ± 2

[1]Mean at 8 sampling times (weekly) rounded to the nearest whole number.

The high helminth egg population was probably due to high poultry manure run-off from the field. Poor sanitation and hygiene on the farm sites may also be a contributing factor to the high helminths population. Different types of helminth eggs were isolated in the irrigation water. These include *Ascaris lumbricoides*, which was the most predominant; *Schistosoma* spp; *Trichuris trichiura*; and *Strongyloides* larvae.

3.2 Microbiological Quality of Lettuce

About 50% of the lettuce analysed showed faecal coliform levels that were more than $1x10^3$ per 100g wet weight (fig. 3) and can be classified according to the International Commission on Microbiological Specifications for Food (ICMSF, 1974) guidelines as undesirable. There was no significant difference observed in the mean faecal coliform concentration in lettuce at different sampling points.

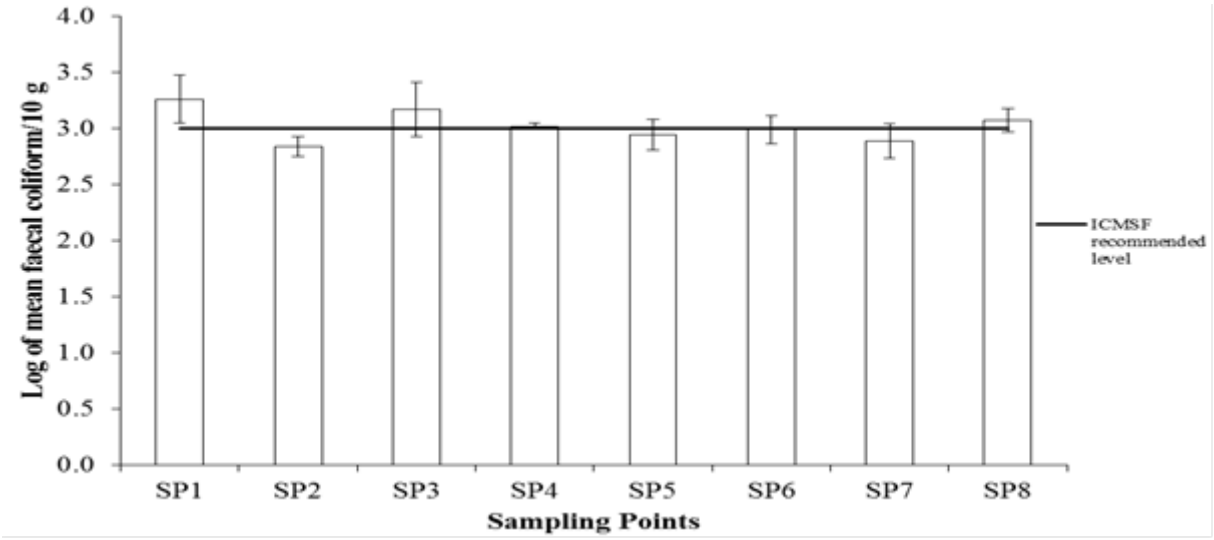

Figure 3. Faecal coliform levels of lettuce from different sampling points in KNUST campus

Sources of faecal coliform contamination of lettuce may include overhead irrigation of lettuce with already contaminated water, planting in contaminated soils and frequent application of poultry manure which was not well composted (Amoah *et al.*, 2005). Faecal coliform on lettuce was relatively less than that in the irrigation water. This may be due to the rapid die-off rate of bacteria on lettuce as a result of exposure of lettuce to sunlight and high temperature (Petterson and Ashbolt, 2001). However, the faecal coliform level can increase if vegetables are washed in irrigation water and decrease if farmers practise cessation of irrigation before harvest (Keraita *et al.*, 2007; WHO, 2006).

Lettuce analysed from different sampling points showed different mean helminth egg populations. The mean helminth egg population ranged from 6 to 19 eggs/100g wet weight (table 2). No significant difference was observed in the mean helminth egg populations recorded in lettuce at different sampling points. This is relatively lower than 1.1 eggs/g wet weight recorded by Amoah *et al.* (2006) in Kumasi and Accra. The relatively high helminth egg population may be as a result of the frequent application of incompletely composted poultry manure and farming on already contaminated soils. This has been confirmed by Amoah *et al.* (2005) and Drechsel *et al.*

(2000). Helminth eggs counted included *A. lumbricoides*, *Schstosoma* spp., *T. trichiura*, and *Strongyloides* larvae with *A. lumbricoides* predominating. *A. lumbricoides* was relatively higher because of their longer persistence in the environment (WHO, 2006).

Table 2: Arithmetic mean of helminth eggs on lettuce

Sampling points	Arithmetic mean[1] of helminth eggs/100 g				
	Average of all species of helminth eggs	Species of helminth eggs			
		Ascaris	*Schistosoma*	*Trichuris*	*Strongyloides*
SP1	19 ± 5	9 ± 1	7 ± 1	0	4 ± 6
SP2	6 ± 3	7 ± 4	0	0	0
SP3	17 ± 7	6 ± 8	6 ± 4	0	6 ± 8
SP4	8 ± 5	0	8 ± 2	0	0
SP5	17 ± 5	8 ± 4	8 ± 2	0	3 ± 4
SP6	7 ± 2	0	4 ± 3	3 ± 4	0
SP7	11 ± 3	7 ± 3	4 ± 3	0	0
SP8	15 ± 6	13 ± 9	3 ± 2	0	0

[1]Arithmetic mean numbers represent the mean of all the different types of eggs as well as *Strongyloides* larvae

3.3 Quantitative Microbial Risk Assessment (QMRA)

3.3.1 Hazard Identification

All pathogens that are excreted in poultry faeces and pathogens from insanitary and unhygienic surrounding environment could potentially be found in irrigation waters and vegetables. A selection of pathogens was made for the risk assessment, representing bacteria (*Salmonella* and *E. coli*) and helminths (*A. lumbricoides*). From epidemiological reviews, helminths and bacteria pose the greatest health risks in wastewater-irrigated agriculture (Blumenthal and Peasey, 2002). The choice of *Ascaris* was due to its predominance in the study and its parasitic infection worldwide with prevalence rate of 52% in Ghana (Hortez *et al*., 2003). *Ascaris* can also persist for months to years in soils under harsh conditions (Jimenez, 2007) thus making it an ideal reference organism for QMRAs in a developing country (Hamilton *et al*., 2006) like Ghana.

3.3.2 Exposure Assessment

The exposures were identified by on-site survey of the farming site and its surroundings. During irrigation, farmers did not wear protective clothing and were in direct contact with the irrigation water. Exposure paths assessed included accidental ingestion of irrigation water and consumption of irrigated vegetables. Lettuce was used for the assessment because it was the largest cultivated vegetable. The following assumptions and scenarios were considered:

Bacteria and *Ascaris* are well dispersed and there is no reduction of these in irrigation water. Farmers will ingest 1 – 2 mL of irrigation water per day, a narrow range based on Ottoson and Strenstrom (2003). The exposure days per year to irrigation water for farmers are 300 days. Also 10 – 15 mL of irrigation water will be left on a 100 g lettuce after harvest (Mara *et al*., 2007; WHO, 2006). Two days is allowed between lettuce harvest and consumption (WHO, 2006). The amount of lettuce consumed per person per day was taken as 100 g at a rate of one lettuce per week per farmer based on survey carried out in this study.

3.3.3 Dose-Response Assessment

For dose-response relationships, the beta-Poisson dose response model described by Haas *et al*. (1999) was used for *E. coli* while the single-hit exponential dose response model was applied to *Ascaris*.

Single-hit exponential model:

$$P_{inf} = 1 - \exp(-rd) \quad (1)$$

Beta-Poisson model:

$$P_{inf} = 1 - \left[1 + \frac{d}{N_{50}}\left(2^{\frac{1}{\alpha}} - 1\right)\right]^{-\alpha} \quad (2)$$

P_{inf} = the probability of infection

r = the empirical parameter assumed to be constant for any given host and given pathogen

d = the mean ingested dose.

N_{50} = the median dose

α and β = slope parameters , which hold when $\beta \geq 1$ and $\alpha \leq \beta$

The annual probability of infection is given by:

$$P_{yr} = 1 - (1 - P_{inf})^n \approx nP_{inf} \quad (3)$$

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

n = number of exposure events per year (event/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.

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

$$\lambda = M_i M_{body} c_{iw} V_{prod} e^{(-kt)} \quad (4)$$

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

3.3.4 Risk Characterisation

The mean annual risk of infection for the accidental ingestion of irrigation water and the consumption of lettuce are illustrated in table 4. 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 ≤10^{-4} per person per year (WHO, 2006; US EPA, 1992).

Table 4: Annual probabilities of *A. lumbricoides*, and *E. coli* infection associated with the ingestion of irrigation water and consumption of lettuce

Pathogen	Irrigation water		Lettuce	
	P_{inf}	P_{yr} (n=300)	P_{inf}	P_{yr} (n=52)
Ascaris	2.0 x 10^{-4}	5.8 x 10^{-2}	1.50 x 10^{-3}	7.51 x 10^{-2}
E. *coli*	3.68 x 10^{-3}	6.7 x 10^{-1}	8.63 x 10^{-3}	3.63 x 10^{-1}

The annual risk of *E. coli* infections in both scenarios (ingestion of irrigation water and consumption of vegetables) is 10^{-1}. That is there will be a risk of one infection of *E. coli* per 10 farmers per year. This is relatively high and exceeded the benchmark in both scenarios by a 3 order magnitude (10^{-3}). Farmers may be at risk of contracting diarrhoeal diseases.

The annual risk of *Ascaris* infection in both scenarios was 10^{-2}; inferring an annual risk of one infection of *Ascaris* per 100 farmers. The recorded value was above the recommended annual risk of infection by a 2 order of magnitude. This is attributed to the relative low levels of *Ascaris* counts in both the irrigation water and 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. An earlier study (Ackerson and Awuah, 2010) in the same study area showed that helminthiais complaints among farmers were relatively low (8%). The study reported that it may be due to the intake of anti-helminth drug by farmers (67%).

A portion of the infected farmers may develop a clinical illness, a disease with clinical signs and symptoms that are recognisable (Asano *et al*., 2007). Other infections will lead to illness and subsequently death. However, there are infections that remain subclinical. Individuals with pre-existing immunity may be protected from infection and illness at low doses (Chappell *et al*., 1999).

Cessation of irrigation before harvest can be adopted to minimise the risk of infection in lettuce consumption. Keraita *et al*. (2007) reported that cessation of irrigation before harvesting reduces microbial contamination of lettuce irrigated with wastewater. Also postharvest practices such as washing, disinfection, and cooking can be adopted. Lang *et al*. (2004) and Bracket (1987) have confirmed the efficacy of washing at removing bacteria.

4. CONCLUSION

Microbiological quality of irrigation water and lettuce were above WHO (10^3 faecal coliform/100 mL and ≤ 1 helminth egg/L) and ICMSF (10^3 faecal coliform/100g) recommendations respectively. Microbial risk in urban vegetable farming to the farmers is relatively high (annual risk of infections of 10^{-2} for *Ascaris* and 10^{-1} for *E. coli*); mainly due to the relatively high levels of pathogens in the irrigation water and on the vegetables.

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