INTERNATIONAL WATER MANAGEMENT INSTITUTE (IWMI)

Fecal exposure pathways in Accra: A literature review with specific focus on IWMI's work on wastewater irrigated agriculture

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1. INTRODUCTION

1.1 Background: Fecal exposure pathways in urban settings[1]

Rapid urbanization has led to a growing sanitation crisis in urban and peri-urban areas of low-income countries. Currently, over half of the global population resides in urban areas and this proportion continues to grow. Cities in Asia and Africa are projected to experience the greatest increase in population (Penrose et al., 2010). In contrast to urban development in developed countries, rapid population growth in developing countries has outpaced existing infrastructure, including water and sanitation systems. Urban populations face the greatest risk of exposure to fecal contamination due to inadequate access to safe drinking water and sanitation. Unsafe water and inadequate sanitation account for 88% of diarrheal disease worldwide (WHO, 2004). In rapidly growing informal settlements, where over 1 billion people reside, under-five mortality rates are generally higher and increasing at a higher rate than those in rural areas (Garenne, 2010; WHO, 2010). Studies have found that the prevalence of soil- transmitted helminths (STHs) can be significantly higher among residents of urban and peri-urban communities than in rural areas (Phiri et al., 2000; Rosewell, 2010)

Despite the considerable sanitation needs of urban and peri-urban communities, few data exists to inform strategies to mitigate risks of fecal exposure in developing countries. Urban settings tend to have complex, inter-related web of fecal exposure pathways and measuring or estimating the health impact of a single intervention poses a considerable challenge (CGSW, 2010). The major fecal exposure pathways in the public domain include: contact with surface water, use of wastewater irrigation or fecal sludge fertilizer for food crops and related food contamination, contact with open drainage or sewers, practice of open defecation or use of public latrines, and contact with soil that as a result of flooding of drains and/or open defecation is contaminated (Labite et al., 2010). Major exposure pathways in the private domain include: household sanitation practices, household water quality and storage and treatment practices, personal and food hygiene practices, and insects or pests. The conceptual framework for environmental exposure pathways (Figure 1) depicts these major fecal exposure pathways. Contact with both human and animal excreta poses a risk of enteric infection and disease to humans, but for sanitation related interventions, the focus is on pathways that involve human excreta.

[1] This section has been drawn largely from the project proposal document (CGSW, 2010).

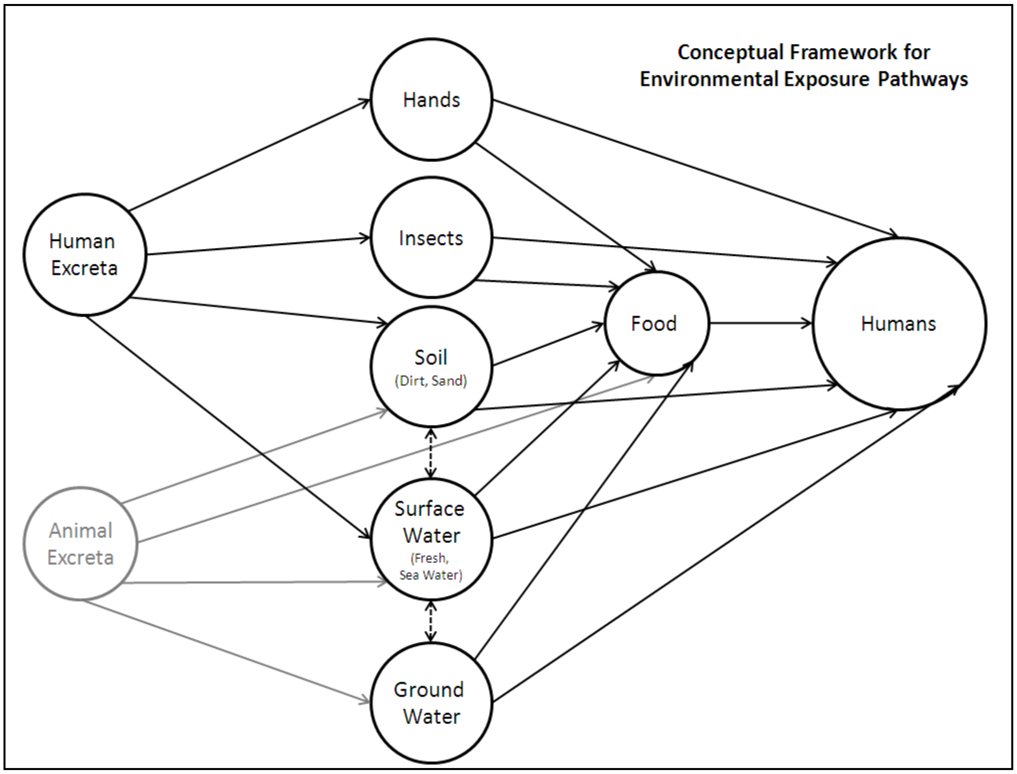

Figure 1. Conceptual framework for environmental fecal exposure
Source: GCSW, 2010

This report presents a literature review on fecal exposure pathways in Accra with a specific focus on wastewater irrigated agriculture and related food contamination, a research focus for the International Water Management Institute (IWMI). An overview of other related studies of relevance are also presented. The report will be a background document to a project entitled, "Assessment of Fecal Exposure Pathways in Low-Income Urban Settings" led by the Centre for Global Safe Water, Emory University and funded by the Bill and Melinda Gates Foundation.

1.2 General description of Accra

Accra is the Capital city of Ghana. The city (Accra Metropolitan Assembly, henceforth referred as to Accra) forms a greater part of the Greater Accra Region, one of the 10 administrative regions in Ghana. Accra covers an area of about 240 km^2 (Obuobie et al., 2006). With an annual population growth rate of about 3.4 % in its current administrative boundary, Accra has an estimated population of 1.66 million (Ghana Statistical Service, 2002). It is interesting to note that about 60 percent of the city's population lives in slums (informal settlements) in its center while the middle and upper class moves to its periphery. Obuobie at al., 2006 further reported that geographically, Accra lies within the coastal-savanna zone with low annual rainfall averaging 810 mm distributed over less than 80 days. The rainfall pattern of the city is bimodal with the major season falling between March and June, and a minor rainy season around October. Rain usually falls in intensive short storms and gives rise to local flooding where drainage channels are obstructed. Mean temperatures vary from 24 ºC in August (the coolest) to 28 ºC in March (the hottest). Natural drainage systems in Accra include streams, ponds and lagoons (e.g., Songo, Korle and Kpeshie). Floodwater drains and gutters are used for grey water, and often drain into the natural system, polluting heavily the lagoons and Accra's beaches. The Accra Metropolitan Area is

made up of six sub metros namely Okaikoi, Ashiedu Keteke, Ayawaso, Kpeshie, Osu Klotey and Ablekuma (Figure 2). Figure 3 shows the slum index which is indicative of the poverty levels in Accra.

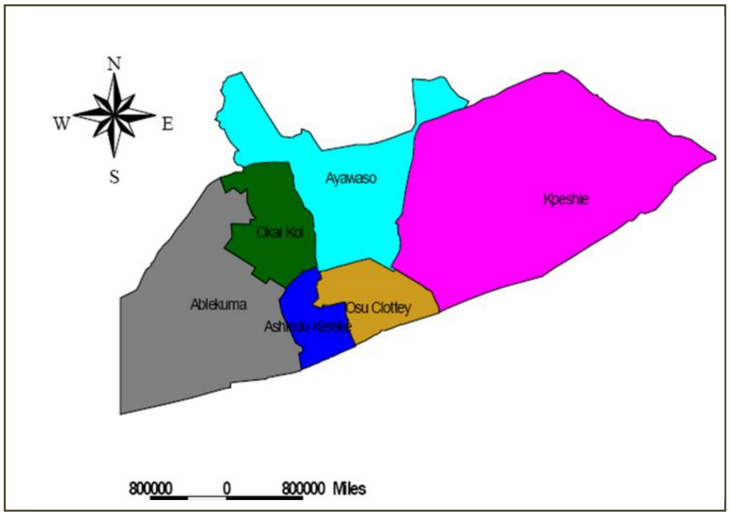

Figure 2. Administrative divisions in Accra
Source: Accra Metropolitan Health Service

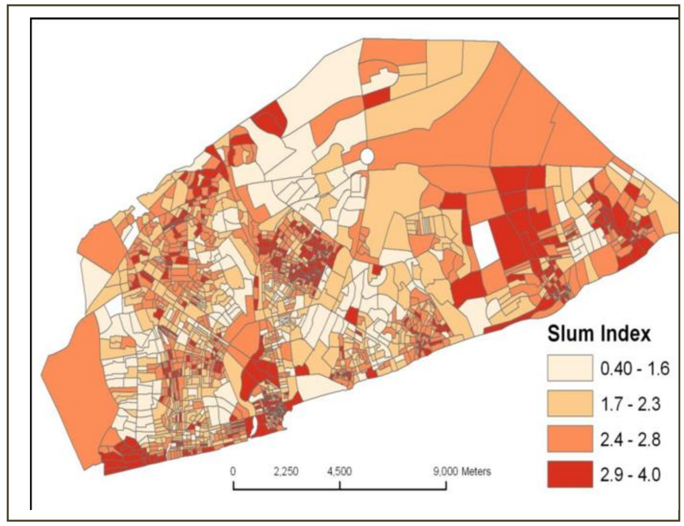

Figure 3. Composite slum index map for Accra
Source: Jankowska (undated)

1.3 State of sanitation in Accra

About 15% of the Accra metropolitan, mainly the central area, is served by a piped waterborne sewerage network and the remaining areas are served by on-site sanitation facilities, in the form of septic tank and pit latrines (AfDB, 2005). The sewerage network has seen very little extension since its construction, in the early 1970. In the newly developing areas, on-site facilities are provided. Due to the limited number of treatment sites, septage from the on-site facilities is either disposed of in receiving water bodies or in nearby drains and open spaces. Increasingly, particularly in flood prone low-lying areas, septic tank effluent is directly discharged into drains. There are also several individual sewerage systems constructed and operated by different organizations, but there capacity is low. Unfortunately, presently, none of these sewerage systems is operational. The effect of neglect and poor maintenance of sanitation and sewerage systems in Accra led to an increasing use of individual on-site sanitation facilities, resulting in an increase in pollution of surface water bodies in the city.

Figure 4 shows sanitation facilities used by households based on the official statistics from the last population and housing census conducted in 2000 (official results from the 2010 census are not yet) (Ghana Statistical Services, 2002). The current proportion of households using pan/bucket latrines could be much lower as the practice was banned in 2000 by the local authorities. The category "WC flush toilet" may include toilets discharging to sewers, to septic tanks, or more or less directly to open drains. A survey of 960 Accra households found that about 37.5% do not have a toilet in their home, of which about 35% relied on public toilets and about 2.5% practiced open defecation (Boadi, 2004). When income was taken into account, about 45% of poor households used public latrines, versus only about 2% of middle-income and wealthy households. The proportion practicing open defection could be higher if numbers of those who are forced to defecate openly due to the long queues in public toilets especially

during the early morning peak period. Stoll (2008) estimated about there are about 150 toilet blocks in Accra, each block containing 20 seats in average.

Like septic tanks, public toilets are emptied by trucks to non-operational treatment plants, and more often of late to Lavender Hill, where it is directly discharged to the sea. Official records in Accra show that in the year 2000, the three then operating fecal sludge treatment plants received about 90,000 m^3 of fecal sludge while 70,000 m^3, could not be absorbed so effectively discharged to open drains, waterways and bushes (Murray and Drechsel., 2011). In 2008, Stoll (2008) estimated that about 1000 m^3 of fecal sludge was generated daily in the city and none was treated. About 80% of the fecal sludge was dumped on the beach and about 20% are discharged at the broken treatment plants that cannot take anymore. As a general practice in Ghana, greywater (water from bathrooms, kitchens, showers etc) is discharged to open stormwater drains. In general, the sanitation situation in Accra could be termed as poor, bearing in mind that the larger proportion of excreta disposal facilities that are used don't meet the JMP criteria for improved sanitation.

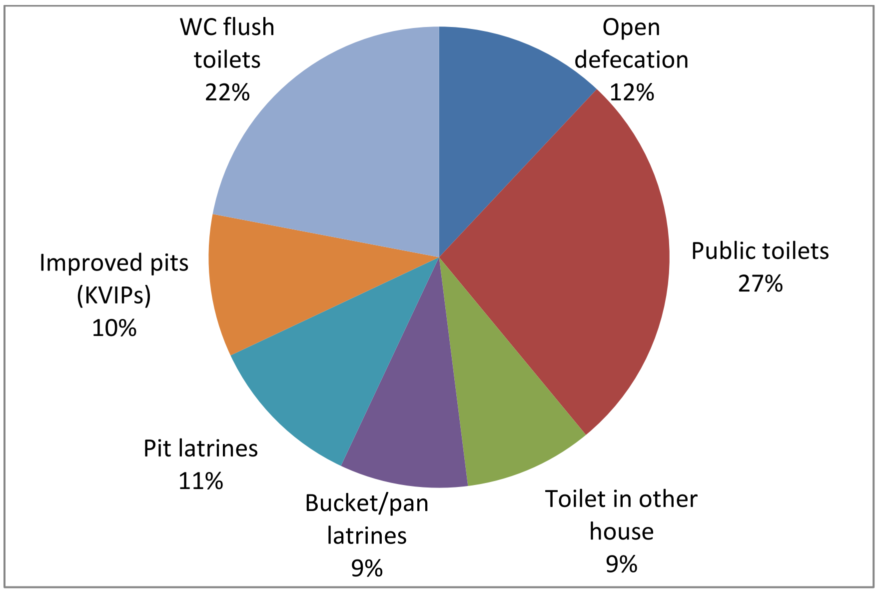

Figure 4. Sanitation facilities used by households in the Greater Accra Region

Adapted from: Ghana Statistical Services, 2002.

However, some improvements could be foreseen in the near future. In April 2006, the Accra Sewerage Improvement Project (ASIP), which is supported by African Development Bank (AfDB) was approved. The project details available in AfDB, (2005) show that two waste stabilization pond based sewage treatment plants will be constructed at the Densu Delta (5,934 m^3/day) and Legon (6,424 m3/day) accompanied by extension of network coverage. The existing household connections will be rehabilitated while 4184 new houses will be connected. The project will also build more 147 new public toilets and 37 septage/night soil reception holding tanks. This will be accompanied with environmental protection works and mitigation of measures. Sensitization campaigns will be done to raise awareness on linkage between improved health and sanitation, as well as to improve environmental sanitation situation in the project areas through promotion of house connections, individual latrines, personal hygiene, and proper maintenance of public toilets. These improvements are expected to change the face of sanitation in Accra.

2. DIARRHEAL DISEASES IN ACCRA

2.1 Significance and aetiology

Diarrhea is the second most common illness among visitors to primary health care centers in Ghana (Osumanu, 2008). Table 1 shows the fecal-oral diseases, aetiology and incidence reported by the Accra Metropolitan Assembly health services (Lunani 2007). The incidence levels in the Table have been adjusted for underreporting (82% underreporting rate). At hospital level, analysis is not done on all aetiological agents so the diarrheal diseases of unknown aetiological agent, are those which are not analyzed at hospital level. In general, these diarrheal diseases of unknown aetiology contributed greatly to the waterborne disease incidence in AMA with an average annual incidence of 1157 cases per 10,000 population. Spatial distribution across Accra's five sub-metros show that the two poorest sub-metros had the highest cases i.e. Ashiedu Keteke with 5302 cases per 10,000 followed by Osu Klottey with 3345 cases per 10,000.

Table 1. Aetiological agents and incidence of fecal-oral diseases in Accra

Disease	Aetiological agent	Incidence (No. of cases per 10,000)
Typhoid	*Salmonella typhi*	82
Cholera	*Vibrio cholerae*	26
Diarrheal diseases	Unknown	1157
Infectious hepatitis	Hepatitis A virus	8
Intestinal worms	Helminths	181

Adapted from: Lulani, 2007.

2.2 Relevant documented studies

Under projects and research institutions, a lot more focus has been given on aetiology of diarrheal diseases. An overview of selected studies done in Accra is presented in Appendix 1 Table A1. These include rotaviruses (Armah et al., 2010; Armah et al., 1994; Dongdem et al., 2010), Cryposporidium (Adjei et al., 2004) and those focusing on more than one agent (Opintan 2010a, b). The Table also shows two publications based on PhD studies (Boadi and Kuitunen, 2005) and Fobil et al., 2011), which were deemed relevant for this review. In general, most of these studies have been conducted by the Noguchi Memorial Institute for Medical Research and the University of Ghana Medical School. These two institutions are increasingly equipping their laboratories with advanced equipment, so better understanding of diarrheal diseases in Accra is expected.

3. WASTEWATER IRRIGATED AGRICULTURE AS A FECAL EXPOSURE PATHWAY

3.1 The human health risks from wastewater irrigation

Wastewater is one of the many inputs that can case fecal contamination to vegetables. At farm level, studies have also pointed out to animal manures as another source of contamination. The review is however on sanitation-related sources so manures have not been covered in this review, hence the focus on wastewater. Extensive studies on human health risks posed by wastewater irrigation especially from pathogen contamination have been done. Most of these studies form the basis of the Guidelines for safe Use of Wastewater in Agriculture, published by the WHO (WHO 2006). Table 2 is a simplified presentation of wastewater related human health risks and the affected groups.

Table 2: Simplified presentation of the main human health risks from wastewater irrigation

Kind of Risk	Health Risk	Who is at Risk	How
Occupational risks (contact)	• Mostly parasitic worms such as Ascaris and hookworm infections • Diarrheal diseases especially in children • Skin infections - itching and blister on the hands and feet • Nail problems such as koilonychias (spoon-formed nails)	• Farmers/ field workers	• They get into contact with irrigation water and contaminated soils • Get into contact with irrigation water and contaminated soils
		• Children playing on the farm	• Contact with irrigation water and contaminated soils
		• Market women	• While harvesting they are exposed to contaminated soils • Some wash vegetables, especially in wastewater
Consumption-related risks	• Mainly bacterial and viral infections such as cholera, typhoid, ETEC, Hepatitis A, viral enteritis which mainly cause diarrheas • And also parasitic worms such as Ascaris	• Vegetable consumers	• Eating of contaminated vegetables, especially those eaten raw
		• Children playing on the farm	• Licking soil

A general conceptual model on health risks from wastewater irrigation is shown in Figure 5. For consumption-related risks, the main concern is vegetables eaten raw, like salads.

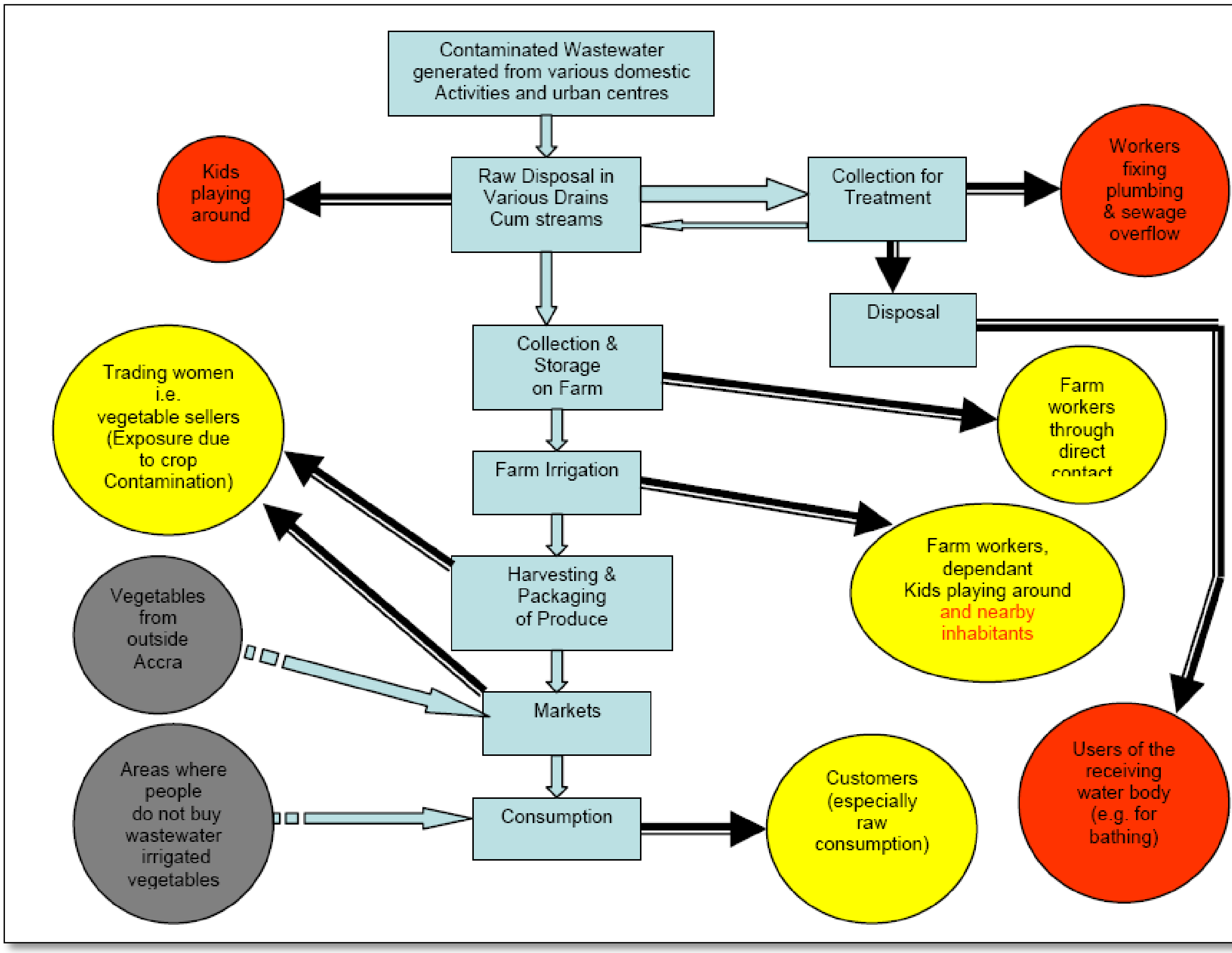

Figure 5: Potential exposure points in chain of events leading to consumption

Source: Suleiman, 2007.

3.2 Consumption-related health risks: Overview of IWMI's "farm-to-fork" research

3.2.1 Research framework and objectives

In the last decade, IWMI, in collaboration with a number of local and international research and academic institutes has done extensive research in Ghana, mainly focused on assessment and management of consumption-related risks. IWMI has largely adapted the multi-barrier approach in the research (see Figure 6). This section gives an overview of studies carried out in Accra, although findings from other cities are reported alongside. The studies were mainly conducted in two phases. The first phase was a preliminary study was undertaken to obtain background information on urban vegetable production in Accra, and two other cities, Kumasi and Tamale. This included the assessment of the microbiological contamination levels of water, soil and crops at farm level, which also extended to postharvest quality of vegetables at market level. The second phase was carried out in Accra and Kumasi to assess the contamination pathways of pathogens (bacteria and helminths) on wastewater

irrigated crops to determine where interventions should be placed and to suggest appropriate health risk reduction strategies at food preparation points.

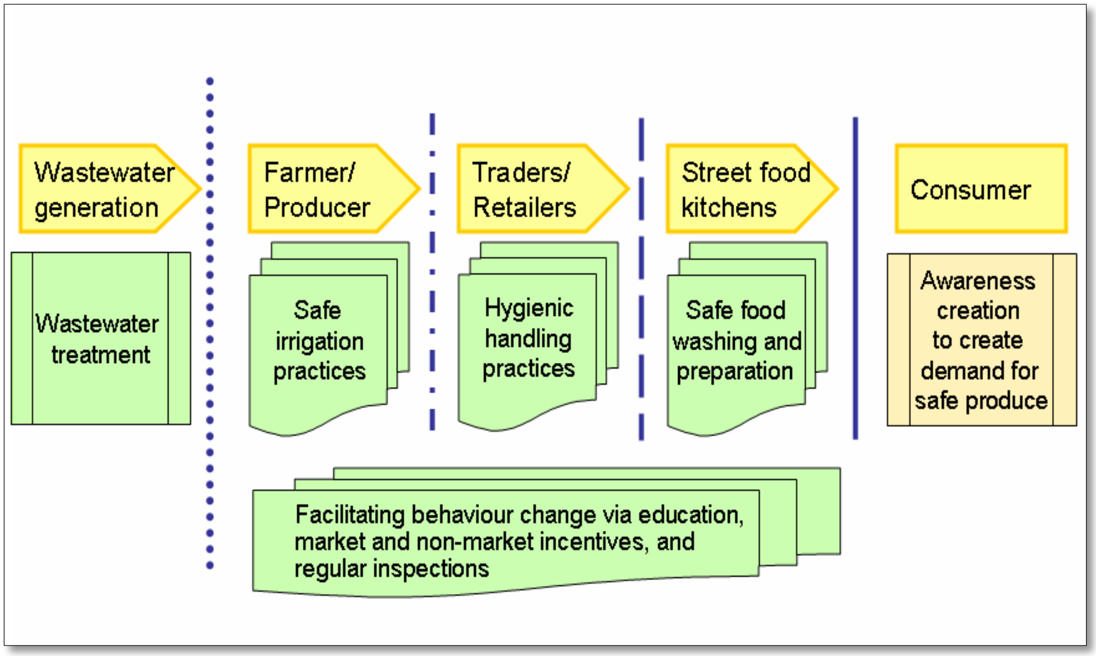

Figure 6. The multi-barrier approach as used in IWMI's studies in Accra.

3.2.2 Hygienic quality of irrigated vegetables produced and sold in three major cities of Ghana

3.2.2.1 Methodology

Samples of wastewater irrigated lettuce, cabbage, and spring onions were collected from selected markets in Accra, Kumasi, and Tamale to determine the current level of exposure of the Ghanaian urban population to hazardous pesticide and fecal bacteria contamination through the consumption of fresh vegetables produced with wastewater. For leafy vegetables (lettuce, cabbage, and spring onions), the contamination is 'distributed' in all its leaves and therefore randomly sub-sampling of whole leaves is a suitable technique (Hayes, 1985). This technique was used for the sampling of field and market vegetables. This study was conducted over 3 months from October to December 2002. A total of one hundred and eighty (180) vegetable (*lettuce, cabbage and spring onion)* samples were collected from nine major markets and twelve specialized individual[2] vegetable and fruit sellers in Accra, Kumasi and Tamale. At each market, samples were collected under normal purchase conditions, from three randomly selected sellers. A minimum of 3 composite samples- *each containing 2 whole lettuces*), 3 bunches of spring onions (*each containing 2 bulbs*) and 3 cabbages, were collected from the upper, middle, and lower shelves of each seller, put in sterile polythene bags and then transported on ice to the laboratory where they were analyzed immediately or stored at 4^{o} C and analyzed within 24 h. Details of laboratory analytical procedures used in IWMI studies are in Appendix 2.

[2] These are sellers with permanent stalls outside designated markets.

3.2.2.2 Results

Most of the vegetable samples showed high fecal coliform contamination levels. The highest level of fecal coliform contamination was recorded in lettuce at a geometric mean count of 1.1×10^7 g^{-1} wet weight. This may be due to the larger surface area exposed to possible sources of contamination. Cabbage and spring onion showed geometric mean counts of 3.3×10^6 and 1.1×10^6 g^{-1} wet weight, respectively**.** All vegetables from the three cities including Accra were fecally contaminated with mean fecal coliform levels exceeding the International Commission on Microbiological Specifications for Foods (ICSMF, 1974) recommended level of 10^3 fecal coliform per gram fresh weight. Helminth egg populations were 1.1 g^{-1} in lettuce, 0.4 g^{-1} in cabbage, and 2.7 g^{-1} (wet weight) in spring onion. No significant difference was observed in the mean helminth egg populations recorded in lettuce and cabbage (P = 0.061; CI = -0.0339 – 1.4862), however, the difference between spring onion and both lettuce and cabbage was significant (P = 0.001; CI = 0.8137 – 2.3831 for lettuce, and 1.5513 – 3.0978 for cabbage). The identification of helminth eggs revealed the presence of *Ascaris lumbricoides*, *Ancylostoma duodenale*, *Schistosoma heamatobium* and *Trichuris trichiura* with *A. lumbricoides* eggs most predominant.

3.2.2.3 Discussion

Several factors may account for the high levels of total and fecal coliform contamination recorded in most of the analyzed vegetables. Among these is the use of polluted irrigation water and fresh poultry manure. Both the irrigation water and the poultry manure are applied directly on the crops. Another contamination source is market-related handling especially where provision for better sanitary standards (e.g. clean water for crop washing and 'refreshing') is lacking. Previous studies in Accra show fecal coliform population of irrigation water sources ranging between 4.8×10^3 and 2.8×10^6 100 ml^{-1} (Cornish *et al*., 1999; Mensah *et al*., 2001; Keraita *et al.,* 2002) which exceed the WHO recommended level of 1×10^3 100 ml^{-1} for unrestricted irrigation. Drechsel *et al*. (2000) reported that fresh poultry litter samples sometimes used without sufficient drying for vegetable production in Kumasi had equally high fecal coliform counts ranging from 3.6×10^4 to 1.1×10^7.

The relatively high total and fecal coliform population recorded on some vegetables was also reported by Johnson (2002) and Armar-Klemesu et al*.* (1998) who analyzed street food and market crops in Accra. These findings *may* explain a number of food-borne and water-related diseases in Accra, such as diarrhoea (sometimes related to typhoid or cholera) as well as intestinal worm infections, but have to be seen also in the context of generally sub-optimal sanitary conditions in parts of the metropolis (Arde-Acquah, 2002). Biologically, the highest health risk is for helminth infections compared with other pathogens since helminthes persist for longer periods in the environment, host immunity is usually low to non-existent and the infective dose is small (Gaspard et al., 1997). *A. lumbricoides* was the most predominant, and observed in 85% of the contaminated vegetables. This could be attributed to its high level of persistence in the environment.

3.2.2.4 Conclusion

The results of this study showed that typical microbiological contamination levels of vegetables in Ghanaian markets pose a threat to human health. Washing or cooking of food before eating is common in Ghanaian households. The comparison of both risk factors shows that efforts for health interventions should focus more on short-term impact of microbiological crop decontamination particularly helminthes. The pesticide problem, despite its dimension, is however less critical for consumers' health.

3.2.3 Microbiological quality of irrigation water from different sources used for vegetable production

3.2.3.1 Methodology

The study was conducted in three Ghanaian cities including Accra. Grab samples were collected at major vegetable growing sites in each city. A total of 23 (including 9 from Accra) samples were collected from the major sites for analysis. Following this a longer study which was to monitor the quality of irrigation water over a longer period of time was conducted in Accra and Kumasi. In each city, two major irrigated vegetable production sites were selected for sampling. The selection criteria were the source of irrigation water and the type of vegetables grown with emphasis on exotic vegetables likely to be consumed uncooked (e.g., lettuce). The sites in Accra use water from drains and streams while those in Kumasi use water from shallow wells and streams. Of course the drains in Accra receive all sorts of waste including untreated wastewater from the neighboring community.

A total of 312 composite water samples were analyzed for total and fecal coliform populations. One composite sample per week was collected from each irrigation water source for 52 weeks from May 2003 to April 2004. In all, six irrigation water sources were sampled - stream, shallow well, and piped irrigation water sources in Kumasi and drain, stream, and piped water in Accra. Sampling for helminth egg quantification (see detailed description in box 1 below) in irrigation water was done twice every month for five months beginning November 2003 to March 2004 at all the selected sites.

3.2.3.2 Results

Feacal coliform contamination levels of irrigation water from different urban sources: The fecal coliform levels observed in all the three cities were between 9.0 x 10^3 and 4.6 x 10^9 100 ml^{-1}). In Accra, fecal coliform levels ranged from 5.0 x 10^4 to 2.3 x 10^6 100 ml^{-1}. The lower values were recorded in areas where farmers used piped water stored in shallow wells while farming sites where farmers used water from urban drains for irrigation recorded higher values. All samples from the three cities exceeded (WHO, 1989) recommended level of 1000 fecal coliform per 100 ml.

Microbiological quality of irrigation water monitored at selected urban sources in Accra and Kumasi: Irrigation water sources in Kumasi and Accra showed considerable variation in total and fecal coliform concentrations. The maximum fecal coliform contamination densities for shallow well and stream samples in Kumasi were 4 x 10^6 and 4 x 10^8 100 ml^{-1}, respectively, while drain and stream samples in Accra showed densities of 9 x 10^6 and 2 x 10^7 100 ml^{-1}, respectively. There was no clearly defined pattern between fecal coliform levels in drain and stream water sources in Accra where the WHO recommended coliform levels were exceeded in the majority of cases

Helminth egg population in irrigation water : A number of different types of helminth eggs were isolated from all irrigation water sources except piped water. These included eggs of *Ascaris lumbricoides, Hymenolepis diminuta*, *Trichuris trichura*, *Facsiola hepatica,* and *Strogyloides* larvae. From all the irrigation water sources in both Accra and Kumasi, *A. lumbricoides* was the most predominant species recorded; population density ranged between 2 to 4 eggs l^{-1} exceeding the recommended level of <1 egg l^{-1} for unrestricted irrigation (WHO 2006).

3.2.3.3 Discussion:

The results confirm earlier reports that low quality water is being used for urban vegetable production in most Ghanaian cities (Cornish *et al.,* 1999; Mensah *et al*., 2001). Other studies carried out in Accra (Armar-Klemesu *et al*., 1998; Zakariah *et al*., 1998; Sonou, 2001) also showed that there are hardly any unpolluted water sources available for irrigation. Vegetable growing sites where farmers used water from urban drains for irrigation recorded the higher values compared to areas where some farmers used piped water stored in shallow wells. The significant differences recorded in fecal coliform levels between the two sources in Kumasi (well and stream) may suggest that shallow well water may pose relatively less risk to farmers and consumers, although the coliform levels exceeded 1000 counts per 100 ml. Similar results have been reported from Kenya (Hide *et al*., 2001) but Cornish *et al*. (1999) recorded in Kumasi temporarily higher fecal coliform population in shallow wells than in nearby streams. This may be due to the fact that probably the wells used in their study were shallower and got more easily contaminated through surface runoff on the field (Drechsel *et al*., 2000).

Shallow wells or "dugouts" might be expected to meet the WHO recommended standard due to the natural filtering of aquifer materials and long underground retention times (Cornish *et al*., 1999) but those used in this study were often not protected against surface inflow and could have easily received pollutants from the surrounding farm environment through runoff. In spite of that, shallow wells in Kumasi had, in general, better quality water than the streams. These were associated with water entering the wells and the extensive use of (fresh) poultry manure in vegetable farming (Cornish *et al*., 1999). There is therefore the need to improve on well systems to avoid run-off entering the wells.
As expected the quality of piped water from Accra and Kumasi had no fecal coliforms during the study period and would pose no health risks to farmers. However, this is rarely an official or reliable option for farming due to its price and/or common supply shortages (Tandia, 2002; Moustier and Fall, 2004). For example, only 40% of residents of Accra have access to clean piped water. On the other hand the availability of marginal quality water affords farmers year-round production with a strong competitive advantage in the dry season. In Dakar, the use of polluted water allows 8-12 harvests of lettuce per year compared with 5-6 harvests by farmers who had no access to wastewater (Gaye and Niang, 2002).

3.2.3.4 Conclusion

The results confirmed that polluted water is mostly used for urban vegetable production in the study sites. This poses high health risks to farmers especially when farmers fetch water without protection from possible contamination. The application of irrigation water (with watering cans) on the leaves of vegetables could also increase pathogen contamination and pose health risk to consumers.

3.2.4 Contamination pathways of pathogens (bacteria and helminths) on wastewater irrigated crops

3.4.1 Methodology

This component was carried out to determine where interventions should be placed along the farm to fork pathway and to suggest appropriate health risk reduction strategies at food preparation points. Over a period of 12 months, starting from May 2003 to April 2004, a total of 1296 lettuce samples were collected at different entry points from farm to the market. There were three main stages from harvesting on the farm to the main retail outlet where consumers were ready to buy: (1) farm, where samples were collected just before harvesting, (2) wholesale market, where sellers converge before finally selling to (3) retailer, where samples were taken 2–3 h after vegetables have been displayed and, in part, refreshed. The original set of lettuce was either irrigated with stream, drain, well or piped

water. The coliform and helminth egg levels of these irrigation water sources were monitored regularly throughout the sampling period.

Twice every month, a minimum of three composite samples (each containing two whole lettuces) from each of the selected farm sites were randomly collected with sterile disposable gloves just before harvesting for sale at the market. These were put into separate sterile polythene bags and labelled as farm samples. The buyer was followed to the wholesale market where another set of samples from the same original stock was collected before being finally sold to a retailer. At the final retail point, three composite samples were again sampled after vegetables have been displayed on the shelves for at least two to three hours which is an average turn-over period at the retail point. Field trials were conducted to identify the different possible sources (wastewater, poultry manure, and soil) of contamination. The experiments were conducted at two vegetable growing sites in Accra in the major rainy season (May to July, 2004) (See details in Amoah et al., 2005).

3.2.4.2 Results

Microbiological quality of water used by farmers during the study period

Apart from piped water, all other sources of irrigation water sampled during the study period showed fecal coliform levels significantly ($p < 0.05$) exceeding a geometric mean count of 1×10^3 100 ml^{-1} recommended by the World Health Organization (WHO, 1989) for unrestricted irrigation.

Microbiological quality of lettuce at different entry points along the production consumption pathway

Table 3 shows the fecal coliform contamination levels of lettuce at different entry points starting from farm to the final retail outlet. Irrespective of the irrigation water source, mean fecal coliform levels exceeded the recommended standard (10^3). For all treatments in both cities, there were no significant differences in the average lettuce contamination levels at different entry points (farm, wholesale market, and retail outlet). Apart from stream water irrigated lettuce from Accra, higher fecal coliform levels were recorded on lettuce from all the other irrigation water sources in the rainy season than in the dry season.

Helminth eggs

Helminth eggs including that of *Ascaris lumbricoides*, *Hymenolepis diminuta*, *Trichuris trichiura*, *Fasciola hepatica* and *Strongyloides larvae* were detected on lettuce samples at the different entry points. The helminth egg population ranged from 1 to 6 egg(s) 100 g^{-1} wet weight and between 50 to 75% of the eggs was viable. In the majority of cases, significantly ($p<0.05$) higher levels were detected in lettuce irrigated with polluted water than those from piped water irrigated sources. However, mean helminth egg populations on lettuce from the same original stock and irrigation water source did not show any significant difference from field to market.

Table 3. Mean fecal coliform contamination levels at different entry points along the production - consumption pathway of lettuce

City	Irrigation water source	Statistic	Log fecal coliform levels (MPN[*]$100g^{-1}$)		
			Farmgate	Wholesale market	Retail
Kumasi	Well	Range (N=216)	3.00 – 8.30	3.10 - 8.50	3.20 - 7.00
		Geometric mean	4.54 (± 1.32)[**]	4.44 (± 1.23)	4.30 (± 1.04)
		Standard error	0.27	0.25	0.18
	Stream	Range (N=216)	3.40 – 7.10	3.60 – 7.20	3.50 – 7.20
		Geometric mean	4.46 (± 0.81)	4.61 (± 0.84)	4.46 (± 0.91)
		Standard error	0.17	0.17	0.19
	Piped water	Range (N=216)	2.30 – 4.80	2.60 – 5.30	2.40 – 5.10
		Geometric mean	3.50 (± 0.70)	3.69 (± 0.84)	3.65 (± 0.82)
		Standard error	0.14	0.17	0.17
Accra	Drain	Range (N=216)	3.40 – 6.00	3.00 - 6.80	3.00 - 6.50
		Geometric mean	4.25 (± 0.74)	4.24 (± 0.86)	4.48 (± 0.78)
		Standard error	0.15	0.18	0.16
	Stream	Range (N=216)	3.20 - 5.70	3.10 - 5.90	3.20 - 5.50
		Geometric mean	4.22 (± 0.66)	4.29 (± 0.62)	4.37 (± 0.59)
		Standard error	0.13	0.13	0.12
	Piped water	Range (N=216)	2.90 - 4.70	2.90 - 4.80	2.80 - 4.50
		Geometric mean	3.44 (± 0.40)	3.46 (± 0.43)	3.32 (± 0.37)
		Standard error	0.08	0.09	0.08

[*]MPN, Most Probable Number
[**]Figures in parentheses are standard deviations

Faecal coliform and helminth egg contamination levels in irrigation water and soil

Mean fecal coliform levels in irrigation water from drains used during the study period were 1.1 x 10^6 for the first, 2.3 x 10^6 for the second, and 1.0 x 10^7 for the third trials. Considerably high fecal coliform contamination levels ranging between 3.9 x 10^3 and 4.1 x 10^5 100 g^{-1} were recorded in the soil samples taken from the lettuce fields. Samples from control plots (*nearby plots with no farming activity*) showed significantly lower fecal coliform levels (ranging between 0 and 2.1 x 10^2 100 g^{-1}).

Faecal coliform and helminth contamination levels on lettuce at harvest

Most (85%) wastewater-irrigated vegetables in all the three trials recorded a comparatively higher fecal coliform contamination levels than the piped water irrigated ones. The difference was however significant only in 33% of the cases. In the wastewater-irrigated plots, there was no relationship between the fecal coliform levels on lettuces and the fecal coliform concentration in poultry manure applied plots. This may be due to the dominant effect of contaminants from the irrigation water. Under piped water irrigation, the effect of the different poultry manure (PM) contamination levels, among each other and in comparison with inorganic fertilizer (F) became more apparent. Generally, higher fecal coliform levels were recorded on plots receiving poultry manure and piped water as compared to those receiving just piped water. However, the differences were only significant in 22% of the cases. Helminth egg contamination in lettuce was significantly higher in 30% of the wastewater irrigated plots than the piped water irrigated ones. Significantly higher levels of helminth egg contamination were observed in only 8% of the piped water irrigated plots than those irrigated with wastewater.

3.2.4.3 Discussion

Household consumption of raw salad is less common in Ghana than its Francophone neighboring countries (Klutse, 2006). However, with increasing fast-food consumption especially in Ghanaian urban centers, "exotic" vegetables, like lettuce, are today a common ingredient of outdoor urban diets. Most lettuce is produced on urban farms along polluted streams and drains. With the exception of piped water, fecal coliform contamination levels in the irrigation water used generally exceeded WHO recommended standard for unrestricted irrigation. This study revealed that the contamination of lettuce with pathogenic microorganisms does not significantly increase through post-harvest handling and marketing. This was not expected in view of the alarming low hygienic conditions, including washing habits, poor display and handling of food as well as limited availability of sanitation infrastructure on market sites. For example, Nyanteng (1998) reported that only 31% of the markets in Accra have a drainage system, only 26% have toilet facilities, and only 34% are connected to pipe-borne water.

Considering lettuce irrigated with water from polluted sources, it seems the initial contamination on the farm was so high to mask the effect of the applied concentrations. However, lettuce irrigated with piped water showed lower on farm contamination which indicates that there is no post-harvest contamination hidden behind huge farm-gate levels. The results on microbiological contamination of lettuce (from field to market) are contrary to results of Armar-Klemesu *et al*. (1998), who attributed the significantly higher fecal coliform levels, found on market than the farm vegetables (*including lettuce*) to handling. Their study did not however establish the produce from the markets had come from the specific farm sources examined in their study.

The study also revealed that even at the farm level, wastewater is only one of several sources of crop contamination, although it can be the major one. Besides irrigation water, other identified contamination sources in the farm are immature manure as well as the previously contaminated soil. Both sources of contamination might be difficult to control (e.g. mulch to reduce splash), which stresses the need for postharvest measures, such as efficient washing practices in markets and at the household level should reduce the contamination considerably. Beuchat (1998) reported that vegetables can become contaminated with microorganisms capable of causing human diseases while still on farm, or during harvesting, transport, processing, distribution and marketing, or in the home. The results of this study however suggest that postharvest contamination is not a major contamination source as compared to contamination on the farm.

To reduce health risk associated with the consumption of contaminated lettuce, it is important therefore to tackle the problem first at the farm level through good agricultural practices, including changes in irrigation methods. However, common guidelines for wastewater use in agriculture are rarely adopted for a variety of reasons. For example, economic constraints limit the level of wastewater treatment that can be provided in developing countries. Also small size and insecure land tenure are significantly constraining farmer's ability to invest in farm infrastructure such as drip irrigation or on-farm sedimentation ponds (Drechsel *et al.,* 2002). Although earlier trials by Keraita *et al*. (2007) show that the contamination levels can be reduced on the farm through minor changes in practices, it is unlikely that contamination can be minimized below the threshold of safe consumption as the data from the use of piped water show.

It will therefore be necessary to wash the crops in addition to on farm techniques designed to reduce health risks. The last stage in the production-consumption chain, where food for home consumption or fast food for street sales is prepared, appears to be a good entry point. Awareness for food safety is generally high in Ghana as more than 90% of the food vendors and consumers wash their salad before serving. However, individual methods vary largely and there is no information on effectiveness of these variations of the methods.

3.2.4.4 Conclusion

The study showed that the much of the microbial contamination of lettuce produced from urban sources in Accra and Kumasi occurs on the farm. The postharvest sector is likely a relatively minor contributor to lettuce contamination. The results confirm that even at the farm level, wastewater is only one of several sources of crop contamination, although it can be the major one. Besides irrigation water, other contamination sources identified in the farm are immature manure as well as the already contaminated soil. From the results, it may be concluded that a focus only on wastewater treatment is insufficient to safeguard consumers' health. This is more so the case where wastewater treatment is inadequate. The reduction of potential health risks resulting from fecal coliform and helminth contamination of urban and peri-urban vegetables thus requires a more holistic approach taking care of various contamination sources.

3.2.5 Phenotypic characterization of the fecal coliform isolates on lettuce irrigated with water from different sources

3.2.5.1 Methodology

This was an attempt made to characterized fecal coliform isolates on lettuce produced with water from different urban sources. Bergey's manual of systematic bacteriology stresses the importance of obtaining a pure isolate before attempting to characterize a species. Techniques described in this manual for the isolation of pure cultures from a mixture of species were strictly followed. Bacteria cultures from farm samples collected during the contamination pathway study were used in this study. The colony characteristics such as shape, colour, size, lactose fermentation were used for the clustering of isolates. From the identified clusters in dendrograms, samples were randomly selected for biochemical analysis using API 20E system (BioMerieux sa 69280 Marcy-l'Etiole/France or bioMerieux, Inc., Hazelwood, MO). Identified *E. coli* isolates were streaked on Chromocult colioform agar (MERCK KGaA, Darmstadt, Germany) and then on Sorbotol MacConkey (SMAC) agar (OXOID, Basingstoke, Hampshire, England) for *E. coli* 0157:H7 identification. SMAC is a selective agar for the direct isolation and differentiation of *E. coli* 0157:H7.
(http://service.merck.de/microbiology/tedisdata/prods/4968-1_00850_0100_0500.html)

3.2.5.2 Results

The identified isolates in both Accra and Kumasi belonged to 9 genera (*Cedecea, Enterobacter, Erwina, Escherichia, Klebsiela, Kluyvera, Aeromonas, chryseomonas and serratia*) . Most of the fecal bacterial isolates from both Accra (50%) and Kumasi (47%) belonged to the genera (Enterobacter and Escherischia). Irrespective of the irrigation water used most of the fecal coliform identified belonged to at least seven of the 9 genera observed.

3.2.5.3 Discussion

The occurrence of foodborne outbreaks associated with fresh produce in recent times has necessitated the examination of food safety measures during growth and processing of these products. The study confirmed earlier studies that irrespective of the quality of irrigation water used, lettuce produced in both Accra and Kumasi were contaminated with fecal coliforms. The isolated bacteria were normal enteric bacteria of both humans and other animals. This implies that both animals and/or humans could be sources of fecal bacteria contamination of vegetables produced in the study areas.

The indication that animals are potential source of fecal coliform contamination is not surprising since the majority of farmers in the study area used poultry manure and cow dung (Drechsel *et al.,* 2000; Mensah *et al.,* 2001; Obuobie *et al*., 2006) as manure. Some studies in Ghana have isolated *Salmonella*, *Campylobacter* and *E. coli* from the cloacal contents of live birds (Sackey *et al*., 2001). Contamination of human faeces is also possible on the farms because of the absence of proper toilet facilities on these farms and the indiscriminate disposal of human excreta from the surrounding communities into drains and streams which serve as sources of irrigation water in most of these farms. Even though tap water used for the irrigation of lettuce during the study period was better than the other sources in terms of coliform populations, it did not affect the specific types of pathogens isolated compared to those irrigated with water from polluted sources.

The absence of *Salmonella* and *Shigella* in all the identified isolates was surprising considering the high levels of fecal coliform population estimates on lettuce, in poultry manure used and the irrigation water sources. However, the selectivity of the most probable number technique (Stiles and Lai-King, 1981) used in the primary isolation process could account for the exclusion of *Salmonella*. Several studies have however isolated *Salmonella* and *Shigella spp* from lettuce and other vegetables (Castro de Esparza and Vargas, 1990, in Peasey *et al.,* 2000; Vaz da Costa Vargas *et al*., 1996; Mensah *et al.,* 2002).
Normally, *E. coli* does not cause disease although some strains frequently cause diarrhoea in travelers (Smith *et al.,* 1997; Spencer *et al.,* 1999) and it is the most common cause of urinary tract infections. One strain designated O157:H7, is particularly virulent and has been responsible for several dangerous outbreaks in people eating contaminated food (Slutsker *et al*., 1997; Mead and Griffin 1998; Michino *et al*., 1998). In some people, particularly children under five years of age, the infection can cause a complication called hemolytic uremic syndrome (HUS). This is a serious disease in which red blood cells are destroyed and the kidneys fail. The isolation of *E. coli* O157:H7 in this study however needs further confirmation (genotypic). A study carried out in the study area isolated *E. coli* O157:H7 on vegetables (Mensah *et al.,* 2002)

Infections associated with *E. sakazakii* have been relatively rare and frequency of reported infections has been very low. Most reported cases of *E. sakazakii* infections are in neonates, infants, and children with few among adults. Despite the low frequency of reported infections, the high mortality rate (33%) and severe neurologic impairment in many survivors has generated a high level of concern for *E. sakazakii* infections. Other studies in Ghana have isolated *E. sakazakii* on vegetables (Mensah *et al.*

2001; Mensah *et al.* 2002). Many experimental, clinical and epidemiological data tend to lend credence to the assertion that *Aeromonas* (rarely in humans) may be etiologically involved in diarrheal illness (Leclerc *et al.,* 2002). Some authors are more cautious and consider that only some strains are likely to be pathogenic, a situation similar to that with *E. coli* and *Y. enterocolitica* (Farmer *et al*., 1992).
Even though most of the fecal coliform isolates identified in this study may be classified as non-pathogenic enterobacteria, some of them could be described as opportunistic pathogens capable of causing diseases/infection like gastroenteritis, meningitis, septicemia, abscesses etc in humans. This suggests that the need for health risk reduction strategies both on farm, market and at food preparation (e.g. effective washing of the vegetables) points to safeguard the health of consumers is real.

3.2.5.4 Conclusion

The study showed that both animals and/or humans could be sources of fecal bacteria contamination of vegetables produced. This is possible due to the use of wastewater and the extensive use of animal (poultry manure) manure as fertilizer. The presumptive detection of *E. coli* 0157:H7 is worrying since this is particularly virulent and could cause dangerous outbreaks. Even though most of the fecal coliform isolates identified may, under normal circumstance, be non-pathogenic, some of them are capable of causing diseases/infection. This means proper handling of these vegetables at all entry points to reduce health risk is necessary to safeguard the health of consumers.

3.2.6. Efficacy trials for common washing methods used in pathogen decontamination of lettuce

3.2.6.1 Methodology

These trials were based on the results of the stakeholder interviews on common washing methods used for washing vegetables. Laboratory analyses were conducted to determine the efficacy of these common practices on fecal coliform and helminth egg decontamination of lettuce. The efficacies of these methods were measured in terms of helminth egg populations and log reductions in fecal coliform. The effect of selected factors (e.g. temperature, pH, sanitizer concentration, and contact time) on the efficacy of the methods was also determined (*see details in Amoah et al 2007*).

3.2.6.2 Results

Efficacy of selected washing methods on faecal colifom and helminth egg populations

Common methods used for washing vegetables in the study areas vary among and within cities. Washing vegetables irrespective of the method used reduced FC levels in lettuce. For locally common methods tested, FC population reductions under a contact time of two minutes ranged from 1.4 to 2.2 log units, while reductions of 0.2 to 1.2 log units were observed when vegetables were washed immediately (see details in Table 4). All the treatments employed could at least reduce helminth egg population by half. Washing under running tap without any sanitizer could reduce helminth egg contamination level from about 9 to 1 egg $100g^{-1}$ wet weight.

Table 4. Summary of methods used and effects on fecal coliform levels on lettuce

Method	Use	Contact time	Mean $\log_{10}$ FC levels before and after treatment						Log reduction
				95% CI of mean			95% CI of mean		
			Before	Lower	Upper	After	Lower	Upper	
Cold water	C	3-4 sec[1].	5.5	4.9	6.1	4.5	3.8	5.1	1.0
	C	2 min.	6.1	5.2	6.9	4.7	4.0	5.4	1.4
Running tap	C	3-4 sec.	5.5	4.9	6.1	5.2	4.7	5.9	0.3
	C	2 min.	6.1	5.2	6.9	3.9	3.3	4.5	2.2
$NaCl_7$[2]	C	3 – 4 sec.	5.5	4.9	6.1	5.0	4.4	5.7	0.5
$NaCl_{23}$	C		5.5	4.9	6.1	4.7	4.2	5.3	0.8
$NaCl_{35}$	C		5.5	4.9	6.1	4.4	3.8	4.8	1.1
$NaCl_7$	C		6.1	5.2	6.9	4.7	4.1	5.2	1.4
$NaCl_{23}$	C		6.1	5.2	6.9	4.6	3.5	5.6	1.5
$NaCl_{35}$	C	2 min.	6.1	5.2	6.9	4.0	3.2	4.9	2.1
$NaCl_7$ + Vin_{6818}	C	3-4 sec.	5.5	4.9	6.1	5.2	4.3	6.0	0.3
		2 min.	6.1	5.2	6.9	4.7	3.9	5.4	1.4
$Vinegar_{6818}$		3-4 sec.	5.5	4.9	6.1	5.3	4.2	5.7	0.2
		2 min.	6.1	5.2	6.9	5.1	3.5	5.6	1.0
$Vinegar_{12500}$	T	5 min.	3.7	3.4	3.8	1.9	0.3	1.3	1.8
		10 min.	3.7	3.4	3.8	0.0	0.0	0.0	3.6
$Vinegar_{21400}$	T	5 min.	4.7	3.8	5.6	0.0	0.0	0.0	4.7
		10 min	4.7	3.8	5.6	0.0	0.0	0.0	4.7
Removal of outer leaves	T	5 min.	4.3	3.8	4.9	3.8	3.4	4.2	0.5
($Vinegar_{12500}$)	T	10 min.	4.3	3.8	4.9	3.4	3.0	3.8	0.9
Laundry Omo™	T	5 min	4.3	3.8	4.9	1.7	0.9	2.4	2.6
(Detergent)	T	10 min	4.3	3.8	4.9	1.9	1.1	2.7	2.4
Eau de javel™	C	5 min	6.4	6.3	6.5	4.0	3.7	4.3	2.4
(Bleach:I65µS/cm)	T	10 min	6.4	6.3	6.5	3.5	3.3	3.8	2.9
Thick Bleach™	T	5 min	6.3	5.7	6.9	3.8	3.1	4.4	2.5
(248µS/cm)	T	10 min	6.3	5.7	6.9	3.8	2.7	4.9	2.5
Power Zone™	T	5 min	6.3	5.7	6.9	4.1	3.8	4.6	2.2
(Bleach:223µS/cm)	T	10 min	6.3	5.7	6.9	3.3	3.1	3.4	3.0
KM_{100}	C	3-4 sec	5.5	4.9	6.1	4.8	4.3	5.2	0.7
		2 min	6.1	5.2	6.9	4.9	4.2	5.4	0.6
KM_{200}	T	5 min	6.4	6.3	6.5	4.4	4.2	4.5	1.0
		10 min	6.4	6.3	6.5	3.9	3.8	4.0	2.5
CL $tabs_{100}$	T	5 min	6.4	6.3	6.5	4.1	3.8	4.3	2.3
	T	10 min	6.4	6.3	6.5	3.7	3.3	4.1	2.7

Source: (Amoah et al., 2007, modified)

[1]Contact time of dipping vegetables in the washing solution; [2]subscript represent sanitizer concentration in ppm; C: Commonly used; T: Tested variation; KM: Potassium permanganate ; CL : Chlorine tablets

3.2.6.3 Discussion

In Ghana salt solution was the dominant method used for washing vegetables, other sanitizers e.g. bleach (sodium hypochloride, NaOCl; *eau de javel*) and potassium permanganate are the main methods used. The fact that all people washed vegetables before consumption suggests that the food preparation points (e.g. household, restaurants, and hotels) are important points for health risk reduction.

Washing lettuce according to common practices showed that it reduces, irrespective of the method used, the bacteria populations. The results of helminth egg populations of washed and unwashed lettuce leaves further confirmed the fact that washing with or without disinfectants can significantly reduce worm load on contaminated vegetables. However for eggs it depends more on physical removal than the use of chemicals. Faecal coliform load decreased significantly after washing under running tap for two minutes confirming reports of other writers (Beuchat, 1998; Elorm, 2002). Washing vegetables in a bowl with potable water, then again washing or rinsing in potable water would aide in removing microorganisms. Additional 10-fold to 100-fold reductions can sometimes be achieved by treatment with disinfectants (Beuchat, 1998). According to Parish *et al.* (2003), the efficacy of the method used to reduce microbial populations is usually dependent upon the type of treatment, type and physiology of the target microorganisms, characteristics of produce surfaces, exposure time and concentration of cleaner/sanitizer, pH, and temperature. Some of these factors (e.g. temperature, concentration, contact or exposure time and pH) accounted for the low fecal coliform reduction levels and are described below.

Generally, the efficacy of all methods tested increased with increasing temperature confirming the sanitizing effect of high temperature. For example, the efficacy of salt and vinegar solutions increased significantly between about 1 to 2 log reduction, when the solutions temperature are increased from 25 ºC to 40 ºC. It should be noted that though increased temperature generally increased sanitizer efficacy, it had a deteriorating effect on lettuce leaves at 40 ºC. This confirms the report that using hot water could adversely affect color and texture of the produce (especially for vegetable likely to be consumed raw) and limit the usefulness of this treatment (Parish *et al*., 2003).

Even though the efficacy of each treatment improved with increased contact time, considerably high levels of fecal coliforms and helminth eggs still remained on the vegetables. This suggests that none of the common methods usually applied in households and kitchens can be relied upon to completely decontaminate wastewater irrigated lettuce. However, their efficacy could be improved significantly if other factors relating like sanitizations are reviewed. Increased contact time in salt (NaCl) solution with concentrations of 23 mgl^{-1} and 35 mgl^{-1} had a considerable deteriorating effect on the lettuce leaves and therefore may not be desirable. At a higher vinegar concentration of 21400 ppm (approximately 1 part vinegar in 2 parts water) the efficacy of the sanitizer completely removed fecal coliform levels in less than one minute. This may be too expensive for the poor households and will have low adoption rate but increasing the contact time at a lower vinegar concentration (approximately 1 part vinegar in 5 parts water) improved its efficacy significantly.

From the above discussions, it is deduced that washing vegetables before consumption is a very important step in the reduction of the potential risks associated with the consumption of contaminated vegetables. However, their efficacy is variable and none are able to ensure total elimination of pathogens. The WHO (2006) has set a health protection level of $\leq 10^{-6}$ DALY (Disability Adjusted Life Years) per person per year. This could be achieved through a fecal coliform reduction of about 6 – 7 log units which is achievable mainly through produce cooking (WHO 2006). Parish *et al.* (2003) reported that there are no known mitigation strategies that will completely remove pathogens after

contamination has occurred while maintaining produce freshness. Consequently it is not possible to rely solely on washing and or disinfection with or without sanitizers to control contamination by pathogens. Beuchat (1998) reported that prevention of contamination at all points of the food chain is preferred over the application of disinfectants. It would therefore be expected that combinations of washing and other intervention methods, at various entry points would be appropriate. For example, strict adherence to Good Agricultural Practices (GAPs), Good Handling Practices (GHPs) and other relevant strategies that prevent contamination from occurring. The concept of using multiple intervention methods at various entry points which is analogous to "hurdle technology" or the multiple barrier approach where two or more preservation technologies are used to prevent growth of microorganisms in or on foods (Leistner and Gorris, 1995; Leistner, 2000; Howard and Gonzalez, 2001).

3.2.6.4 Conclusion

The study revealed that washing vegetables irrespective of the methods used reduces the fecal coliform population levels. Common methods vary widely and are often applied ineffectively because of lack of information or appropriate instructions. None of the common methods used in the household could be relied upon to remove any significant amount of fecal coliforms populations on vegetables. However, the efficacy of these methods could be improved by using the correct sanitizer concentrations and for desirable contact times. The study showed that there is a high potential in Ghana to strengthen health risk reduction efforts through improved vegetable washing before consumption.

3.2.7 Ongoing/upcoming research at IWMI on wastewater irrigated agriculture

IWMI has some ongoing collaborative research on wastewater irrigation. Some of the ongoing research which may be of interest to the proposed study include:

3.2.7.1 Microbial characterization and farm based risk mitigation, with specific focus on viruses

Ms. Andrea Silverman, a PhD at the University of California, Berkeley has been working on various aspects of risk assessment and farm-based management measures in Accra, with a specific focus on viruses. On risk assessment she is working on assessing concentrations of fecal indicator bacteria (*E. coli*, *Enterococci* and thermotolerant coliform) and bacteriophage (F+ and somatic coliphage). She uses quantitative PCR (qPCR) to determine concentrations of human pathogens and human-specific *Bacteroidales* (proposed fecal indicator bacteria) such as Adenovirus, Norovirus GII, and the two extraction controls (MS2 and Pph6); there has been preliminary work on Enterovirus and Norovirus GI assays. On risk mitigation, she is working for example on UV disinfection of irrigation water in on-farm ponds.

3.2.7.2 Emerging contaminants

Ms. Senorpe A-Hiablie, a Norman E. Borlaug Fellow, has just her research focusing on emerging contaminantsin wastewater irrigated aquaculture and agriculture. She is working on Endocrine Disrupting Compounds (EDCs) specifically synthetic estrogen EE2 on fish, crop, soil and water).

3.2.7.3 Improving QMRA models

Ms. Fiona Barker, a research scientist, at the University of Melbourne is working with IWMI to improve on QMRA models developed in her university using data collected from IWMI's wastewater research in Accra.

3.2.8 Other related IWMI studies in other cities in Ghana.

Other than wastewater irrigation in Accra, IWMI has been involved in wastewater irrigation studies in other cities in Ghana. In Kumasi, substantial work has been done in developing low-cost risk reduction measures (refer to publications by Keraita 2005- 2010; PhD thesis 2008). Employing a participatory approach measures such as on-farm ponds, filtration techniques, water application techniques were developed and tested. WHO-FAO-IDRC also involved IWMI and other local partners to test the implementation of the WHO (2006) guidelines in the Ghanaian context. In northern Ghana for example, Prof. Kranjac-Beri- savljevic worked with farmers on low-cost drip irrigation kits as a risk management measure for fecal contamination from wastewater. Beyond wastewater, Dr. Olufunke Cofie led research on risk assessments and management of fecal sludge for use in agriculture. This research covered a number of aspects including pathogen deactivation using co-composting (in Kumasi) and sun drying in Northern Ghana where farmers apply untreated fecal sludge directly to farms. The sun drying studies as a risk management measure was continued by Dr. Razak Seidu, under his PhD studies (Seidu, 2010).

3.3 Other non-IWMI commissioned wastewater irrigation studies

In Accra, researchers from the University of Ghana have been conducted some studies on the topic. For example, Donkor et al (2010) carried out to assess the rate of internalisation of microbes in common Ghanaian vegetables. Standard microbiological methods were employed in microbial enumeration of vegetables collected at the market and farm levels, as well as irrigation water and soil samples. In the study, the overall mean counts of vegetables were 4.0 x 10^3 cfu g^{-1}; 8.1 x 10^2 cfu g^{-1}; 2.0 x 10^2 cfu g^{-1}; 3.5 x 10^2 cfu g^{-1} for total bacteria, coliform counts, fecal coliform counts and yeast counts, respectively. They found the rate of internalisation of coliforms in vegetables irrigated with stream/well water to be 2.7 times higher than those irrigated with pipe water. The mean coliform counts (4.7 x 10^7 cfu g^{-1}) and fecal coliform counts (1.8 x 10^6 cfu g^{-1}) of soil samples were similar to those of stream water suggesting both sources exerted similar contamination rates on the vegetables. The study showed no significant variations between the rates of internalisation of microbes at the market and farm levels, indicating that internalisation of microbes in the vegetables mainly occurred at the farm level. The study concluded that microbial contamination of vegetables in Ghana is not limited to the external surface, but internal vegetable parts could harbour high microbial loads and pose risk to consumers. So, Safety practices associated with the commodity should therefore not be limited to external washing only. Some

Other internalization has been done in Kumasi and Accra but more to heavy metals (see Mensah E, PhD thesis). Studies done on general microbial contamination have been on animal manures (see Mensah et al., 2001; Sackey et al., 2001). Several studies also conducted on wastewater irrigation in other cities and have been reported as university projects and thesis, but have few sample sizes, descriptive and don't show significant differences with work carried out by IWMI.

4. NON-IWMI COMMSIONED STUDIES DONE IN ACCRA ON OTHER FECAL EXPOSURE PATHWAYS

4.1 Exposure from street-vended foods

Food borne illness caused by microbial contamination of foods is an important international public health problem and is known to be a major cause of diarrhea diseases especially in developing countries (Harris et al., 2003; Nguz, 2007, Mensah, 1997). Untreated wastewater is just one of the many transmission routes of foodborne illnesses. This section focuses on the broader food contamination and safety studies done in Ghana.

In Ghana, specific health concerns have been on street foods, i.e. ready-to-eat foods prepared and or sold at public places such as schools, market places and along the streets, which are common sources of food in urban low-medium income households (Mensah et al., 2002). These foods are relatively cheaper in cost and at easily accessible places. Furthermore, it offers the traditional meals and preparations of a number of them are quite laborious and time consuming. Tomlins (2000) estimated that the street foods sector employs over 60,000 people with an estimated annual turnover of over US$100 million and an annual profit US$24 million. Several studies show that these health concerns mostly relate to unhygienic nature of preparation, transportation and storage, handling at selling points, unsanitary nature at selling points and also unhygienic vendors (Yeboah-Manu et al, 2010).

4.2 Exposure from flooding

The frequency of flooding and the damage caused by urban flood events have increased over the past decades, mainly due to accelerated urbanization (Ashley et al., 2005). When urban flooding occurs, flood water is likely to be fecally contaminated and may pose health risks to citizens exposed to pathogens in these waters. In Accra, flooding is a serious environmental issue, and with rising sea levels it may become an even greater problem. It is expected that an increased level of cyclonic storms to a great extent and storm surges to a lesser extent will be associated with future climate change and may increase flood occurrence in spatial patterns similar to those of the present (Rain et al., 2011). Accra floods currently are usually of short duration and are caused by heavy rains that generally occur in June and July. Significant flood events have been recorded in 1973, 1986, 1995, 1999, 2001, and 2002. Along with property damage and loss of lives, flood waters have the ability to spread pollution from solid waste, industrial waste, and sewage is an important health and environmental issue particularly in poor areas. Several factors contribute to the flooding problem. These include

- Rapid growth of the city has increased the extent of impervious surfaces such as roads, rooftops, sidewalks, bedrock outcrops and compacted soil, which prevent infiltration of water into the soils. This leads to increased discharge that overloads drainage channels.
- Flaws in the drainage network as most of it is undersized, unconnected or improperly channeled drains.
- Poor development controls, limited garbage collection and disposal block channels and sewers, which slow drainage through the city. There is substantial uncontrolled development occurs in low-lying or unsafe areas – often immediately adjacent to and even directly over drainage channels.

A simple flood model developed for Accra that shows impacts and possible property damage and pollutant spread in Accra is shown Figure 7. The figure illustrates the estimated amounts of overflow for each channel resulting from a 10-year 24-hour rainfall total (167.6 mm). This water would overflow the stream channels and affect the immediately adjacent areas. The figure also shows the populations at

risk to flooding in Accra, where a conservative figure of 172,000 people based on the 2000 census was obtained. The figure shows that a large portion of the population that would be impacted by flooding tend to be the slum dwellers, who are often the poorest.

4.3 Other exposures

Literature from other fecal exposures drinking water, solid waste and flies as sources of human health risks are few and rather unsystematic. Table A3 in Appendix 1 shows some two studies done on drinking water and another one on cockroaches. On the public domain of drinking water the exposure could be more from sachet water than tap water (Kwakye-Nuako, 2007; Addo et al., 2009). However, there has been frequent reporting in Ghana media when sewage pipelines cross-contaminate piped water. Cases of malfunctioning of the water treatment plants have been aslo reported especially when there is frequent power failure. In general, the domestic domain (point of use) of drinking water (in terms of quality, even quantity) as a risk factor for diarrheal diseases in Accra is least studied and remains as a study gap that needs urgent attention. The same can be said for solid waste and flies at household and community level.

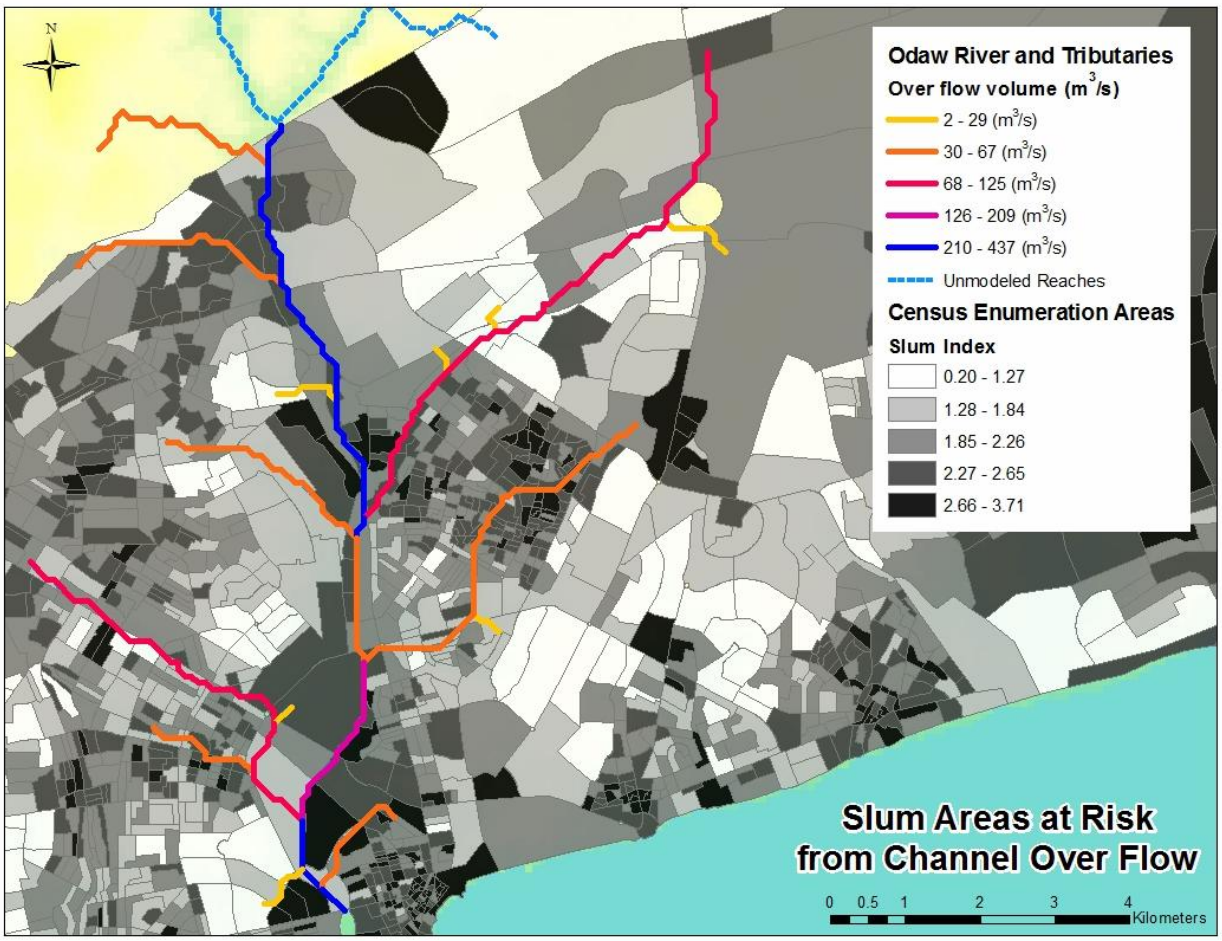

Figure 7. Areas in Accra most vulnerable to floods from the Odaw drain

Source: Ludlow C (2009)

5. QMRA FOCCUSSED STUDIES

The WHO has promoted an integrated, preventive management framework and risk-benefit approach rather than set international standards in response to the growing crisis of pervasive fecal exposure in the urban environments (WHO, 2006). This risk assessment and management approach employs quantitative microbial risk assessment (QMRA) as a critical element of the WHO framework and guidance for policy makers. QMRA models are frequently used to estimate disease risk associated with fecal contamination, and have been used in risk assessments for drinking water, waste management, and most recently, for high-risk populations that come into contact with wastewater (Howard et al., 2006; Mara, 2007; Schönning et al., 2007; Seidu et al., 2008). However, it is challenging to apply challenging to apply QMRA to developing-country contexts where limited data on exposures and specific pathogens exists (Hamilton et al., 2008).

Nevertheless, few QMRA based studies have been conducted in Accra focusing on environmental fecal contamination. An overview is given in Appendix 1Table A4. Two studies quantify health risks from wastewater irrigated agriculture in Accra (Suleiman, 2007; Seidu et al., 2007), two others go further to estimate cost-effectiveness of intervention measures (Drechsel and Seidu, 2011; Labite, 2008), while one study estimates risks in multiple environmental exposures (Lunani, 2008). While the studies clearly demonstrate the importance of QMRA studies Accra, a number of assumptions to make estimates and refining them through more in-depth environmental sampling will help in giving better estimates. They also show the need to conduct broader relative risk studies, considering both public and domestic domains, as these studies are more focused on public domains.

6. SUGGESTIONS FOR FURTHER STUDIES

Based on the previous studies reviewed in this report and considering the scope of the proposed study, the following gaps could be addressed.

i. Further studies on wastewater irrigation

- Further research on the contamination pathway should be carried out using other vegetables (e.g. cabbage, spring onions, cauliflower etc.) as a test crops in addition to lettuce. This is because handling of these crops may be different and could further determine how much handling from farm to market contributes to the microbiological contamination of vegetables and hence exposing consumers to potentially harmful pathogens
- Developing more risk mitigation measures especially at market and household levels. For example, the tested washing methods should be simulated by food vendors and caterers to ascertain their efficacy in real life situations and also determine the effect of some sanitizers on the sensory nature of the vegetables washed. Or, studies that will include constructing of a water point or toilet with a - facility in the market and testing impacts could be feasible within research projects.
- Though a general framework and methodology has been developed in assessing and developing risk management measures in low-income urban settings, the studies have a geographical limitation. For example, irrigation practices differ, so mitigation measures

will also differ. These studies should be up-scaled to other similar cities in Ghana and even more importantly to other sub-Saharan African countries.

ii. **Detailed environmental sampling using actual pathogens:** Detailed and systematic environmental sampling is required across all fecal exposure pathways to get more reliable data to improve precision of QMRA estimates. This will also help identifying where management measures are most needed. These studies should go beyond indicator organisms and focus on actual pathogens. Such studies could include:
    - The review shows limited studies in the domestic domain in urban settings. Data on fecal contamination at household level from drinking water, use of latrines, foods is scarce. This is a huge gap that needs to be addressed.
    - In spite of their relatively lower importance in disease transmission in most developing countries, viruses are highly and immediately infectious and virtually one cell could suffice for infection. Further investigation into their presence and numbers in irrigation water and on crops is still required.
    - The high fecal coliform contamination levels on the vegetables analyzed in both is alarming especially when piped water was used for irrigation although the water is not the source in this case. However, it will be helpful if the specific fecal coliform strains are quantified in further studies to ascertain the real danger of consumption of contaminated vegetables from irrigated urban sites in developing countries
    - Test local links between common indicator organisms used in this study and pathogen occurrence and revise indicators if needed (norovirus as recommended example).

iii. **Comparative risk assessment:** While such studies are complicated due to many confounding factors, it is important that such studies are initiated, even at community level. The relative importance of fecal exposure pathways can only be quantified if all exposure pathways will be compared in systematic studies. This is particularly important in urban settings.
    - The comparative risk assessments done so far did not especially consider the private domain.
    - Comparison between food and water-borne pathways for diarrheal diseases.

iv. **Epidemiological studies:** Reliable epidemiological studies on the risk of the used water with marginal quality to vegetable consumers and to farmers should be conducted in developing countries. This is because present knowledge is mostly limited to studies conducted in developed countries.

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Appendix 1

Table A1. Overview of selected studies on diarrheal diseases in Accra.

Authors	**Study description**	**Key findings/conclusions**
Armah, et al. (1994)	***Objective:*** To study human rotavirus (HRV) infection and its seasonal distribution was studied over a 12- month period in Ghana. ***Method*****:** Collection and analysis of a total of 561 stool samples, 447 diarrhoea stools and 114 non-diarrhoea stools (controls), obtained from children attending three polyclinics in Accra.	***Results***: Rotavirus was detected during 10 of the 12 months and showed a seasonal trend. It was high during the relatively cool dry months and low during the wet season. Peaks of infection were in February (26.2%) and September (24.5%). HRV was detected in 67 of 447 of the diarrhoea stools (15.0%) and in eight of 114controls (7.0%). The HRV isolation rate was highest (20.2%) in the under-18-months age group. The RNA electropherotype of the HRV isolates was predominantly (83.6%) of the long type. Non-group A HRV was detected in 14.9% of the HRV-positive samples.
Dongdem et al., (2010)	***Objective:*** Investigate levels of rotaviruses on tap water from Weija Water Works, the major source of drinking water in Accra ***Method:*** Treated water samples were collected from five zones within the distribution network of Weija Water Works. Two litres of each sample were concentrated 4,000 fold and the viral particles extracted. Viral RNA was extracted from all concentrates using phenol/chloroform, purified with the RNaid®kit and reverse transcribed. The cDNA was amplified by semi-nested PCR using P and G genotype-specific primers and analyzed an agarose gel.	***Results:*** Rotavirus was detected in 48.1% of samples. Nine rotavirus P-types and 12 G-types were detected. The detection pattern showed increased viral pollution with distance from the treatment plant and in areas with high human activity. ***Conclusions*****:** Water treated and distributed by the Weija Water Works was found to contain several strains of rotavirus. The level of viral contamination increased with distance from the plant. The prevalence of rotaviruses was also found to increase in areas of high human activity. Disinfection methods currently in use do not seem to be adequate in inactivating viruses in the water. Increase in chlorine dosage which will in turn raise residual chlorine levels in the water should be considered by water companies. Legislative measures for regular viral monitoring as part of microbial risk assessment in drinking water should also be considered by the national quality monitoring bodies
Adjei et al., (2004)	*Objective:* Assess prevalence of *Cryptosporidium* Spp., among Children at Korle Bu teaching Hospital, Accra, Ghana. Methods: Stool samples taken from 227 children	***Results****:* Prevalence rates were 27.8 and 15.6% in children with and without diarrhea, respectively. Cryptosporidium infection was found to be high in children between the ages of 6 and 24 months. Cryptosporidium spp. was more common in malnourished children, but was not isolated in children under 6 months of age who were exclusively breastfed.

	with diarrhea and 77 without diarrhea, aged less than 5 yrs and analyzed for *Cryptosporidium* Spp, *Shigella*, *Salmonella* and yeast-like organisms.	Neither the presence of domestic animals, abdominal pain, blood in stool, nausea, vomiting, nor the consumption of untreated water was associated with Cryptosporidium spp. infection. Shigella, Salmonella, and yeast-like organisms were the most frequently identified enteropathogenic bacteria ***Conclusion:*** There is frequent occurance of *Cryptosporidium* among Ghanaian children with or without diarrhea, so the disease should be considered as an important childhood diarrheal disease in Ghana.
Opintan et al., (2010a)	***Objective:*** Find out aetiological agents of diarrhoea include different categories (pathotypes) of diarrhoeagenic *Escherichia coli* (DEC) ***Method***: Case–control study. Stool specfimen taken from 72 adults and children aged over 3 years, who presented with diarrhoea at a hospital in Accra, Ghana, and 72 matched controls. In addition, stool specimen taken from 31 infants (<2 yrs) with diarrhoea and 22 without. All analyzed for DEC.	***Results:*** DEC are commonly recovered from healthy individuals above three years and also DAEC are associated with diarrhoea in this age group. Pathotypes frequently detected among older individuals – EAEC, EPEC and ETEC – although not associated with disease in older individuals were also isolated from children with diarrhea. ***Conclusion***: It is probable that older individuals serve as reservoirs for strains that could threaten the health of vulnerable children, emphasizing the need to promote hand washing and safe feeding practices and to further characterize DEC with the ultimate goal of developing pathogen-specific interventions
Opintan et al., (2010b)	***Objectives:*** (i) to determine the prevalence of EAEC, *Shigella* spp., *Cryptosporidium* spp., *E . histolytica*, and *Giardia lamblia* in children 5 years of age with and without diarrhea in southern Ghana. (ii) determine whether these enteropathogens were associated with intestinal inflammation ***Methods:*** Anthropometric datawere collected from 274 children with (*N* = 170) and without (*N* = 104) diarrhea. Stool specimens were analyzed by conventional culture, polymerase chain reaction for enteroaggregative *Escherichia coli* (EAEC), *Shigella* , *Cryptosporidium* , *Entamoeba* , and *Giardia* species, and by enzyme-linked immunosorbent assay for fecal lactoferrin levels	***Results:*** About 50% of the study population was mildly to severely malnourished. Fecal lactoferrin levels were higher in children with diarrhea (*P* = 0.019). Children who had EAEC infection, with or without diarrhea, had high mean lactoferrin levels regardless of nutritional status. The EAEC and *Cryptosporidium* were associated with diarrhea (*P* = 0.048 and 0.011, respectively), and malnourished children who had diarrhea were often co-infected with both *Cryptosporidium* and EAEC. ***Conclusion:*** the use of DNA-biomarkers revealed that EAEC and *Cryptosporidium* were common intestinal pathogens in Accra, and that elevated lactoferrin was associated with diarrhea in this group of children.

Boadi and Kuitunen (2005)	***Aim:*** Examining two weeks incidence of diarrhea among children under six years in Accra and the risk factors. ***Methods:*** Qualitative study 489 children under the age of six. Data collected using a detailed structured questionnaire where mothers were respondents	***Results:*** Household economic status and education of the mother are significant determinants of diarrhea. The study found a significant association between diarrhea morbidity and access to water and sanitation facilities, hygiene practices, flies infestation and the regular consumption of street food. ***Conclusion:*** Integrated child health intervention programs including provision of facilities, maternal hygiene education and environmental health awareness have to be strongly implemented in order to reduce the high incidence of childhood diarrhea.
Fobil et al., (2011)	***Objectives.*** To investigate the relationship between malaria and infectious diarrhea mortality and spatially varied neighborhood environmental quality conditions in a low-income economy. ***Design and Methods.*** A one-time point spatial analysis of cluster-level environmental conditions and mortality data using principal component analysis (PCA), one-way analysis of variance (ANOVA) and generalized linear models (GLMs). Environmental variables were extracted from the Ghana Census 2000 database while mortality data were obtained from the Ghana Births and Deaths Registry in Accra over the period 1998–2002.	***Results***. Whereas there was a strong evidence of a difference in relative mortality of malaria across urban environmental zones of differing neighborhood environmental conditions, no such evidence of mortality differentials was observed for diarrhea. In addition, whereas bivariate analyses showed a weak to strong evidence of association between the environmental variables and malaria mortality, no evidence of association was found between diarrhea mortality and environmental variables. ***Conclusion***. We conclude that environmental management initiatives intended for infectious disease control might substantially reduce the risk of urban malaria mortality and to a less extent that for urban diarrhea mortality in rapidly urbanizing areas in a low-income setting.

Table A2. An overview of food safety related studies conducted in Accra

Authors	**Study description**	**Key findings/conclusions**
Tomlins, (2000).	***Objective:*** to assess and prioritise the economic and social importance, and safety and quality constraints of street-vended foods in peri-urban locations and to make recommendations on quality and safety risks associated with street-vended foods made, and identify areas where new knowledge is required. ***Method:*** A socio-economic survey involving 334 vendors and a a food safety study screened 96 case study street-vended food samples (waakye, fufu and salad) from Accra for heavy metals, pesticides, micro-organisms and mycotoxins and 48 raw material samples from primary and secondary markets for heavy metals and mycotoxins.	***Results:*** 40% of waakye samples contained the heavy metal lead above the draft Codex recommended maximum limit of 0.2 mg/kg and 70% contained the organophosphorous pesticide chloropyriphos. Lead contamination can result in learning difficulties and behaviour problems in children. Low but non-hazardous levels of mycotoxins and the heavy metal cadmium were detected in many street vended food samples. Some laboratories in Ghana, however, were not able to use current analytical methods such that the results for pesticides were indicative. Institutional support of laboratories is needed if reliable analysis of pesticide residues is available in Ghana. No mycotoxins or heavy metals were detected in the raw materials from primary and secondary markets. ***Recommendation:*** Need to improve access to water, sanitation and refuse disposal for street food vendors.
Addo *et al*., (2007)	**Objective**: A pilot study to investigate food and its preparation conditions in ten hotels in Accra **Methods:** Microbiological laboratory analysis of 184 samples made of 105 swabs of kitchen working surfaces and cutlery and 79 foods and drinks	**Results:** All the surface swabs, food and drink samples tested negative for *Salmonella, Staphylococcus* and *E. coli*. Total aerobic bacteria high (> 103 cfu/ml) in 35.2% of swab samples and in all fruit juices (orange, punch, mango, pineapple) except the ginger drink **Conclusion:** Food and water served in hotels are generally safe but fruit juices recorded presence and growth of coliform organisms suggesting that methods employed in the preparation of these juices should be improved.
Mensah et al.(2002)	**Objective**: To investigate the microbial quality of foods sold on streets of Accra and factors predisposing to their contamination **Methods:** (i) Structured questionnaires were used to collect data from 117 street vendors on their vital statistics, personal hygiene, food hygiene and knowledge of foodborne illness. (ii). A total of 511 food samples examined for aerobic mesophilic bacteria (total counts), *B. cereus*, *S. aureus* and Enterobacteriaceae.	**Results**: Most vendors were educated and exhibited good hygiene behaviour. Diarrhoea was defined as the passage of $\geq$3 stools per day by 110 vendors (94.0%), but none associated diarrhoea with bloody stools; only 21 (17.9%) associated diarrhoea with germs. The surroundings of the vending sites were clean, but four sites (3.4%) were classified as very dirty. The cooking of food well in advance of consumption, exposure of food to flies, and working with food at ground level and by hand were likely risk factors for contamination. Mesophilic bacteria were detected in 356 foods (69.7%): 28 contained Bacillus cereus (5.5%), 163 contained Staphylococcus aureus (31.9%) and 172 contained Enterobacteriaceae (33.7%). The microbial quality of most of the foods was within the acceptable limits but samples of salads, macaroni, fufu, omo tuo and red pepper. **Conclusion:** Street foods can be sources of enteropathogens. Vendors should therefore receive education in food hygiene. Special attention should be given to the causes of diarrhoea, the

		transmission of diarrheal pathogens, the handling of equipment and cooked food, hand-washing practices and environmental hygiene.
Addo et al., (2009)	**Objective**: Assess microbial quality of bottled water in Accra **Methods**: 70 samples of bottled water from 7 companies analyzed for total coliforms, fecal coliforms and *E. coli*	**Results**: All water samples were within the acceptable limits set by WHO guidelines (<2 counts). **Conclusion:** Bottled water is generally safe for consumption. However, more extensive surveillance of the bottled water industries and stringent regulations should be developed and enforced to ensure that the standards recorded in this study are maintained.
Tay et al., (2008)	**Objective**: Determine levels of trace metal contamination in fish from coastal waters **Methods:** 124 samples of three shell fish species and 250 samples of 7 fin fish species analyzed for concentrations of zinc, copper, manganese, iron, lead and cadmium in some coastal and inland waters in Ghana	Results: Compared to World Health Organization limits, the levels of Zn, Pb, Cu, and Cd in the fish species were within acceptable limits for human consumption. The study also showed that all the fish species, except *Sardinella eba*, *Solar crumophthalmus* and *Panulirus regius* are safe for human consumption with respect to Zn, Cu and Fe. **Conclusion**: As a health risk factor, heavy metal contamination on fish from coastal Accra is of lesser significance
Yeboah-Manu et al., (2010)	***Objective:*** Determine the microbial quality of ready-to-eat foods being sold in the open (street foods) and those from restaurants on the university of Ghana campus ***Method:*** 27 foods were sampled from the 5 sites and analyzed for Aerobic Colony Count (ACC), total Enterobactereacea (EC), presence of *Escherichia* coli and other Enterobacteriaceae and the presence of *Salmonella* sp. and *Shigella* sp.	***Results***: Foods within acceptable limits ($<10^4$ cfu g^{-1}); 48% for ACC and 59.3% for EC. Salads were most contaminated. Bacterial species were isolated were: *E. coli*, *Klebsiella pneumoniae*, *Streptococcus* sp., *Enterobacter cloacae*, *Bacillus* sp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus* sp., *Streptococcus agalactiae* and *Enterococcus faecalis*. ***Conclusion***: Need for stricter implementation of food sanitation practices to reduce the possible risk of transmission of infection on consumption of these foods in future. In addition there is the need for educating the vendors and hired helps on safe food handling practices and proper hygienic practices, particularly proper hand washing.
Aye-Kumi et al., (2009)	***Objective:*** Determine the prevalence of intestinal parasites associated with food vendors in Accra and assess the risk for consumers of street-food. ***Methods:*** Random sampling was used to select 204 food vendors from 7 metropolitan areas in Accra. The parasitological profiles of stool samples from the vendors sampled were developed using direct smear, formalin-ethyl acetate sedimentation method, modified Ziehl Neelsen, and trichrome staining techniques.	***Results:*** The overall prevalence of parasitic infection was 21.6%, with helminthic (15.2%) predominating over protozoan (6.4%) infections. Seven different parasites were identified: *Ascaris lumbricoides (*5.0%), *Strongyloides stercoralis* (4.4%), *Enterobius vermicularis* (4.1%), *Cryptosporidium parvum* (2.5%), *Giardia lamblia* (2.0%), *Ancylostoma duodenale* (2.0%), and *Entamoeba histolytica* (2.0%). ***Conclusion:*** The study indicated high levels of gastrointestinal parasitic infection among food vendors in the metropolis, and raised the need for education on safe handling of food, and improved sanitation and personal hygiene, to avert potential health threats to patrons.

	Vendors were also interviewed using a questionnaire.	
Donkor et al., (2009)	***Objective:*** To apply the WHO five keys to safer food in an evidence based training programme for food vendors to improve the safety of street food or ready-to-eat food in a poor community in Accra. ***Methods:*** (i) Structured questionnaire where 127 street food vendors were interviewed (ii) structured observations (iii) Stool specimens were collected from all the vendors and screened for enteric pathogens of bacteria and parasites by standard microbiological methods (iv) training of vendors on WHO's 5 keys	***Results:*** Low prevalence of intestinal/ diarrheal pathogens among the vendors was low (1%) but high (51.4% annually) incidence of diarrhoea among vendors. Assessment of personal and environmental hygiene of the vendors showed that 4.7% and 16.5% of the food vendors had poor personal and environmental hygiene, respectively. Impact assessment of workshop showed 67.6% of the vendors had acquired some knowledge from the workshop and were putting it into practice ***Conclusion:*** Food vendors have information on food safety such as hygiene and disease prevention. However, they require an impulse such as a training workshop to put knowledge on food safety into practice. Lack of facilities among food vendors in poor resource communities could be a major constraint to the employment of good food safety practices.

Table A3. Drinking water studies

Authors	Study description	Key findings/conclusions
Addo et al., (2009)	**Objective**: Assess microbial quality of bottled water in Accra **Methods**: 70 samples of bottled water from 7 companies analyzed for total coliforms, fecal coliforms and *E. coli*	**Results**: All water samples were within the acceptable limits set by WHO guidelines (<2 counts). **Conclusion:** Bottled water is generally safe for consumption. However, more extensive surveillance of the bottled water industries and stringent regulations should be developed and enforced to ensure that the standards recorded in this study are maintained.
Kwakye-Nuako et al., (2007)	**Objective:** To assess the safety of sachet drinking water. **Methods:** Twenty seven different brands of 500ml sachet water samples randomly selected and purchased from various vendors in Accra were subjected to microscopic examinations to determine the presence of parasitic protozoa.	***Results:*** Seventy-seven percent of the samples contained infective stages of pathogenic parasitic organisms. Common pathogens identified include, Microsporidia sp 14/27 (51.2%), Cryptosporidium parvum 17/27 (63.0%), Cyclospora cayetenensis 16/27 (59.3%), Sarcocystis sp. 18/27 (66.7%). Rotifers 5/27 (18.5%), and Charcoat Leyden crystals 12/27 (44.4%). Ninety-three percent of the samples contained unidentified impurities/artifacts. 29.6% of the samples contained at least one type of parasite, 14.8% contained at least 2 types of parasites, 25.9% contained at least three types of parasites, while 29.6% contained four types of parasites. **Conclusion**: The study indicated the presence of contaminants of feacal and zoonotic origin in some of the sachet water examined. This has grim public health implications as the organisms identified can cause water related diseases which have serious complications in children and adults particularly immunocompromised individuals. Sachet water should be constantly monitored for its microbial quality.
Agbodaze & Owusu (1989)	**Objective**: To demonstrate that cockroaches could play an important role in the transmission of pathogenic organisms (diarrheal pathogens), especially in our environment. **Method**: Characterisation of cultures of enteric bacterial pathogens from bodies and intestinal contents of 208 cockroaches (Periplaneta americana), collected from kitchens in Accra and some surrounding villages.	**Results:** Six of them harboured three different serogroups of Salmonella, one had Shigella dysenteriae, 64 had Coliforms, 13 had Proteus species, two had Pseudomonas species and the rest (122) carriednone of the bacterial species mentioned above. **Conclusion:** The presence of Salmonella species Shigella dysenteriae and Coliforms in these insects, which were collected from kitchens where foods are kept, points to the facts that these insects could play an important role in the transmission of these pathogenic organisms, especially in our environment. Permanent solution to these bacterial diarrhoea disease problems could only be solved when food, animals and the environments are free of these microbes.

Table A4. QMRA focused studies

Seidu et al. (2008)	***Method*: ** Quantitative Microbial Risk Assessment (QMRA) models with 10,000 Monte Carlo simulations were applied to ascertain the risks of rotavirus and Ascaris infections for farmers using different irrigation water qualities and consumers of lettuce irrigated with the different water qualities after allowing post-harvest handling	***Results***: A tolerable risk (TR) of infection of 7.7 $X10^{-4}$ and 1 x 10^{-2} per person per year were used for rotavirus and Ascaris respectively. The risk of Ascaris infection was within a magnitude of 1022 for farmers accidentally ingesting drain or stream irrigation water; ≈10^{0} for farmers accidentally ingesting farm soil and 10^{0} for farmers ingesting any of the irrigation waters and contaminated soil. There was a very low risk (10^{5}) of Ascaris infection for farmers using pipe water. For consumers, the annual risks of Ascaris and rotavirus infections were 10^{0} and 10^{-3} for drain and stream irrigated lettuce respectively with slight increases for rotavirus infections along the post-harvest handling chain. Pipe irrigated lettuce recorded a rotavirus infection of 10^{-4} with no changes due to post harvest handling ***Conclusion:*** The assessment identified on-farm soil contamination as the most significant health hazard.
Labite (2008)	***Objective:*** assess the microbial risks generated in the Accra urban water system based on primary data and to recommend some effective interventions with a given budget. ***Method:*** Assessment of pathogen levels, estimation of risks using QMRA and estimating cost-effectiveness of risk interventions	***Results:*** • The concentration of E. coli were and Salmonella in the storm drain water and recreational water were respectively 8.0 and 4 log10CFU/100 ml; however, Salmonella was not detected in seawater. The measured concentrations of these pathogens in the sand were respectively 6 and 6.7 log10 CFU/g of dry weight. The helminth eggs count in the open drain was low (0.77 eggs/ l). • The total disease burden of Accra urban water system was 37, 000 Disability Adjusted Life Years (DALYs) and the contribution of sanitation was about 88%. The DALYs per person per year (pppy) was 5*10-3, which was above the WHO reference value (10-6). The most hazardous was the open drain with the contribution of 55% of total burden disease. • In order to improve the burden of waterborne disease, five available options were listed: option A (separate sewerage), option B (sewerage treatment plant) option (sewerage network associated to treatment plan), option D (coverage of the storm

		water drain) and option E (improvement of the water supply system). The selection criterion was to avoid people to get in contact with waste water. The calculation showed that among the suggested options, the separate network associated to the sewerage treatment would avert 32, 466 DALYs per year. However taking into account the financial aspect, the coverage of the drain (option D) appeared economically more feasible due to its low initial investment.
Lunani (2007)	***Objective****:* Microbial risk assessment was used to estimate the public health risks of the Accra urban water system (AUWS). **Methods:** Use of QMRA to estimate pathogen concentrations and health risks.	***Results****:* The potential waterborne disease exposure routes from the sanitation system were identified as; recreational swimming, flooding of the Odaw drain, open drainage channels, UASB treatment plant and fecal septage disposal place; while from the water supply as; contaminated water distribution system and water treatment plants due to errors in the treatment processes. The predicted disease burden from the AUWS was 28,531 DALYs per year due to *Campylobacter*, rotaviruses, *Cryptosporidium* and *Ascaris;* with 91% contribution from the sanitation pathway and with rotaviruses dominating. Comparing the predicted risks with the background endemic waterborne disease incidence showed that the AUWS contributed 75% of the rotavirus cases; 37% of the *Campylobacter* cases; 21% of the *Ascaris* cases and 3% of the *Cryptosporidium* cases. ***Conclusion:*** The sanitation pathway was judged to be of more importance compared to the water supply pathway. The AUWS system is not safe for its citizens and therefore, it should be addressed in order to minimize the risks.
Suleiman (2007)	***Objective***: assess the microbial risks of urban wastewater irrigation in Accra and to suggest sustainable risk management approach while maximizing the health benefits. ***Methods:*** Interviews, observations and use of QMRA to make pathogen concentrations and risk estimations	***Results:*** Estimations of illnesses from various pathogens show wastewater contributes to the actual cases of diarrheal diseases in Accra. Consumers of raw lettuce could be at the highest risk of infection/illness. This is followed by street food vendors, farmers at various sites, vegetable sellers, children playing around wastewater storage sites and finally harvesters of vegetable. ***Conclusion:*** Need for considering the risk of water/food borne (especially gastrointestinal) illness in Accra in the context of total risk from all possible exposures (i.e. drinking water, recreational water contact, and other contaminated foods apart from the vegetable considered). This facilitates making risk-management decisions that address the greatest risks.

Drechsel and Seidu (2011)	***Objective:*** demonstrate the importance of risk reduction measures in wastewater irrigation considering cost effectiveness and to provide a framework for analyzing pertinent costs and risk parameters ***Methods:*** Combined approach that integrates quantitative microbial risk assessment (QMRA), disability-adjusted life years (DALYs) and cost-effectiveness analysis.	***Results:*** Most practices appear highly cost-effective, although only a few are likely to avert more than 80% of the DALY burden. As compliance will always be a challenge, the results support the need for a multi-barrier risk-management approach that, where possible, combines treatment and non-treatment interventions.

Appendix 2. Laboratory analytical procedures used by IWMI

A2.1 Methods for helminth egg quantification

Several methods are available for the enumeration of Helminth eggs in both liquid and soil samples. Among these are the concentration method (Schwartzbrod 1998) and Ayres and Mara (1996). The Schwartzbrod method (*see details in box 1 below*) is a modified US-EPA method, but the same principle (floatation ⁄ sedimentation) compared with Ayres and Mara (1996). In both methods similar reagents e.g. ZnSO4 solution (specific gravity, d = 1.2), ether or ethyl acetate, detergent solution (e.g. Tween) and a buffer solution are used. However, the buffers are different, acetoacetic buffer (Ayres and Mara) and acid ⁄ alcohol buffer solution (H2SO4 at 0.1 N at 35% ethanol; Schwartzbrod 1998); different centrifugation speeds are used and the ZnSO4 solutions are applied at different stages in both methods.

Schwartzbrod 1998 method for quantification of helminth eggs on lettuce

- About 100 g of lettuce leaves are thoroughly washed in about 1 l of sterile distilled water containing two to three drops of Tween 20.
- The washed leaves are rinsed with sterile distilled water and rinsings added to the washing water in a 2-l container and allowed to stand overnight to enable the eggs to settle completely.
- As much of the supernatant as possible was sucked up and the sediment transferred into 15-ml centrifuge tubes. The 2-l containers are rinsed two to three times with deionised water and the rinses are transferred into the centrifuge tubes. The tubes containing the sediments are then centrifuged at 1450 rpm for 3 min. The sediments in the centrifuge tubes for each sample were pooled into one centrifuge tube and centrifuged again at 1450 rpm for 3 min.
- The supernatant is poured away and the deposit is re-suspended in about 150 ml ZnSO4 solution (specific gravity = 1.2). The mixture is homogenised with a sterile spatula and centrifuged at 1450 rpm. At a specific gravity of 1.2 (ZnSO4), helminth eggs float leaving other sediments at the bottom of the centrifuge tube.
- The ZnSO4 supernatant (containing the eggs) is poured into a 2-l flask anddiluted with at least 1 l of distilled water. This is allowed to stand overnight or for at least 3 h for the eggs to settle again. As much supernatant as possible is sucked up and the deposit is re-suspended by shaking.
- The deposit is then transferred into centrifuge tubes. The 2-l container is rinsed two to three times with de-ionised water and the rinsed water added to the centrifuged tubes and centrifuged at 1600 rpm for 3 min.
- The deposit is pooled into one tube and centrifuged again at the same speed and time. The deposit is re-suspended in 15 ml acid ⁄ alcohol buffer solution (H2SO4 at 0.1 N at 35% ethanol, i.e., 350 ml ethanol and 5.16 ml H2SO4) after sucking up much of the supernatant, about 5 ml ethyl acetate is added.
- The mixture is shaken and the centrifuge tube occasionally opened to let out gas before centrifuging at 2200 rpm for 3 min. After the centrifugation, a diphasic solution (aqueous and lipophilic phase representing the acid ⁄ alcohol and ethyl acetate, respectively) solution is formed.
- With a micropipette, as much of the supernatant as possible (starting from the lipophilic and then the aqueous phase) is sucked up leaving about 1 ml of deposit. The deposit is observed on a Sedgwick-Rafter cell under the microscope and the eggs counted. (for viability continue from here.

A2.2 Viability of helminth eggs

After removing lipophilic and the aqueous phases as described above the following steps should be followed to determine the viability of the eggs:

- Remove the residual ethyl acetate by washing with 10ml 0.1 N H2SO4 and centrifuge at 1450 rpm for 8 monutes
- Remove the supernatant and dilute the deposit with 4 ml 0.1 N H2SO4 and incubate at 26oC for three (3) weeks.

The disadvantage here is the long waiting time (three weeks) before results are known and the preparation has to be closely monitored to ensure that the mixture (containing the eggs) does not dry out during the three weeks incubation period.

Alternatively, the viability of the helminth eggs can be determined using the safranine dying method developed by de Victorica and Galván (2003). The sediment obtained from the last centrifugation (as described above) is stained by adding 2 to 3 drops of safranine O (2.5% in H_2O) to the sediment, and the tubes are then filled with distilled water and centrifuged for 5 minutes at about 2900 rpm. The supernatant is poured off, the pellets are re-suspended with water, and the tubes centrifuged again. This process is repeated 3 times. The sediment is then diluted with 0.1N H_2SO_4 and the total eggs are counted in the Sedgwick-Rafter cell, as described above. If the dye had penetrated the Ascaris eggs they were counted as non-viable. The determination of the viability of helminth eggs using this method is not without problems. Sometimes it is extremely difficult to see, whether the safranine had really penetrated into the egg, or whether it was just sticking to the outer shell, making the egg look colored. This makes it difficult to evaluate the public health implications of the helminth egg populations detected. Also, in some of the cases the eggs are embedded in plant fibers which could have prevented an effective dyeing.