

Original Research Article

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Seasonal Disparity in Microbial and Soil Transmitted Helminths Concentrations in Irrigation Samples

A. Abubakari^{1,2*}, E. Degraft-Johnson³, J.A. Larbi¹ and RC. Abaidoo¹

¹Department of Theoretical and Applied Biology, ³Department of Mathematics,
Kwame Nkrumah University of Science and Technology, Kumasi-Ghana

²Department of Laboratory Technology, Faculty of Health Sciences, Kumasi Technical
University, Kumasi-Ghana

*Corresponding author

ABSTRACT

This study was conducted to assess the concentrations of microbial pathogens and Soil Transmitted Helminths (STH's) as an initial step in the health risk reduction and reuse strategies in wastewater irrigation practices. Kumasi where the study was conducted is the second largest city in Ghana having significant number of farmers who engage in unrestricted wastewater irrigation practices. Field and laboratory analysis was carried out within 40 weeks covering both the dry and the wet seasons for almost all the irrigation sites within the city. Samples collected and analyzed included wastewater used for irrigation, Manured soil and Lettuce irrigated with wastewater. Samples analyzed in the laboratory resulted in identification and quantification of microorganisms with *E. coli* and Total coliforms presenting mean levels exceeding WHO recommendations $1 \times 10^3/100\text{ml}$ and ≤ 1 egg/L for unrestricted irrigation. This study showed significant difference between the concentrations presented within the dry and wet seasons with the *P*-values far less than 0.05 (< 0.05). With that notwithstanding, *E. coli* O157:H7 showed no significant difference between the seasons for manured soil ($P = 0.107$). For the STH's With the exception of *T. trichiura* which had its concentrations in both seasons ≤ 1 egg/L which conforms to WHO recommendations, *A. lumbricoides* and *A. duodenale* were in excess of 1 or 2 eggs. This result therefore calls for strict measures and policies for farmers who engage in unrestricted irrigation practices.

Keywords

Wastewater irrigation, Microbial, Seasonal and Soil transmitted helminths

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Introduction

Rapid urbanization and population growth has put a lot of pressure on food security and fresh water supply. Due to this challenge, farmers in developing world use polluted water for irrigating their crops. In Ghana studies revealed that most of the surface water

used for irrigation are heavily polluted and therefore not appropriate for unrestricted irrigation practices (Mensah *et al.*, 2001). It was also established by Cornish *et al.*, (2001), that in Kumasi alone, about 12,000 farmers are involved in vegetable farming during the dry seasons. Farmers rely on the urban wastewater as inputs for production which

also allows them to grow multiple cycles in a year. Urban wastewater has rich essential plant nutrients, therefore given farmers the opportunity to gain high yields with only little financial input. However, the uncontrolled reuse of wastewater poses serious threats to public health because of the fecal-oral transmission route. Wastewater contains various pathogens including helminth, bacteria and viruses that were passed by the infected host's excreta (Toze, 2006). These pathogens can survive in water for weeks to month, putting the farmer and all field workers at risk of infection. However, not only farmers and field-workers are at risk, crops grown with wastewater can be contaminated with pathogens, thus also placing the consumers at risk. Farm workers and other population exposed to wastewater irrigation practices have high prevalence of infection with *Ascaris*, hookworm and other enteric infections (Ensink *et al.*, 2005; Blumenthal and Peasey, 2002). A cross sectional study conducted in Faisalabad in Pakistan, revealed a significant *E. coli* outbreaks may well have been linked to leafy salad vegetables but were not proven, e.g., an outbreak in 1995 involving leafy lettuce occurred in Montana affecting more than 70 people (Ackers *et al.*, 1998), and another one in Minnesota in 2005 infected 12 people who ate bagged salads (Anonymous, 2005). A study revealed increase in risk of STH infections in wastewater farming households than those that do not engage in wastewater irrigation practices (Ensink *et al.*, 2005).

Objectives of study

This study sought to; 1) identify the multiple exposure pathways linked to wastewater irrigation practices 2) measure the concentration of pathogens in irrigation water, manured soil and lettuce irrigated with wastewater relation to seasonality.

Materials and Methods

Study area and design

This study was carried out in the Kumasi Metropolis from August to June covering both the dry and the wet seasons. The study area was chosen because; it is the second largest city in Ghana with a population size of 2,035,064 and a household size of 3.8 and an annual growth rate of 2.7% (Ghana Statistical Service, 2010). Kumasi located in the Ashanti region of Ghana experiences 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° and 30.7°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 unrestricted irrigation all year round. Vegetable Irrigation sites within the City were initially mapped out. There were about 150 farmers with an average of 3 field assistants helping them in their individual farms with others having to be helped by relatives and friends. In all 8 sites were identified and worked with in the study. Each site has approximately 1200 beds with 18-20 farmers. Pretested quality farm-based questionnaires were administered to farmers who participated in the study.

Sampling

Irrigation water was sampled with 1.5 liter sterile plastic bottles with screwed caps early morning at 0600 hours. Manured soil, Lettuce from irrigation farms were aseptically sampled in triplicate at random into plastic bags (Stomacher^(R)) from lab system from Seward, UK kept in cooling boxes and sent to the laboratory for analysis within the same day.

Laboratory analysis

Total coliforms and *E. coli*

Samples were weighed and homogenized appropriately with distilled water with known volumes. 1 millilitre of homogenized samples were picked and serially diluted and 10 ml of the diluents added to a beaker containing 90ml of distilled water mixed with one pack of colisure powder (Quanti-Tray®/2000). The mixture was allowed to stand for some time while shaking it in between to give room for total mixture. It was then poured into the Quant- tray and heat sealed with the Quanti-tray sealer. Incubation was done at $35^{\circ}\text{C} \pm 0.5$ and the result read after 24 hours using the protocol provided. If sample contained total coliforms then, original yellow wells turned red or magenta. *E. coli* present in the sample wells metabolized colisure nutrient and fluoresced in a dark room when 6 – watt, 365m, UV light was passed through the surface of the sample and wells that gave purple colour were counted as *E. coli* colonies per 100 ml of sample using the most probable number (MPN) table (IDEXX, Westbrook, USA).

Salmonella spp.

Samples were weighed and blended and then 1ml which is equivalent to 1g of sample were picked with a sterile pipette 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 Oxoid Salmonella Test Kit which is 100% sensitive and between 97.2%-100% specific (Oxoid Limited, 2012)

E.coli 0157:H7

Samples were 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 agar (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 from Oxoid, England which has a specificity and sensitivity 100% and 99% respectively (Oxoid Limited, 2012).

Soil transmitted helminth eggs enumeration

Helminths eggs were enumerated using a combination of the floatation and sedimentation methods (Schwartzbrod, 1998). Samples were diluted up to 2ml in 4L container and allowed to stand overnight to enable the eggs to fully sediment. Series of sedimentation and floatation was carried out according to the protocol. With the aid of a micropipette, as much of the supernatant as possible was sucked up leaving approximately 1ml of deposit which was picked with a pipette and examined under the microscope. Helminthes eggs were identified using their morphological characteristics compared with the bench aids for the Diagnosis of Intestinal Parasites (WHO, 1994) and expressed as eggs per gram (EPG).

Results and Discussion

Microbial identification and quantification

Samples which include, lettuce, wastewater for irrigation and manured soil were assessed quantitatively for *E.coli*, Total coliforms, *salmonella*, *E.coli* 0157:H7 and helminthes eggs. The results obtained were normalized by log transformation of the coliform unit before an independent t test was applied to find the significance of differences due to seasonality concentrations. Wet and Dry seasons data were compared and analyzed for the concentration level using the log average

of the log transformation figures. Most data (*E.coli* and total coliforms) samples recorded in all seasons showed mean levels exceeding $1 \times 10^3/100\text{ml}$ recommended by WHO (2006) for unrestricted irrigation, moreover, the presence of helminthes egg were evident in all the different samples for the study, and also exceeded the ≤ 1 egg recommendation by the WHO (2006).

Bacterial concentrations

Bacterial concentrations were higher in all the different medium (Table 1 and 3), they exceeded WHO level in excess of 3/4 logs/cfu of the concentration levels in all seasons. On the seasonality effects on the concentration level, the dry seasons were lower compared to the levels of concentration in the wet season. However, concentration levels of *E.coli* 0157:H7 and *Salmonella* (Table 2 and 4) were lower and meets the WHO level of between 3 to 4 logs/cfu, *E. coli* 0157:H7 and *Salmonella*

were found to have lower concentration levels. Nevertheless, the concentration levels of bacteria on lettuce, manure soil and wastewater in the seasons showed a significant difference ($p < 0.05$) among them with the exception of *E.coli* 0157:H7 in manure soil (Table 2). *E.coli* 0157:H7 also recorded significant different levels of concentration for lettuce and wastewater only (Table 2). *Salmonella* (Table 4) showed a significant difference in all the different samples indicating different concentration levels due to seasons.

Helminthes egg

Helminthes eggs presented with high concentration levels in lettuce, manure soil and wastewater in both seasons, the arithmetic mean concentration levels were high for *Ascaris* spp, and *Ancylostoma* spp. *Trichuris* spp had <1egg in all seasons (Table 4) which meets acceptable levels of ≤ 1 egg.

Table.1 Concentration of total coliform, *E. coli*, *Salmonella* spp and *E. coli* 0157:H7 in irrigation water during the wet and the dry seasons

Microorganisms/ seasons	Mean microbial log concentrations in cfu/ml \pm SD	t-statistic	P-value
Total coliform			
Dry season	7.557 \pm 0.201	8.36	0.000
Wet Season	7.915 \pm 0.371		
<i>E. coli</i>			
Dry season	6.619 \pm 0.260	19.04	0.000
Wet Season	7.488 \pm 0.356		
<i>Salmonella</i> spp			
Dry season	2.524 \pm 0.984	2.93	0.017
Wet season	3.692 \pm 0.388		
<i>E. coli</i> 0157:H7			
Dry season	1.100 \pm 1.060	5.57	0.001
Wet Season	3.282 \pm 0.333		

SD – Standard deviation

Table.2 Concentration of total coliform, *E. coli*, *Salmonella* spp and *E. coli* O157:H7 in manured soil during the wet and the dry seasons

Microorganisms/ seasons	Mean microbial log concentrations in cfu/mg ± SD	t-statistic	P-value
Total coliform			
Dry season	7.684 ± 0.212	12.62	0.000
Wet Season	7.847± 0.281		
<i>E. coli</i>			
Dry season	6.653 ± 0.274	18.96	0.000
Wet Season	7.417± 0.257		
<i>Salmonella</i> spp			
Dry season	1.190 ± 1.02		
Wet season	3.694 ± 0.246	7.53	0.000
<i>E. coli</i> O157:H7			
Dry season	1.330± 1.360	2.81	0.107
Wet Season	3.551± 0.135		

SD – Standard deviation

Table.3 Concentrations of total coliform, *E. coli*, *E. coli* O157:H7 and *Salmonella* spp in lettuce during the wet and dry seasons

Microorganisms/ seasons	Mean log microbial concentration in cfu/ml ± SD	t-statistic	P-value
Total coliform			
Dry season	7.382 ± 0.170	12.62	0.000
Wet season	7.842 ± 0.307		
<i>E. coli</i>			
Dry season	6.456 ± 0.283	21.15	0.000
Wet Season	7.379 ± 0.279		
<i>E. coli</i> O157:H7			
Dry season	0.845 ± 0.775	8.72	0.000
Wet Season	3.538 ± 0.259		
<i>Salmonella</i> spp			
Dry season	0.796 ± 0.591	10.56	0.000
Wet season	3.644 ± 0.296		

SD – Standard deviation

Table 4 Mean egg concentrations and standard deviation of STH's in wastewater, manured soil and lettuce

Samples/season	Mean egg cocentrations ± standard error		
	<i>Ascaris lumbricoides</i>	<i>Ancylostoma duodenale</i>	<i>Trichuris trichiura</i>
Wastewater			
Wet season	2.11±0.14	0.44±0.07	0.03±0.02
Dry season	1.58±0.18	1.14±0.13	0.15±0.06
Manure Soil			
Wet season	2.82±0.23	1.26±0.13	0.36±0.07
Dry season	2.70±0.19	2.06±0.17	0.40±0.08
Lettuce			
Wet season	2.36±0.25	0.87±0.16	0.14±0.05
Dry season	1.52±0.13	0.99±0.11	0.15±0.04

Agricultural practices using wastewater need thorough planning supported with careful hygiene protocols. If this is adhered to, most health risk problems associated with this practice could be minimized. More especially among poor countries with pronounce lack of portable water for irrigation and minimal policies on the use of wastewater. The World Health Organization (WHO, 1989) made 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. 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 by Rosewell (2010) reported a high prevalence of STH's within urban and peri-urban regions compared to rural dwellings which was attributed to using untreated wastewater for irrigation in these sittings.

This study showed *E. coli* and Total coliforms concentrations in the examined samples to be

high for both seasons with mean levels exceeding the 1×10^3 /100 ml recommended by WHO (2006) for unrestricted irrigation. There were excesses of 3 to 4 logs in all seasons which could be attributed to the fact that about 90 % of wastewater generated even in the capital cities of Ghana are not treated and eventually mixed up with storm drain which most farmers rely on for unrestricted irrigation (Scott *et al.*, 2004). The results also confirmed studies by Gupta *et al.*, (2007) which reported high levels (concentrations) of *E. coli* in vegetables irrigated with wastewater. Amoah *et al.*, (2006) also confirmed in their studies that, drains and streams highly contaminated with faecal coliform concentrations above the WHO acceptable level was the primary water source used in irrigation of most vegetables in Accra. On the assessment of seasonal effects, the dry season samples had lower concentrations of *E. coli* and total coliform concentrations compared to those reported for the wet season which confirms the studies reported by Seidu *et al.*, (2015). Seidu *et al.*, (2015) indicated that, lettuce from wastewater irrigated farms

had a six time chance of *E. coli* O157:H7 contamination during the wet season compared to the dry season which is similar to the observation from this current study. However, this study observed low concentrations of *E. coli* O157:H7 and *Salmonella* spp for both seasons meeting the WHO limits of between 3 to 4 logs (WHO, 2006). Although they were present in low concentrations, *Salmonella* spp and *E. coli* O157:H7 are reported as having low infectivity dose of 10-100 cells (Wall *et al.*, 1994) and calls for the need for caution. In this study, the low concentration level of *E. coli* O157:H7 also confirms the study of Donkor *et al.*, (2008) who obtained only 1 (0.4%) *E. coli* O157:H7 isolate from *E. coli* isolates in irrigation water, manured soil and livestock feces in Accra. Often reports indicate that fresh produce is responsible for about 22% of all food borne disease infections in the United States from 1999-2004 (Anon, 2006). Research reported by Sivapalasingam *et al.*, (2004) indicated a strong link between lettuce and *E. coli* O157:H7. Their report further indicated that 29% (5 out of 17) of all lettuce-related outbreaks were linked to *E. coli* O157:H7 and 38% (five out of 13) of all *E. coli* O157:H7 outbreaks with fresh produce were associated with lettuce. Their observation defers slightly from results obtained from this study which could be due to the high temperatures (24.7 °C-37.2 °C) experienced during the study which could inhibit the growth of these organisms to some extent. The concentration of *Salmonella* spp was observed to be a little higher compared to *E. coli* O157:H7 since it was observed that majority of farmers use poultry manure as organic fertilizer on their farms which has been found to contain high concentrations of *Salmonella* sp. (Orji *et al.*, 2005). Food-borne disease outbreaks have mostly been linked to consuming vegetables unrestrictedly irrigated with wastewater (Gould *et al.*, 2013). The safety of food depends on a series of events

beginning at the level of farm production to consumption with each of such events being essential. Estimates of contaminated food illnesses in developing countries presents a major challenge since infections are mostly treated at home and often not documented (GMJ, 2005). *Escherichia coli* O157: H7 has been identified as a cause of the life threatening haemolytic uremic syndrome (Banatvala *et al.*, 2001). Although *E. coli* O157:H7 is primarily associated with animals, contamination of 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 the presence of *E. coli* O157:H7 in wastewater irrigated lettuce. *Salmonella* spp has also been significantly present in the same lettuce samples in which *E. coli* O157:H7 were detected. Other studies have noted that *E. coli* O157:H7 and *Salmonella* spp infect over 1.6 million humans within United States costing the nation some \$15 billion yearly (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 directly via contaminated foods or indirectly via 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 is in line with this current study that has detected microbial pathogens in high levels in the manured soil of irrigation fields. It was also revealed during one on one interaction with the farmers that their source of manure was from the major poultry farms within the Kumasi Metropolis which were eventually used untreated. This study observed that farmers mostly used manure from livestock on their farms but these serve

as pathogen reservoir and consequently serves as a channel for the transmission of pathogens which also agrees with a similar report by Oliveira *et al.*, (2012). Faecal matter of grazing livestock was also frequently observed close to the irrigation areas. These eventually are washed into the nearby irrigation water during a rain fall event increasing contamination by pathogens greatly as also reported by Karesh *et al.*, (2012).

Soil transmitted helminth eggs were detected in all the samples analyzed in the study, and the concentration exceeded the ≤ 1 egg/L recommended by the WHO (2006) for unrestricted irrigation. In both seasons, *Ascaris lumbricoides* and *Ancylostoma duodenale* presented high concentration levels on lettuce, in manured soil and wastewater, with equally high mean concentration levels. This probably explains the 52% prevalent rate of *Ascaris* infection in Ghana as reported by Hotez *et al.*, (2003). Again, there is a generally acceptable notion from research studies that, farmers irrigating with wastewater have higher rates of helminth infections than farmers using freshwater (Trang *et al.*, 2006) which could be right due to their constant interaction with these organisms. Additionally they are prone to developing skin and nail problems as a result of these infections when using wastewater for irrigation (Hoek *et al.*, 2002; Trang *et al.*, 2007). High concentrations of microorganisms in wastewater are largely dependent on the level of pollution within the study community (Amann *et al.*, 1998). The high concentrations obtained from this study for both microbial cells and helminthes eggs indicated that the wastewater used by famers for urban and peri-urban agriculture irrigation in some communities within Kumasi may be polluted to some extent. The results from this study confirm the study of Mensah *et al.*, (2001) which found that significantly polluted

water is used for irrigation in Ghana. Another study in Kumasi by Keraita *et al.*, (2003) has shown that, the microbiological contaminants in irrigation water sources in most cases exceeded the WHO (1989) guidelines significantly. Other reports from previous researchers (Amoah *et al.*, 2005 and Obiri-Danso *et al.*, 2005) indicated that urban rivers and streams in Kumasi which are the main sources of irrigation water for vegetable production are extensively polluted with microbial pathogens. Furthermore, according to Drechsel *et al.*, (2000), the streams in Kumasi metropolis are often contaminated with waste from agricultural lands, abattoirs, brewing and wood processing industries. The presence of helminths eggs in the wastewater was expected as previously reported by Toze (2006). Gastrointestinal infection and weakness which were reported by farm workers during our interaction with them could be related to helminths infection and anaemia due to hookworm infection (Ensink *et al.*, 2005). However, farmers who were engaged in the study during interaction did not admit that their irrigation practices posed threat of acquiring helminths infection which could possibly result in diarrhoea disease cases. This notwithstanding, Pullan *et al.*, (2014) revealed that ascariasis is the most common of the STH's and is endemic in Africa, Latin America, and the Far East and an estimated 133 million people suffer from high-intensity ascariasis infections, which often lead to severe consequences, such as cognitive impairment, severe dysentery and anaemia. The perceptions of farmers have been reported in an earlier study by Rutkowski *et al.*, (2007) in which farmers refused to associate their irrigation practices with infection and diseases. This study detected significant levels of *Ascaris* spp in lettuce sampled for the study. This is in accordance with the report by Oboubie *et al.*, (2006) which indicated high levels *Ascaris* and faecal coliforms in lettuce normally

consumed uncooked compared with other equally eaten raw leafy vegetables irrigated with wastewater in Accra. One other aspect that needs critical attention is the use of manure on the farm; most of the farmers enrolled in this study apply poultry manure on their farms which are sometimes poorly treated or untreated (Drechsel *et al.*, 2000) but may not be aware of its potential health risk. Organic manures contain valuable quantities of nitrogen, phosphate and potash, which act as sources of plant nutrients and are very essential for vegetable growth but may also contain high levels of microbial concentrations and therefore pose health risk to those who directly or indirectly come into contact with them. Such a high level of concentration and continuous exposure of such wastewater and vegetables irrigated with wastewaters to farmers and consumers respectively could lead to a high risk of infection and increases the burden of diseases. An example has been given in a similar study conducted in Malamulele in South Africa (Gumbo *et al.*, 2010) which revealed large numbers of faecal coliform bacteria and helminth eggs among people exposed to wastewater used for irrigation compared with the control group presenting lower levels of faecal coliform bacteria and helminths. This study therefore presented results indicating that microbial loads and helminth eggs assessed surpassed the Tolerable level (TL) of the WHO guidelines which were established to provide protection to irrigation farmers and consumers of crops grown with wastewater (WHO, 1989).

In conclusion, concentration of pathogens show diversity of the presence of microbes and helminth eggs in all forms of the substrates assessed.

Wet seasons recorded high concentration of pathogens compared to the dry seasons which might be due to the prevailing environmental

and climatic conditions pertaining within a particular seasons.

In as much as there were differences in the result for the two seasons, there was a high level of pathogen concentration for all the microorganisms from all the different samples used for the study with the exception of the concentration of *E.coli* 0157:H7 on lettuce during the dry season.

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