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Assessment of Bacteria and Heavy Metals Contamination in Lettuce at Farm Gate and Market in the Accra Metropolis

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Authors' contributions

This work was carried out in collaboration between all authors. Each Author contributed to write, and edit the drafted manuscript, as well as manage literature search and perform statistical analysis of the study.

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ABSTRACT

The bacteria and heavy metals contamination of lettuce from two vegetable growing farms irrigated with either stored municipal, stream or polluted drain water were assessed on farm and in the market. A total of 120 irrigated lettuce samples consisting of sixty samples from each farm and the market were collected. All samples were analysed using standard methods. The total coliform levels of lettuce from the farm ranged from 5.63 to 9.38 log MPN/100 g, 5.32 to 10.38 log MPN/100 g, and 6.38 to 10.38 log MPN/100 g when irrigated with stored municipal water, stream water and drain water, respectively. Irrespective of the irrigation water used on the lettuce, the total and faecal coliform levels were above the ICMSF recommended levels of 1×10^{3} 100 g^{-1}. Significant difference ($p=0.05$) was observed between the lettuce irrigated with municipal water and drain water. Thirteen gram-negative bacteria species were identified for irrigated lettuce both on farm and at the market. One bacterium of pathologenic concern, *Klebsiella pneumoniae* occurred to a lesser extent, on both farm and market-derived lettuce depending on the source of irrigation water. The heavy metals concentration in all lettuce samples examined were far below FAO/WHO recommended

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levels for safe vegetables consumption. It is therefore recommended that lettuce bought from farm gates or markets be washed properly to reduce the bacteria contamination before it is consumed.

Keywords: Irrigation water; heavy metal; total coliform; faecal coliform.

1. INTRODUCTION

Urban Agriculture is one of the ancient practices that have gained recognition in the developing world over the years. The rapid increase in urban population and the associated demand for food and jobs, have introduced various agricultural production systems in and around major cities of West Africa. In Ghana, this agricultural production system popularly known as urban and peri – urban agriculture (PUA) is normally established throughout the year [1]. Their production requires constant rainfall or irrigation for a good yield. However, the erratic rainfall pattern and the unavailability of potable water have forced farmers to use waste water for irrigation. Many countries including some developed countries reuse wastewater for vegetable production. Different water sources such as municipal water stored in shallow wells and contaminated stream water are used for vegetable production in some vegetable growing sites in Accra. Wastewater, depending on the source, may contain high concentrations of excreted pathogens: bacteria, viruses, protozoa, and the helminthes (worms) that cause diseases to man. It is also noted that, the transmission of faecal bacterial infections like *Escherichia coli, Salmonella* and other gram negative bacteria to farmers, consumers and those living close to wastewater irrigated fields is a major human health-related risk which has been established based on epidemiological evidence [2,3]. In addition, heavy metals tend to accumulate in the soil and with time becomes bioavailable to crops with the continuous use of irrigation wastewater containing them [4]. Fresh vegetables have been implicated as vehicles for the transmission of microbial foodborne disease worldwide [5]. According to [6], wastewater used in the irrigation of lettuce at Dzorwulu and Korle-Bu in the Accra metropolis contains unacceptably high loads of faecal coliform bacteria above the WHO recommended levels. The qualities of water used in vegetable irrigation consequently have implications on the quality of vegetables produced. Additional sources of contamination, especially, bacteria may arise with harvesting, transportation and post-harvest handling of the harvested vegetables. Vegetables are usually consumed in relatively small quantities as a side-dish or as a relish with staples [7]. The objective of this paper is to assess the heavy metals and bacterial contamination of lettuce irrigated with three different water sources sampled from the farms and Markets in the Accra Metropolis.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in two urban vegetable farming sites, Dzorwulu and Korle-Bu in Accra, Ghana. Dzorwulu is one of the major vegetable growing areas in the Accra metropolis. It has a total land area of about 12 ha [8]. The sources of water for irrigation are municipal water stored in shallow wells. Water from the Onyasia stream which is a tributary of the Odaw River was also used. This stream is polluted with wastewater generated from neighbouring urban settlements. Korle-Bu is another major vegetable growing area in Accra with a total land of about 10 ha [9]. The main source of irrigation water is drain water from houses of hospital staffs and surrounding communities, channeled into dugouts. Different types of vegetables are cultivated at these sites but for purpose of this study lettuce was used. According to [10], lettuce is eaten raw, widely cultivated and the most contaminated. All these vegetable farm sites use poultry manure as a source of nutrient for the vegetables.

2.2 Sampling and Data Collection

A total of 120 lettuce samples consisting of lettuce irrigated with three water sources: municipal water stored in shallow wells, stream and drain water were collected. Sixty samples each were collected from the two vegetable growing sites and same quantity from one major market (Abgogbloshie) in Accra Ghana during 2007 and 2008. On the farm, lettuce samples were collected just before they were harvested for sale at the market. In the market, displayed lettuce irrigated with the three water sources were identified and samples collected and analysed monthly. All the 120 lettuce samples were analysed for bacteriological quality. Thirty two samples consisting of six lettuces each from the different water sources were analysed for heavy metal concentrations to determine their safety for human consumption.

2.2.1 Quantification of total and faecal coliform on lettuce samples

A total of 120 lettuce samples were analysed for bacteriological quality. The Most Probable Number (MPN) method was used to determine the number of total and faecal coliform populations on the samples. Twenty grams (20 g) each of samples was weighed and carefully cut into 180 ml of phosphate-buffered saline solution and rinsed vigorously. A further ten-fold serial dilution and a set of triplicate tubes containing sterile MacConkey broth (MERCK, Darmstadt, Germany) were inoculated from each dilution and incubated at 37ºC for total coliform and 44.5ºC for faecal coliform for 24 to 48 hours respectively. The number and distribution of positive tubes (acid and/or gas production) from incubated samples were used to obtain the population of coliform bacteria from MPN Table [11].

2.2.2 Isolation of pure cultures of bacteria and their identification

One positive tube of total coliform from all the samples were randomly selected after MPN value had been determined. A loopful of the bacteria culture from the selected positive tubes were then inoculated onto nutrient agar slants and incubated at 35-37ºC for 24-48 h. These were later stored for further biochemical tests.

Bacteria from the slants were later suspended into nutrient broth to resuscitate them and incubated at 35-37ºC for 18-24 h. Sterile inoculation loop was then used to transfer a loopful of the bacteria culture onto selective media plates made up of MacConkey agar and incubated at 35-37ºC for the isolation of gram negative bacteria present [8].

2.2.2.1 Determination of isolates characteristics

The importance of obtaining pure isolates from a mixed species culture before proceeding to characterize a species has been highly emphasised in the Bergey's manual of systematic bacteriology [12]. In view of this, techniques from the book were strictly adhered to.

In colony morphological characterization, the size, elevations, forms and margins of bacterial colonies and their pigmentation (lactose fermentors or non-lactose fermentors) on the media were observed. Bacterial colonies differing in their morphological characteristics were randomly selected and purified for at least three times. The colonies were purified by picking a bacterial colony which appeared to be composed of one cell type using an inoculating loop. This was then streaked onto the selective agar plates and was incubated at 37ºC for 18-24 h. After incubation, re-streaking of isolated colony was carried out. This was repeated twice in order to obtain identical colonies. The purity of the bacterial colonies was then confirmed by Gram staining.

2.2.2.2 Biochemical characterization of isolated colonies

A total of thirty pure colonies were randomly selected from all the lettuce samples for identification using the Analytical Profile Index (API) 20E system (BioMerieux sa 69280 Marcy-l'Etiole/France or bioMerieux, Inc. Hazelwood, MO). This is an identification system for Enterobacteriaceae and other non-fastidious Gram-negative rods which use 23 standardized and miniaturized biochemical tests and a database from isolated colonies of bacteria on plating medium. The manufacturer's recommended procedures were strictly adhered to and the interpretation of the results were done using the API Analytical Profile Index [13].

2.2.3 Heavy metal analysis

The lettuces were oven dried for 2 days at a temperature of 80ºC after which they were ground and homogenized and finally sieved to remove all debris. An amount of 0.2 g each of the sieved lettuce samples was weighed into Teflon tubes. A volume of 2 mL concentrated Nitric acid (HNO_3) was added to the tubes. The tubes were closed and placed in stainless steel bombs. The bombs were then placed on a hot plate and heated at 110ºC for 1 h and then 150ºC for 3 h. The bombs were opened after they had been allowed to cool to room temperature and the pressure released. The digested samples were then transferred into a polypropylene graduated tubes rinsing three times with deionised water. The rinsing water was also added to the polypropylene tubes. These solutions were diluted to the 25 mL mark with deionised water and mixed thoroughly. The particles were allowed to settle overnight and samples stored for analysis.

The concentrations of Fe, Mn, Cu, Zn, Pb and Cd were determined using AAS (Atomic Absorption Spectrophotometer) Unicam 699 after double distilled water has been used to zero the instrument, the concentrations of Fe, Mn, Cu, Zn, Pb and Cd in the blank were also measured and then followed by the determination of the concentrations of Fe, Mn, Cu, Zn, Pb and Cd in the digested samples.

2.3 Data Analysis

SPSS for windows version 15 (SPSS Inc. Chicargo IL, USA) was used to analyse the data. Faecal and total coliform populations obtained from MPN were normalized by log transformation before analysis of variance (ANOVA) was performed. Significant differences in both the bacteriological and heavy metal concentrations were analysed by the multiple comparisons procedure of Least Significance Difference (LSD) using a level of significance at $p<0.05$.

3. RESULTS

3.1 Total and Faecal Coliform Levels on Lettuce from the Three Water Sources

The bacteriological quality of lettuce from the different irrigation water sources showed considerable variations in the total and faecal coliform levels for both the on-farm and market-samples with total coliform levels being higher than the faecal coliform. The total coliform levels of lettuce from the farm ranged from 5.63 to 9.38 log MPN/100 g, 5.32 to 10.38 log MPN/100 g, and 6.38 to 10.38 log MPN/100 g for municipal water stored in shallow well, stream and drain water, respectively. The mean total coliform levels determined in the municipal water stored in shallow well-irrigated lettuce were observed to be the lowest and significantly different ($P<0.05$) from the highest levels recorded for the drain-irrigated lettuce (Fig. 1). A similar trend of total coliform contamination was observed for the market samples, with municipal irrigated lettuce being the lowest (7.14 log MPN/100 g) and drain irrigated lettuce (8.35 log MPN/100 g) the highest. The levels of total and faecal coliform on lettuce samples irrigated with the three water sources were slightly higher in the market samples than from the farm (Fig 2). The differences observed in the levels at the farm and the market were however not significant.

3.1.1 Bacteria flora on irrigated lettuce

Thirteen gram-negative bacteria species were identified for the isolates that were obtained from lettuce both on farm and at the market for samples irrigated with the three water sources.

The results showed a total of 10 bacterial species on lettuce sampled on farm, of which only one species, *Citrobacter freundii* was found on lettuce irrigated with all the three water sources. Furthermore, *Chrysemonas luteola* was present on lettuce irrigated with municipal and drain water but absent on stream irrigated lettuce samples. Chromobacterium violaceum and Pseudomonas aeruginosa occurred on only the municipal -irrigated samples, *Enterobacter agglomerans, Erwinia amylovora*. *Serratia plymuthica* and *Serratia rubidaea* occurred on

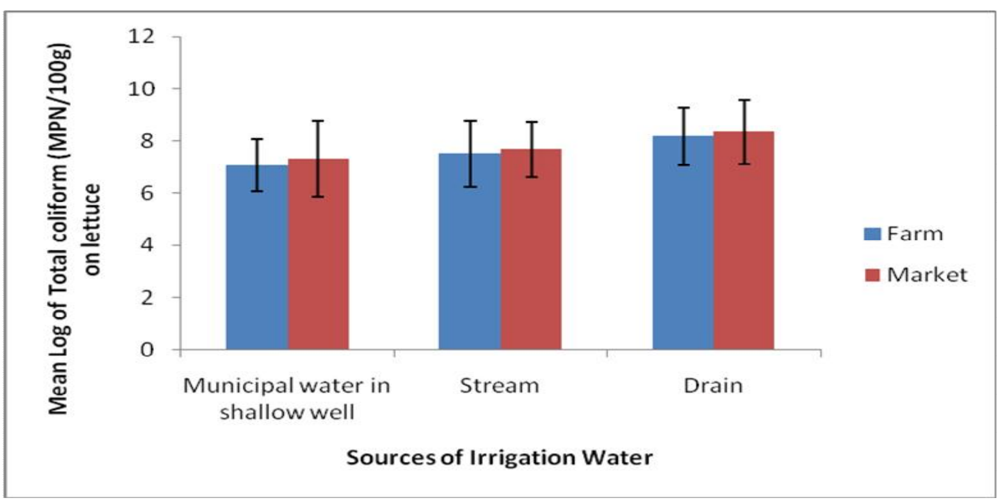

Fig. 1. Mean Log of total coliform count in irrigated lettuce on farm and market

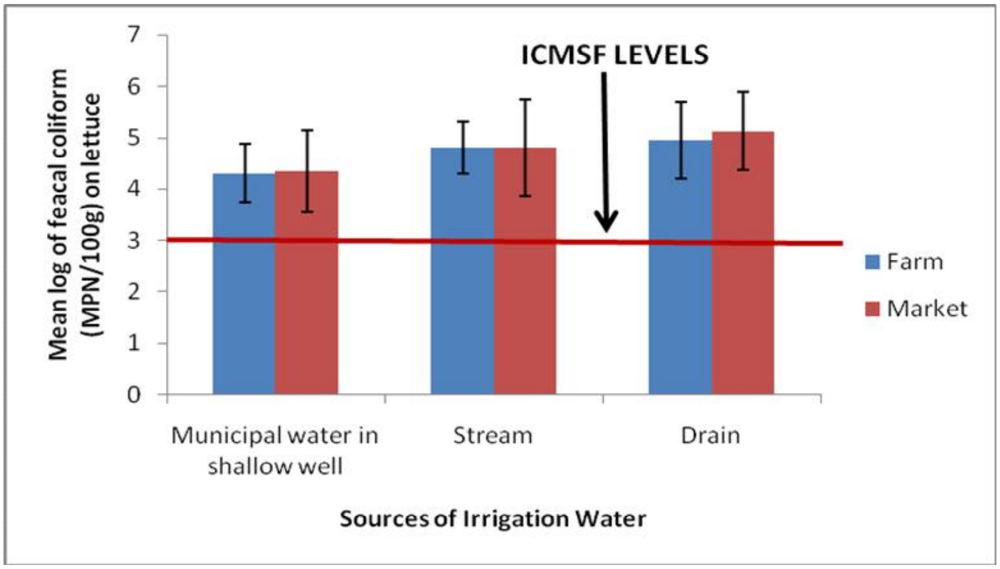

Fig. 2. Log mean of faecal coliform count in irrigated lettuce on farm and market

the Stream-irrigated sample and *Klebsiella pneumoniae* and *Pseudomonas putida* on only the Drain-water-irrigated lettuce.

Overall, the Municipal-irrigated lettuce was contaminated with four bacterial species, *C. violaceum, P. aeruginosa, C. freundii* and *Chrysemonas luteola*. The stream-irrigated lettuce by five species, *C. freundii, Enterobacter agglomerans, E. amylovora*. *Serratia plymuthica* and *S. rubidaea.* Also four bacterial spp., *C. luteola, C. freundii, K. pneumonia* and *Pseudomonas putida* for the Drain-water-irrigated samples all on the farm.

A fewer number of bacteria species (five) were however, isolated from the market samples. From these samples no single bacterial species of the five occurred on all three irrigated lettuce samples as was observed in the farm samples.

While *Enterobacter sakazakii* occurred on the municipal water and Stream-water-irrigated lettuce, *Proteus mirabilis* was isolated from the stream and drain-water-irrigated lettuce.

For the market samples, *C. freundii, E. sakazakii* and *Enterobacter cloacae* were bacterial species isolated on the municipal-water-irrigated samples. In addition, *K. pneumonia* was isolated from lettuce sampled from the farm and market which were irrigated with drain water.

3.2 Heavy Metal Analysis in Irrigated Lettuce

The results from the heavy metal analysis showed varied concentrations of heavy metals in lettuce irrigated with the three water sources. However the differences between concentrations of the metals for the three water sources were not significant (Table 1). Also, concentrations of the various metals in lettuce irrigated with different water sources sampled were statistically similar for the on-farm and market samples ($p>0.05$). Lead and Cadmium levels in all the samples were below detection limit, while concentrations of the metals investigated on the lettuce were far below FAO/WHO recommended maximum levels for vegetables.

4. DISCUSSION

Irrespective of the source of irrigation water used on the lettuce crops, total and faecal coliform levels were found to be above the International Commission on Microbiological Specification for Food (ICMSF) recommended level of $1x10^3$ 100 g^{-1} (Log 3 100 g^-1). High prevalence of coliform on lettuce may indicate irrigation as a possible source of food-borne illness since it is eaten uncooked. It is possible that the means of water storage prior to use and the watering cans used for irrigation contributed to the microbial contamination of the lettuce. Also, splashing of contaminated soil onto the crop during irrigation as observed on the field could increase the contamination level on the lettuce.

Table 1. Concentrations of heavy metals in lettuce irrigated with different water sources both on farm and market

Irrigated lettuce source	Heavy metals	Farm		Market		Recommended levels (mg/kg)[a]
		Range (mg/kg)	Mean	Range (mg/kg)	Mean	
Lettuce irrigated with municipal water in well	Fe	167-178	170 (±2.8)	148.25-175.25	168.85 (±3.3)	**425.5**
	Mn	57.75-76.75	62.85 (±3.3)	49.50-69.00	60.28 (±5.2)	**500**
	Cu	7.50-8.03	7.70 (±0.17)	7.48-7.73	7.61 (±0.76)	**73.3**
	Zn	34.00-58.50	44.12 (±6.38)	29.50-58.00	40.79 (±7.22)	**100**
Lettuce irrigated with stream	Fe	157.00-192.50	171.40 (±12.1)	157.25-175.75	165.02 (±5.9)	**425.5**
	Mn	55-76	61.72 (±6.2)	54.75-63.75	58.18 (±3.5)	**500**
	Cu	7.33-9.23	7.91 (±0.62	7.05-9.03	8.27 (±0.64)	**73.3**
	Zn	31.50-48.50	38.07 (±5.26)	29-46	35.26 (±5.39)	**100**
Lettuce irrigated with drain water	Fe	162-190	171.40 (±7.6)	163-176	169.21 (±5.1)	**425.5**
	Mn	61.50-78.25	68.67 (±6.2)	60.25-74.50	67.71 (±6.4)	**500**
	Cu	6.03-8.33	7.08 (±0.76)	6.95-8.80	7.40 (±0.56)	**73.3**
	Zn	32-52	38.79 (±6.14)	23.88-46.75	37.44 (±7.45)	**100**

The faecal coliform levels were significantly lower in lettuce irrigated with municipal water stored in well than those irrigated with stream and drain sources. This agrees with work done by [14] where lettuce irrigated with municipal water had lower coliform levels than those irrigated with stream and drain water. According to [6], irrigation water from these sources had faecal coliform counts above the WHO recommended level of 1 x 10^3/100 ml. In a related study in Kumasi, for 60% to 70% of the sampling periods for lettuce irrigated with municipal water, significantly lower coliform counts were obtained than the wastewater used. The relatively lower coliform counts in the lettuce irrigated with stored municipal water in this study could be attributed to the low coliform levels in the municipal water as a result of runoff from nearby farmlands into the well. This suggests that the type of water used in irrigation contributes so much to the microbial contamination of the crops irrigated. The continuous application and mode of poultry manure application (broadcasting) to the soil for lettuce cultivation could also contribute to the lettuce contamination [15,10].

The bacterial count difference in the lettuce samples from the farm and the market indicates that during transporting and display of lettuce on the shelves at the market there is an additional bacteria load on the lettuce although as our results showed, may not be that significant. The contamination could have come from the containers used in the transportation and handling before, during and after display on the shelves and tables for sale.

All the bacteria isolated from the lettuce samples were opportunistic pathogens in that they can cause diseases in humans especially for individuals with compromised immune systems. *Citrobacter freundii* was isolated in all the lettuce on-farm irrespective of the irrigation water used .This bacterial species is known to be present in soil, water, sewage, food and the intestinal tracts of animals and humans [16]. Transfers of pathogens onto the lettuce were more likely to come from the surrounding soil as well as the irrigation water. The presence of *Klebsiella pneumoniae and C. freundii* on lettuce from both the on-farm and market samples irrigated with drain and municipal water could have come from either the water source or the soil, or both used for growing the lettuce. [6] reported the occurrence of *K. pneumoniae* in drain water used for irrigating vegetable. The reduction of bacterial species from ten to five in the farm and market lettuce samples, respectively, could be attributed to structural or metabolic injuries as a result of the change in environmental conditions. *Chromobacterium violaceum, Chrysemonas luteola* and *Pseudomonas aeruginosa* in the farm lettuce irrigated with municipal water and their absence in the market lettuce samples could be attributed to stress and destruction of the bacteria as a result of change in environmental conditions. Moisture availability and pH changes according to [17] can change microbial community composition on fresh produce. Similar reasons could be attributed for the occurrence of *Chrysemonas luteola, C. freundii, Enterobacter agglomerans, K. pneumonia, Pseudomonas putida, Serratia plymuthica* and *Serratia rubidaea* in farm lettuce irrigated with stream and drain water and their absence in the market samples. Subsequently, the presence of bacteria species such as *Proteus mirabilis*, *Enterobacter sakazakii* and *Enterobacter cloacae* in the market lettuce samples and their absence in the farm samples indicate some form of contamination through post handling as vegetables are transported from the farm gate to the market. These bacteria were observed in lettuce and other vegetables in a similar study carried out in Accra [18]. The concentrations of the metals were in the order of Fe > Mn > Zn > Cu in all the lettuce samples. The results also indicate that, there was no additional heavy metal contamination on lettuce at the market which could pose health risk to consumers. According to [19], the main sources of heavy metals on vegetable crops are their growth media. The study showed that the heavy metal concentrations in all the lettuce sampled on the farm and market were within safe limits. Furthermore, the non-detection of lead and Cadmium in the samples makes them suitable for consumption as far as heavy metals are concerned.

5. CONCLUSION

The bacteriological quality of lettuce sampled both on-farm and market place were above the ICMSF recommended levels of 1 x 10^3 100 g^{-1} (Log 3 100 $g^{-}1$). The concentration of the heavy metals investigated on the lettuce samples were far below FAO/WHO recommended maximum levels for safe vegetables consumption. It is therefore recommended that lettuce bought from farm gate or market be washed properly to reduce the bacterial contamination before they are consumed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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