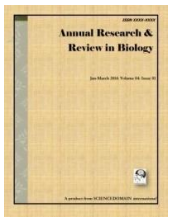

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Potential Links between Irrigation Water Microbiological Quality and Fresh Vegetables Quality in Upper East Region of Ghana Subsistence Farming

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Potential links between irrigation water microbiological quality and fresh vegetables quality in subsistence farming in Tono in the Upper East Region of Ghana were investigated. Water samples from Tono and Nanglakinia dam and six different types of vegetables (collected from Nanglakinia, Bonia, Korania), irrigated with this water were analysed for microbiological qualities. The study was carried out within a month. *Bacillus cereus, Clostridium perfringes, Escherichia coli, Salmonella and Shigella, Streptococcus* spp, *Staphylococcus spp* and Yeast sp. were enumerated using plate count method while Total coliform bacteria were enumerated using Multiple Fermentation Tube Method. In water samples, B*acillus cereus* counts ranged from 34 x 10^5 to 49 x 10^5 cfu/ml , *Staphylococcus* spp. counts ranged from 1 x 10^5 cfu/ml to 26 x 10^5 cfu/ml. *Clostridium perfringes* had bacteria counts 55 x 10^5 cfu/ml to 66 x 10^5 cfu/ml. *Escherichia coli* counts ranged between 51 x 10^5 cfu/ml to 79 x 10^5 cfu/ml. *Salmonella* spp. ranged from 8 x 10^5 cfu/ml to 47 x 10^5 cfu/ml. Yeast sp. also had counts ranging from 21 x 10^5 cfu/ml to 70 x10^5 cfu/ml. Total coliform counts ranged from 460 MPN/100ml to >1100 MPN/100 ml. In the vegetable samples, *Bacillus cereus* counts ranged from 3 x 10^5 cfu/g to 74 x 10^5 cfu/g. *Staphylococcus* spp. counts ranged from 0 to 21

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x 10^5 cfu/g, *Clostridium perfringes* counts ranged from 37 x 10^5 cfu/g to 80 x 10^5 cfu/g. *Escherichia coli* counts ranged from 4 x 10^5 cfu/g to 80 x 10^5 cfu/g. *Salmonella* spp. counts ranged from 0 to 70 x 10^5 cfu/g. Yeast sp. also had counts ranging from 0 to 75 x 10^1 cfu/g. *Streptococcus spp.* was also tested for but there were no bacteria counts recorded. The microbial loads found in the water were similar to those on the fresh produce which showed potential links of the organisms.

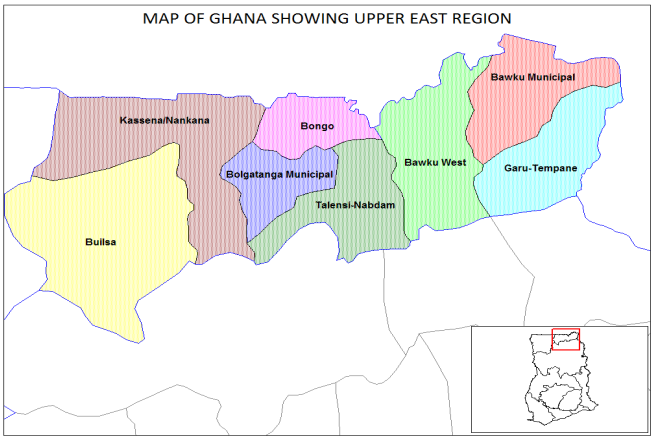
Keywords: Irrigation water; Tono; Nanglakinia; Bonia; Korania; Kasena Nankana District; Upper East Region of Ghana UER; fresh vegetables, subsistence farming; microbes.

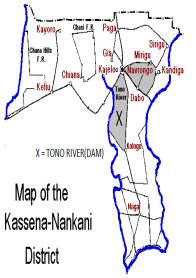
1. INTRODUCTION

Fresh fruits and vegetables are important to the health and well being of man. To lead a healthy lifestyle, it is essential to have a diet that includes fresh and minimally processed fruit and vegetables [1]. Some vegetables and fruits can be consumed raw, some may be eaten cooked, and some must be cooked in order to be edible. Traditional vegetables have high contents of protein, calcium, phosphorus, iron, potassium, carotene and vitamins A, B and C complementing the nutritional value of basic staple foods [2] and fiber that may help protect one from chronic diseases. Vegetables also contain a great variety of phytochemicals, some of which have been claimed to have antioxidant, antibacterial, antifungal, antiviral and anticarcinogenic properties [3,4].

Vegetables are very important nutritionally for contributing vitamins, roughage and flavour to human diets. In every Ghanaian household, practically vegetable crops especially egg plant, okra, onion, pepper, tomato and leafy vegetables such as kontomire, kenaf and African spinach are used in the homes (southern and northern parts respectively). They serve to thicken soups and increase the bulk of stews [5].

Urban vegetable farmers in Ghana use different water sources for irrigation, depending on the location of their farming sites. In many rural areas especially in the Upper East Region between February and April, there is little food around on the farms and people do not have enough money to assure a well-balanced diet [6]. Also, due to rapid population growth, urban development and climate change, the availability and quality of water sources are diminishing [7]. In the dry season, farmers irrigate their vegetables farms using any type of water. Most of the vegetables are produced on open spaces and surface water is most commonly used as it is easily accessible and thus most economical. Farmers use water from streams, storm water drains and gutters to water their farms. The use of adjacent surface water such as from streams, lakes and dams as well as wastewater for irrigation poses potential risks due to the presence of microbial pathogens that have entered the environment via the faeces of infected livestock or human hosts [8]. Low income countries such as Ghana use irrigation water heavily polluted with untreated wastewater [6]. This is mainly due to poor sanitation in urban areas [9]. [10] found that some irrigation water used on urban vegetable farms in Ghana had high levels of microbial contamination that exceeded World Health Organization (WHO) recommendations for unrestricted irrigation [11,12,13] as overhead irrigation is common in Ghana. Increasing contamination of irrigation water sources makes this practice a major risk factor for public health, especially as most vegetables grown are consumed raw. A high level of microbial contamination in the water used for this type of irrigation is therefore raising concerns with regards to public health [14]. Another more attractive option for irrigation is through the use of low cost plastic water tanks to collect rainwater run-off during the rainy season [15]. However, there are risks involved with this method as animal fecal contamination (such as from birds) can still occur. Over the last several years, the detection of outbreaks of food borne illness associated with fresh fruits and vegetables has increased [16]. In the U.S., it is estimated that as many as 76 million people contract some type of food borne illness each year. As a result, over 325,000 are hospitalized and about 5,000 deaths occur. *Salmonella* on tomatoes and cantaloupes, *E. coli* 0157:H7 on lettuce and in apple juice, hepatitis A on strawberries, and Cyclospora on raspberries have shaken consumer confidence in the safety of fruits and vegetables [16]. However, how much food borne illness originates from the water (on the farm)? *No one knows*. This work is therefore to ascertain the potential link of





possible contamination or transfer of pathogenic microbes from the water to the fresh produce on the farm during the irrigation process with the view of advising farmers to desist from using such water sources.

2. MATERIALS AND METHODS

The study was conducted in three farming sites (Nanglakinia, Bonia and Korania) in the Tono Irrigation catchment area in the Kasena Nankana District of the Upper East Region of Ghana.

The principal source of water for the area is the Tono Irrigation Dam and its associated canals. Many animals around this place drink water from the dam and the associated canals. Three samples of irrigated water and six samples of vegetables from each farm were collected into sterile containers between the hours of 9:00 and 11:00 hours GMT and they were immediately transported to the laboratory on ice chest at 4ºC within two hours. Vegetable samples such as African spinach, kenaf and lettuce were collected from the farm together with fruit vegetables such as tomato, pepper, and okra from the environs of the Tono irrigation dam catchment area. Water samples and vegetable samples were collected thrice at different time and were subjected to bacteriological analysis.

Various selective media were prepared following strictly the manufacturer's specifications and subsequently used in the pour plate method. 1ml of dilution 10^{-5} of each sample was transferred into a sterile petri-dish / plate using pipette and about 15 - 20 ml of each medium was poured onto it. The plates were swirled for about 1 minute to ensure thorough mixing of the content. The plates were then left to set, inverted and incubated at 35ºC for 24 hours for bacteria colonies counts and 25ºC- 27ºC for 24 hours for yeast counts.

For Total coliform counts and fecal coliform counts (*E. coli*), multiple fermentation tube method was used. Three tubes of Double Strength Lactose Broth (DSLB) and Six tubes of Single Strength Lactose Broth (SSLB) were prepared following the manufacturer's specifications. Durham tube was inverted into each tube containing the lactose broth. Each tube was labeled according to the amount of water sample that is to be added to it; 10 ml, 1 ml and 0.1 ml respectively. The water samples were mixed using a vortex mixer and 10 ml was added to each of the DSLB tubes and 1ml and 0.1ml were dispensed into each three tubes of the SSLB tubes respectively. The tubes were then incubated at 37ºC for total coliform bacteria and 44ºC for faecal coliform bacteria (*E. coli*) for 24 hours. Each tube was examined after the 24 hours and the number of tubes in each set that have about 10% gas or more were recorded. Most Probable Number (MPN) of coliform in the water samples were then determined by referring to an MPN statistical table.

For the vegetables, 10 g of the vegetables were weighed and macerated into 100 ml of sterile the peptone water and mixed thoroughly using vortex mixer for about 5 minutes to achieve thorough homogenization. The peptone water drained out into centrifuge tubes and centrifuged for (1000 rps) for 5 minutes. The supernatant was discarded and the sediments were mixed thoroughly and used for the analysis. 1 ml of dilution 10^{-5} of each sample was made and was inoculated into culture media for microbial loads using standard pour plate methods and multiple fermentation tube method for fecal coliform

bacteria counts (*E. coli*). The plates were swirled for about 1 minute to ensure thorough mixing of the content. The plates were then left to set, inverted and incubated at 35ºC for 24 hours for bacteria colonies counts, 25ºC- 27ºC for 24 hours for yeast counts and 44ºC for fecal coliform bacteria counts (*E. coli*). The media used to enumerate the micro-organisms were Clostridium perfringes agar media, Mac-conkey agar media, Bismuth sulphite agar media, Bacillus cereus agar media, Staphylococcus agar media, Azide dextrose agar media, Sabouraud dextrose agar and lactose broth.

3. RESULTS AND DISCUSSION

3.1 Results

The fresh vegetable samples collected from three different farms are presented in Table 1. Six different fresh vegetables were collected and these vegetables are found and planted in each farm. Mean microbial counts of water samples used to irrigate the farms is presented in Table 2. These farms are found using the water close to them to irrigate their farms. Mean microbial counts of vegetable samples in farm 1 is shown in Table 3. Table 4 and Table 5 showed the mean microbial counts of vegetable samples in farm 2 and farm 3 respectively. The micro-organisms enumerated in the study which are presented in the tables are total coliform bacterial, *Bacillus Cereus, Salmonella* spp., *Clostridium perfringes, E. coli, Staphylococcus* spp and Yeast spp.

From Table 2, water samples in farm 1 and farm 2 were highly contaminated compare to farm 3. The mean microbial counts for Total Coliform (>1100 MPN/100 ml), *B. cereus* (49 x 10^5 cfu/ml), *Salmonella* spp (47 x 10^5 cfu/ml) and *Staphylococcus* spp (20 x 10^5 cfu/ml) were higher in farm 1 while the mean microbial counts of E. coli (79 MPN/100 ml) was higher in farm 2. In farm 3 Cl. perfringes and Yeast sp had the highest mean counts of 66 x 10^5 cfu/ml and 70 x 10^5 cfu/ml respectively. In Table 3, from farm 1, African spinach had the highest mean counts of *Salmonella* sp (70 x 10^5 cfu/g) and *Staphylococcus* spp (21 x 10^5 cfu/g). kenaf had the highest mean counts of B. cereus (65 x 10^5 cfu/g), pepper had the highest mean counts of Cl. perfringens (80 x 10^5 cfu/g), lettuce had the highest mean counts of *E. coli* (70 x 10^5 cfu/g) and okro had the highest mean counts of yeast (70 x 10^5 cfu/g). Farm 2 in Table 4, tomato had the highest mean counts of *B. cereus* (70 x 10^5 cfu/g), African spinach had the highest mean counts of *E. coli* (80 x 10^5 cfu/g), *Salmonella* (63 x 10^5 cfu/g) and yeast (50 x 10^5 cfu/g). Lettuce had the highest mean counts of Cl. perfringens (73 x 10^5 cfu/g). African spinach, lettuce and kenaf had the highest mean counts of Staphylococcus spp (3 x 10^5 cfu/g). In Table 5 and from farm 3, tomato had the highest mean counts of B. cereus (72 x 10^5 cfu/g), *Salmonella* spp (60 x 10^5 cfu/g), *E. coli* (65 x 10^5 cfu/g) and yeast (75 x 10^5 cfu/g). Lettuce had the highest mean counts of Cl. perfringens (68 x 10^5 cfu/g) while kenaf had the highest mean counts of *Staphylococcus* spp (5 x 10^5 cfu/g).

3.2 Discussion

Most of the pathogenic organisms in water sources were found in the vegetables plant irrigated with this water. This is in agreement with [17] which indicated that pathogenic organisms are one of the main health risks when wastewater is used for irrigation. Washing of human excreta and animals defecating into the water bodies can transfer pathogenic microbes to fresh vegetables. This could subsequently cause diseases in immune-compromised consumers. Irrigated vegetables with contaminated water may be contaminated by *E. coli, Salmonella* spp*., Clostridium perfringes, Bacillus* spp*., Staphylococcus* spp*. and* Yeast through the water used in watering them. This showed that there were possible transfers of these microbes from the water to the vegetables. This was also well established by [18]. Both field and laboratory studies conducted by [17] demonstrated that pathogens present in raw wastewater can survive extended periods of time in soil and on crops, thereby allowing some of these pathogens to survive harvesting and subsequent processes such as packaging to finally reach the consumer. The results revealed the presence of *Bacillus cereus, Salmonella* spp*., Clostridium perfringes, Escherichia coli,* Yeast sp., and *Staphylococcus spp.* in both the water samples and vegetable samples. According to the study of [19], similar results were obtained in which there were presence of E. coli and some other microbes in both water and fresh produce. Farm 1 had the highest level of contamination followed by farm 2 whereas farm 3 had the lowest. This could be as a result of the nature and location of the water source. It is also known that farm 1 is closed to the Tono irrigation dam and located within the catchment area. Also Farm 1 is a large dugout in the area where so many activities are undertaken. People do fishing in the water and

the same water is also used for irrigating vegetable farms nearby. Animals are also allowed to drink from the water and pigs and children from around the community swim in it. However, a recent study indicated that *Salmonella sp*. present on the surface of plants cannot only survive for extended periods [20] but might overcome innate immune response of plants [21] and actively enter the plant via the stomata as was demonstrated for lettuce [22]. According to [23] the survival of *Bacillus* spp. depends on several factors such as resistance to new environments, nature of the organism and their ability to form spores. Among all the vegetables under study, pepper recorded the lowest counts; this could be due to some antibacterial properties that inhibit the establishment of these pathogens. Meanwhile, the leafy vegetables recorded the highest *E. coli* counts in farm 1. This could be as a result of human and animal activities within the area. Defecation in and around farm 1 and 2 may transfer fecal coliform bacteria (*E. coli*) on to the leafy vegetables and this leafy vegetable may absorb this bacteria into its body as in case of lettuce. From the study it was observed that the organisms that were detected in the water sample were also found on the vegetable samples. This observation then suggested that there is a possible transfer of pathogenic microbes from the water samples to the vegetables as it is used in watering the vegetables.

Table 1. Vegetable samples collected

No.	Local name	Common name	Scientific name	Family name
1.	Kanzaga	Kenaf	*Hibiscus cannabinus*	Malvaceae
2.	Aleefi	African spinach	*Amanranthus cruentus*	Amaranthaceae
3.	Lettuce	Lettuce	*Lactuca sativa*	Asteraceae
4.	Kamantos	Tomato	*Lyco-persicon esculentum*	Solanaceae
5.	Nanzua	Pepper	*Capsicum annuum*	Solanaceae
6.	Mahna	Okro	*Abelmoschus esculentus L.*	Malvaceae

Table 2. Mean microbial counts in water samples from the three farms

Water samples	Total coliform MPN/100 ml	*B. cereus* x 10^5 cfu/ml	*Salmonella* spp. x 10^5 cfu/ml	*Cl. perfringes* x 10^5 cfu/ml	*E. coli* MPN/100 ml	Yeast spp x 10^5cfu/ml	*Staphylococcus* spp. x 10^5 cfu/ml
F1W1	>1100	49	47	55	74	69	26
F2W2	1100	34	38	64	79	21	3
F3W3	460	40	8	66	51	70	1

Table 3. Mean microbial counts in the vegetable samples from farm 1

Samples	*B. cereus* x 10^5 cfu/g	*Salmonella* spp.x 10^5 cfu/g	*Cl. perfringes* x 10^5 cfu/g	*E. coli* x 10^5 cfu/g	Yeast spp x 10^5 cfu/g	*Staphylococcus* spp. x 10^5 cfu/g
Tomato	34	65	37	64	62	11
African spinach	62	70	60	68	55	21
Pepper	32	18	80	60	56	5
Lettuce	60	64	75	70	61	20
Kenaf	65	60	68	17	64	8
Okro	64	12	52	57	70	13

Table 4. Mean microbial counts in the vegetable samples from farm 2

Samples	*B. cereus* x 10^5 cfu/g	*Salmonella* spp. x 10^5 cfu/g	*Cl. perfringes* x 10^5 cfu/g	*E. coli* x 10^5 cfu/g	Yeast spp x 10^5 cfu/g	*Staphylococcus* spp. x 10^5 cfu/g
Tomato	70	59	61	42	40	2
African spinach	53	63	11	80	50	3
Pepper	3	0	70	21	0	0
Lettuce	24	36	73	30	6	3
Kenaf	60	57	52	34	38	3
Okro	53	1	66	58	32	2

Table 5. Mean microbial counts in the vegetable samples from farm 3

Samples	*B. cereus* x 10^5 cfu/g	*Salmonella* spp x 10^5 cfu/g	*Cl. perfringes* x 10^5 cfu/g	*E. coli* x 10^5 cfu/g	Yeast x 10^5 cfu/g	*Staphylococcus* spp. x 10^5 cfu/g
Tomato	72	60	60	65	75	1
African spinach	58	41	55	61	68	1
Pepper	13	1	51	8	65	2
Lettuce	30	42	68	36	72	3
Kenaf	7	5	22	7	60	5
Okro	50	3	19	4	55	1

4. CONCLUSION

The study revealed that pathogenic organisms from water sources which were used to irrigate the farms were transferred and found in the vegetable plants. Hence pathogenic organisms from water sources can be linked to microbial quality of vegetables plants. The presence of potential food borne pathogens in irrigated vegetables raises food borne safety concern and threat to consumers' health.

5. RECOMMENDATIONS

Farmers should endeavour to minimize wild and domestic animals from entering water bodies meant for irrigation purposes.

Consumers should wash vegetables and fruits well with potable water and ensure proper cooking methods.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

Results showing multiple tube fermentation test for total coliform in the water samples

Water sample	Appearance	Gas	Number of tubes									Reading	MPN per 100 ml	Range 95% probability	
			Double strength			Single strength									
			10 ml			1 ml			0.1 ml						
			1	2	3	1	2	3	1	2	3			Lower	Upper
F_1W	Slightly turbid	Present										3-3-3	>1100	-	-
		Absent													
F_2W	Clear	Present										3-3-2	1100	150	4800
		Absent													
F_3W	Clear	Present										3-3-1	460	71	2400
		Absent													

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