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Bacterial Contamination Levels of Lettuce Irrigated with Waste Water in the Kumasi Metropolis

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Abstract

Consumption of vegetables contaminated with pathogenic micro organisms, particularly, in areas where urban waste water is used to irrigate vegetable crops is suspected to be a factor in outbreaks of some public health diseases. The microbiological quality of lettuce on three farms where waste water is used in the Kumasi Metropolis in Ghana was therefore studied for their Total colifoms, faecal coliforms, enterococci, *E. coli* and *Salmonella* CFU levels using standard methods. Total coliforms on the lettuce varied from 4.93×10^4CFU to 6.17×10^4CFU. Faecal coliforms ranged from 3.48×10^3CFU to 4.66×10^4CFU and *E. coli* 2.98×10^3CFU to 3.86×10^4CFU. *Samonella* and enterococci levels ranged from 2.50×10^2CFU to 2.72×10^2CFU and 0.68×10^0CFU to 2.05×10^0CFU respectively. In most cases the highest bacterial contamination was associated with lettuce grown at Atonsu and the lowest was at Karikari farms located within the Kumasi Metropolis. The differences in total colifom counts at the two locations were significant ($P < 0.001$). Differences in bacteria counts for faecal colifoms ($P < 0.000$) and *E. coli* ($P < 0.000$) were significantly higher than counts associated with the other bacteria from all the three farms. In general, bacterial counts on farm lettuce exceeded the recommended World Health Organization (WHO) and International Commission on Microbiological Specifications for Food (ICMSF) standards of 10^3. Wastewater use on farms therefore, could be the main contributor to lettuce contamination and outbreak of communicable diseases. Education on use of effective de-contamination methods before eating will help reduce the risk associated with the consumption of such contaminated vegetables.

Key Words: lettuce, contamination, bacteria, Kumasi, Ghana.

1.0 Introduction

Lettuce (*Lactucasativa*) is a plant that is usually eaten raw. Its cultivation takes about a month and it is ready for the market. The vegetable is eaten raw especially when prepared in the form of salad. It has high nutritional content which is very good for human consumption and the general upkeep and well-being of the human body. Most people in the Kumasi metropolis enjoy salad and with the upsurge of fast foods, lettuce can never be done away with. What comes to mind is, are we really enjoying lettuce wholesomely or consuming other harmful things into our bodies?

Urbanization in the developing world is proving to be one of the greatest challenges of the 21st century with an annual growth rate of 5.8% in Sub-Saharan Africa whilst in Ghana, the urban population is growing at an estimated annual rate of about 2.3%, with only 4-5% of the population linked with sewage systems and sewerage treatment plants (World Bank, 2011)

Urbanization coupled with population growth and increase in food demand especially perishable vegetables in major Ghanaian cities has resulted in increased demand for resources such as land and water. This demand is not seasonal and therefore necessitates all year round production approach that is heavily dependent on wastewater use. Studies in Kumasi by Keraita *et al.,* (2003a) have shown that the microbiological contaminants in irrigation water sources in most cases exceed the WHO (1989) guidelines significantly. Pearlier researchers have shown that bacterial contamination levels on lettuce at the three sites namely - Atonsu, Gyinyaase and Karikari farms which was chosen for the study was very high. Other sources of potential health risk are the use of organic manure especially poultry litter *(Amoah et al., 2005)*, methods of transportation of produce to the markets, and handling at the markets and consumption points *(Drechsel et al., 2000; Sonou, 2001*). Public health concerns due to possible crop contamination with pathogens such as *Escherichia coli* or Thermotolerant coliform and Salmonella, among others especially where vegetables are eaten uncooked have therefore been raised by *Sonou, 2001*and *Amoah et al., 2006.*

In spite of the associated health risks, urban and peri-urban farming contributes significantly to some of the millennium development goals relating to poverty alleviation, job creation employment and food security to the extent that in Kumasi, this farming practice has contributed remarkably to food supply and is an important source of livelihood not only for the farmers but also for vegetable sellers and others in the post-harvest chain *(Danso et al., 2002).*

Pescod, 1992 viewed that as adequate wastewater treatment infrastructure is not likely in the near future and wastewater continues to play a major aspect of irrigated urban agriculture, with adequate treatment to reduce health risks, wastewater use for irrigation is a realistic policy option. Though efforts have been made towards interventions to produce safer vegetables and reduce health risks associated with the production and consumption of vegetables along the production-consumer chain in Ghana, most of these studies ended at the market (whole sale and retail) with little situational analyses on street foods and associated production-consumer health risk factors *(Armar-Klemesu et al., 1998; Sonou, 2001; Amoah et al., 2007).*

This study was designed to assess indicator bacterial numbers (Total coliform,Faecal coliform, *Salmonella, Escherichia coli* and *Enterococci*) on lettuce farms.

1.1 General Objectives

The study assessed the extent of bacterial contamination of waste water irrigated lettuce on-farm by checking the level of contamination at the production site. Since lettuce is highly perishable and usually consumed raw, producers as well as consumers and sellers are highly susceptible to endemic parasitic diseases caused by these microorganisms.

1.1.1 Specific Objectives

1. To determine faecal contamination levels in lettuce with thermo-tolerant coliforms, salmonella enterococci and at selected production sites (farms) within urban areas in Kumasi.

2. To identify and assess the risk factors associated with faecal contamination of lettuce selected at production sites (farms) within urban areas in Kumasi.

3. To assess the presence and level of *salmonella* in lettuce at selected production sites (farms) within urban area in Kumasi.

1.2 Methodology

1.2.1 The Study Area

The Kumasi Metropolis is centrally located in Ghana and is the second largest city with a population of 1,517,000 and annual growth rate 2.6 %.(Ghana statistical service, 2010). Kumasi is located within moist semi-deciduous forest vegetation. The climate of the area is tropical maritime which is characterized by wet and dry seasons. The major rainfall season occurs between March and September peaking in June and August. November to March is the main dry season. The soils are well drained, porous and loamy. Agriculture and trading are the backbone of the region's economy.

1.2.2 Sampling Sites

Three different farm sites were selected for the study. These are Karikari farms, Gyinyaase and Atonsu all in the Kumasi metropolis.

The Gyinyaase Farm is one of the largest urban vegetable-farming sites in Kumasi. It is located next to and behind Kumasi High School. This site has a diversity of crops and farmers here practise organic and inorganic farming. Shallow and hand dug wells located along the ridges on the beds and at convenient points on the farm are used in irrigating the crops. Karikari Farms is located a few meters away from Karikari poultry farm at Gyinyaase. This site is low land area with water available throughout the year. Type of crops grown includes carrots, green pepper and lettuce. Irrigation water sources include hand dug shallow wells which are shared by all farmers. Irrigation is usually by direct application of water over crops using watering cans. The Atonsu Farm is located at some meters away from the Kumasi High School. Farmers at this site grow quite a number of vegetables. These include cabbage, green pepper, carrots and lettuce. Farmers at this site use poultry droppings mainly as manure to enrich the soil. Pipe borne water is used to irrigate the crops at this site.

1.2.3 Lettuce Sampling

During sampling of lettuce, a bed was selected at random on each site and three lettuce heads were harvested from top to middle and bottom of the bed. Each sample was immediately placed separately into a labelled sterile plastic bag without washing. Samples were then transported in a cool box to the KNUST Microbiology Laboratory.

Plate 1 Miss Kwarteng Abena Serwaa, harvesting lettuce samples from the Atonsu farm near the waste water source for laboratory assessment of microbial contamination levels.

1.3 Microbiological analysis

Thermo tolerant coliforms were estimated using a three-tube Most Probable Number (MPN) method according to standard procedures (Anon, 1992). Ten grams of the lettuce sample was placed in a stomacher bag and pulsified in 90 ml of 0.9 % NaCl MQ-water for 30 seconds using a pulsifier (PUL 100E; Stuart Scientific Co. Ltd, U.K). Serial dilutions of 10^{-1} to 10^{-11} were prepared from 1 ml of the stomacher bag content. One millilitre of each dilution was inoculated in triplicate into 5 ml of mineral modified Glutamate medium. Tubes showing acid and gas production after incubation for 24 h at 44°C were confirmed by transferring a drop of liquid from positive tubes into 5 ml test tube of tryptone water and incubated at 44^{o}C for 24 h. A drop of Kovacs' reagent was then added to the tube of tryptone water. All tubes showing a red ring colour development after gentle agitation were recorded as positive for thermotolerant coliforms. Estimated counts were obtained from MPN tables (APHA-AWWA-WEF, 2001) and results expressed on per gram wet weight for lettuce and 100 ml of waste water supernatant.

Enterococci were enumerated by placing 1 ml volumes of the same serial dilutions prepared for the thermotolerant coliforms directly onto set plates of Slanetz and Bartley agar. These were allowed to dry and then incubated for 4 hr at 37^{o}C and for 44 h at 44^{o}C. Red, maroon, or pink colonies were counted as presumptive enterococci. Presumptive colonies were confirmed on MacConkey No. 2 (Scharlaw 02-120). All counts were expressed as colony forming units (cfu) per gram wet weight for lettuce leaves and per 100 ml for water. *Salmonella* was enumerated by the MPN technique (Anon, 1992) using Buffered Peptone water as pre-enrichment and selenite broth as enrichment medium and sub cultured on modified brilliant green agar (Cogan *et al.,* 1999).Laboratory data were analysed using Statistical Package for Social Scientists Windows Version 16.0.

1.4 Results

Ranges of Bacterial numbers (100-1 ml) from the three sampling sites in Kumasi Metropolis

Lettuce leaves sampled from the three different sampling sites shows various degree of contamination. Indicator organisms such as total coliforms, feacal coliforms, *Escherichia coli,* Salmonella and faecal enteroccoci numbers were determined on lettuce leaves and shown in the figures 1- 6.

1.4.1 Total Coliform Bacteria Loads on Lettuce Leaves

The mean total coliform bacteria numbers on lettuce leaves from all the three sites ranged

from 4.93× 10^{7} to 6.17× 10^{7}. The lowest value was recorded at Atonsu and the lowest at Karikari (figure.1).The differences in total coliform contamination levels at the three sites were statistically significant ($P < 0.001$). A site-by-site multiple comparison for samples in terms of total coliform contamination levels between Atonsu and Gyinyase and Atonsu and Karikari were statistically significant ($P < 0.002$ and $P < 0.001$) respectively. However, total coliform contamination of lettuce between Gyinyase and Kakari sampling sites was not statistically significant ($P < 0.738$).

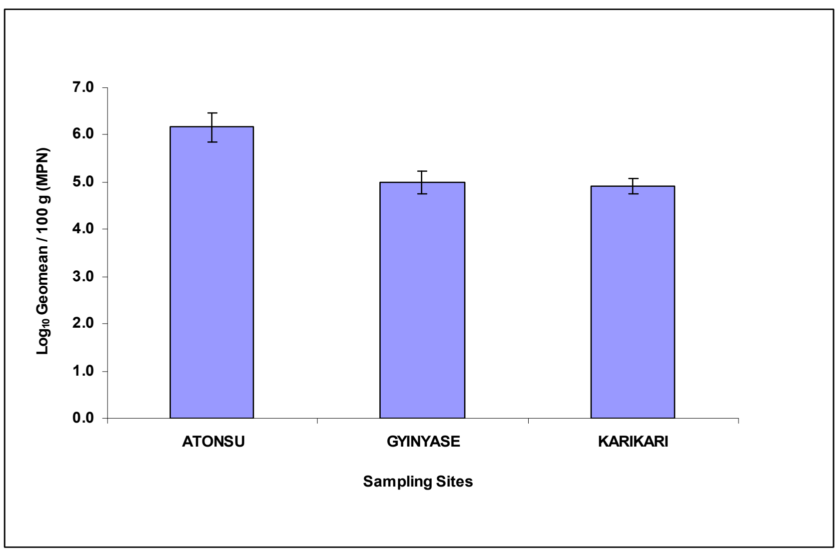

Figure 1: Total coliform levels on lettuce sampled from the different sites (n=36)

This shows that samples analysed from Atonsu were much contaminated with total coliform even though Gyinase and Karikari farms were almost having the same level of contamination.

Faecal Colifom Bacteria Loads on Lettuce Leaves

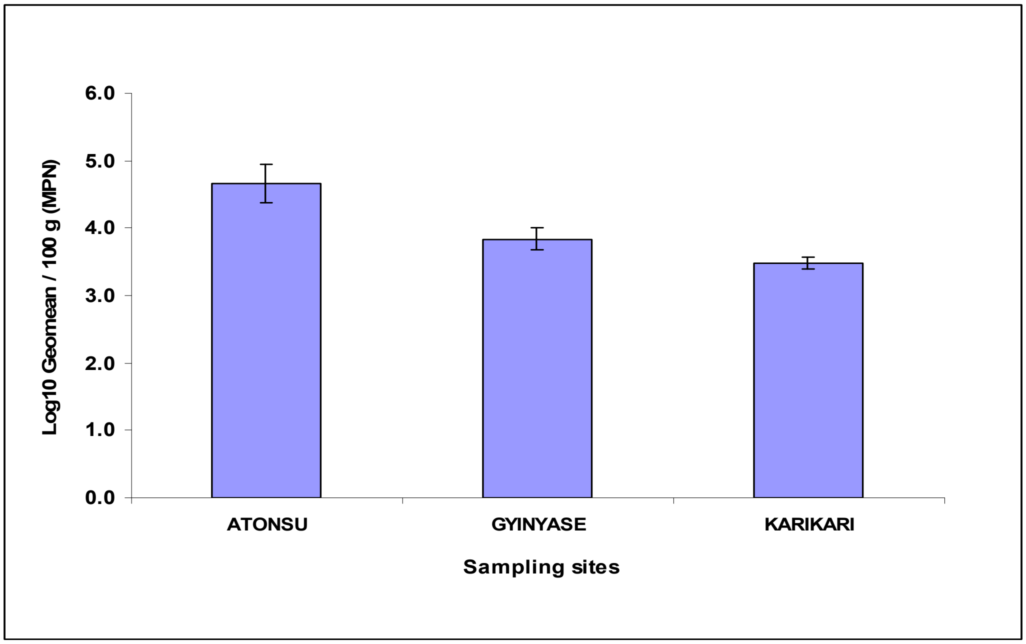

Figure 2: Faecal coliform levels on lettuce sampled from the different sites (n=36)

The mean values of faecal coliform bacteria contamination on lettuce leaves from all the three sites ranged from 3.48×10^5 to 4.66×10^5. Similarly, the highest value was recorded at Atonsu and the lowest at Karikari (figure 2). The faecal coliform contamination levels at the three sites were statistically significant ($P < 0.000$). A site by site multiple comparison for samples in terms of faecal coliform contamination levels between Atonsu, Gyinyase and Atonsu and Karikari were statistically significant ($P < 0.003$ and $P < 0.000$) respectively. Faecal coliform contamination of lettuce between Gyinyase and Kakari sampling sites was however, not statistically significantly different ($P < 0.177$). This shows that samples analysed from Atonsu were much contaminated with faecal coliform as compared to Gyinase and Karikari farms (figure 2).

1.4.2 *Escherichia coli* Bacteria Loads on Lettuce Leaves

The mean values of *Escherichia coli* bacteria contamination on lettuce leaves from all the three sites ranged from 2.98×10^3 - 3.86×10^3. Similarly, the highest value was recorded at Atnonsu and the lowest at Karikari (figure 3). The faecal coliform contamination levels at the three sites were statistically significant ($P < 0.000$). A site by site multiple comparison for samples in terms of *E. coli* contamination levels between Atonsu and Gyinyase, Atonsu and Karikari, and Gyinyase and Kakari were all statistically significant ($P < 0.025$, $P < 0.000$ and $P < 0.001$) respectively. This shows that there was relatively a gradual and significant level of decline in *E. coli* numbers on lettuce leaves from Atonsu site to Kakari (figure 3).

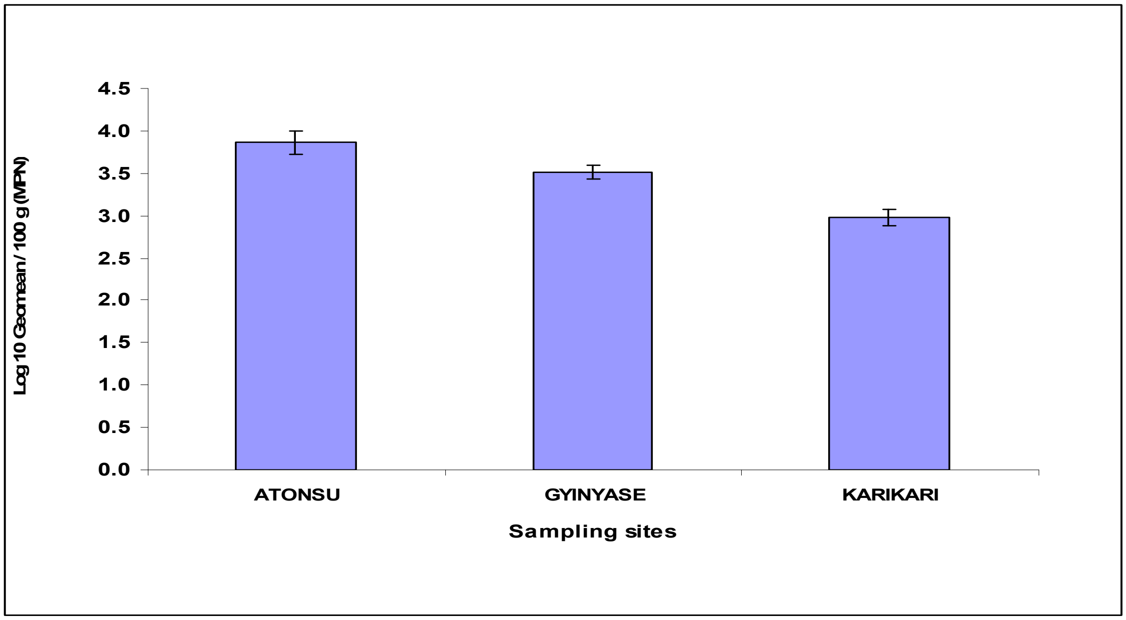

Figure 3: *E. coli* contamination levels on lettuce sampled from the different sites (n=36).

1.4.3 Salmonella Bacteria Loads on Lettuce Leaves

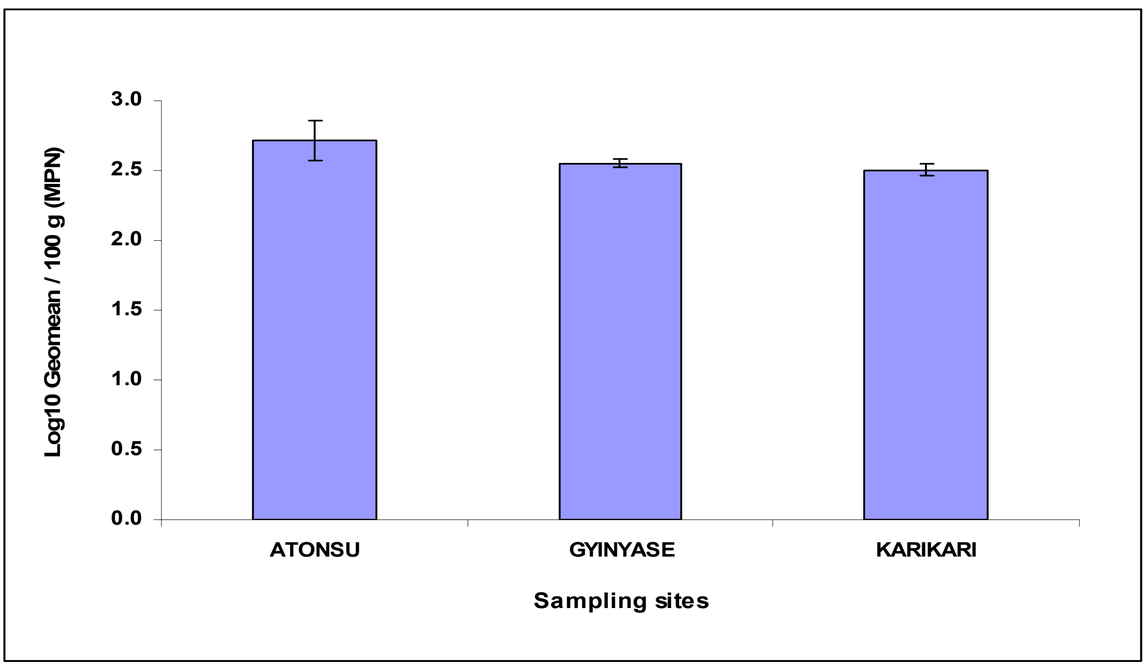

Figure 4 Salmonella contamination levels on lettuce sampled from the different sites (n=36).

The data from figure 4.4 shows the salmonella contamination levels at the three farms sites. Mean values of salmonella bacteria on lettuce leaves from all the three sites ranged from 2.50×10^2 to 2.72×10^2. Similarly, the highest value was recorded at Atonsu and the lowest at Karikari (figure 4.4). The salmonella contamination levels at the three sites were however statistically insignificant ($P < 0.111$).

A site by site multiple comparison for samples in terms of salmonella contamination levels between Atonsu and Gyinyase, Atonsu and Karikari, and Gyinyase and Kakari were all statistically insignificant ($P < 0.105$, $P < 0.050$ and $P < 0.711$) respectively. However, there was relatively a gradual but insignificant level of decline in salmonella numbers on lettuce leaves from Atonsu site to Kakari (figure 4).

1.4.4 Faecal *Enterococci* Loads on Lettuce Leaves

Figure 5 shows faecal *enterococci* contamination levels from the three farms. Geometric mean values of faecal *enterococci* bacteria on lettuce leaves from all the three sites ranged from 0.68×10^0 to 2.05×10^0. The highest value was recorded at Atonsu and the lowest at Gyinyase (figure 5). Faecal *enterococci* contamination levels at the three sites were however statistically insignificant ($P < 0.182$). A site by site multiple comparison for samples in terms of faecal *enterococci* contamination levels between Atonsu and Gyinyase, Atonsu and Karikari, and Gyinyase and Karikari were relatively insignificant ($P < 0.075$ and $P < 0.197$ and $P < 0.607$) respectively.

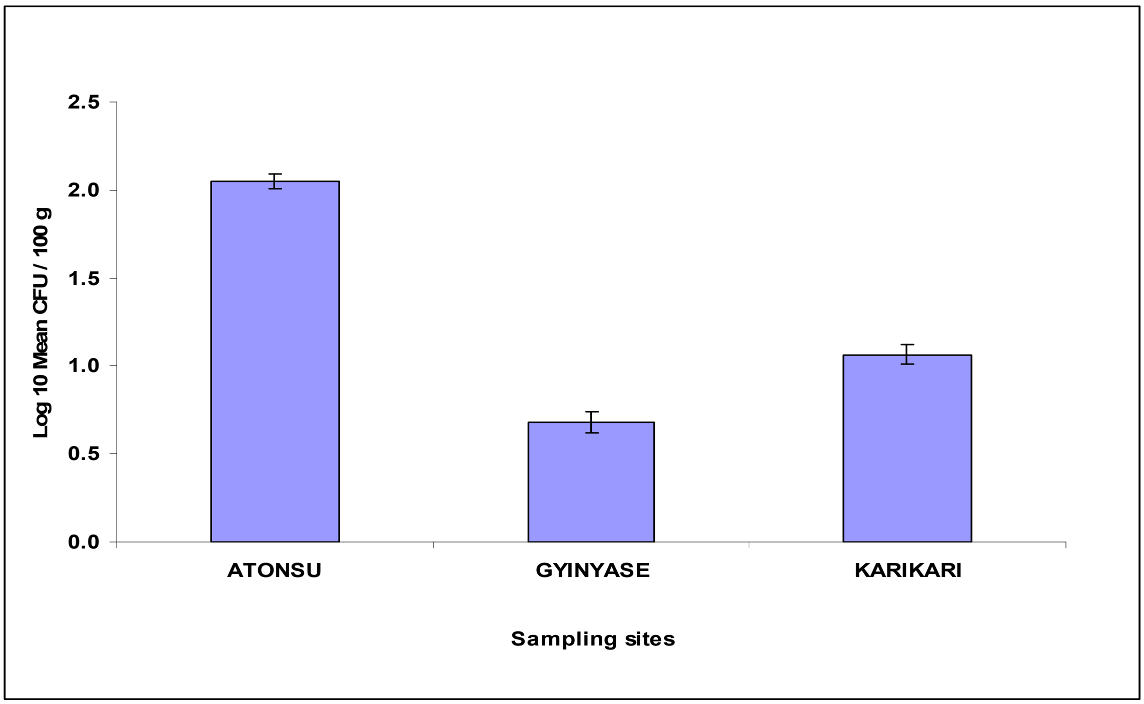

Figure 5: Faecal *enteroccoci* contamination levels on lettuce sampled from the different sites (n=36).

However, from the absolute geometric mean values for each site, there were marginal variations which probably accounted for the type of graphical representation from logged mean values shown on figure 5. However, there was relatively a gradual but insignificant level of decline in salmonella numbers on lettuce leaves from Atonsu site to Karikari (figure 5).

1.4.5 Comparative Total Bacteria *Loads* on Lettuce Leaves from the three sites in Kumasi Metropolis.

The differences in levels of contamination of lettuce leaves with total coliform, faecal coliform, *Eschericha coli*, salmonella and faecal *Enterococci* bacteria strains from Atonsu, Gyinyase and Karikari farms are comparatively shown on figure 4.6.

The mean total coliform bacteria load on lettuce from all the three sites ranged from 4.93×10^4 to 6.17×10^4. The lowest value was recorded at Karikari whilst the highest at Atonsu. Faecal colifoms loads on lettuce ranged from 3.48×10^3 to 4.66×10^4. The lowest value was recorded at Karikari whilst the highest at Atonsu. *Escherichia coli* loads on lettuce on the other hand, ranged from 2.98×10^3 to 3.86×10^4 and the lowest value was recorded at Gyinyase whilst the highest at Atonsu. It ranged between 2.50×10^2 to 2.72×10^2 for the lowest and highest values of *salmonella recorded* at Karikari and Atonsu sites respectively.

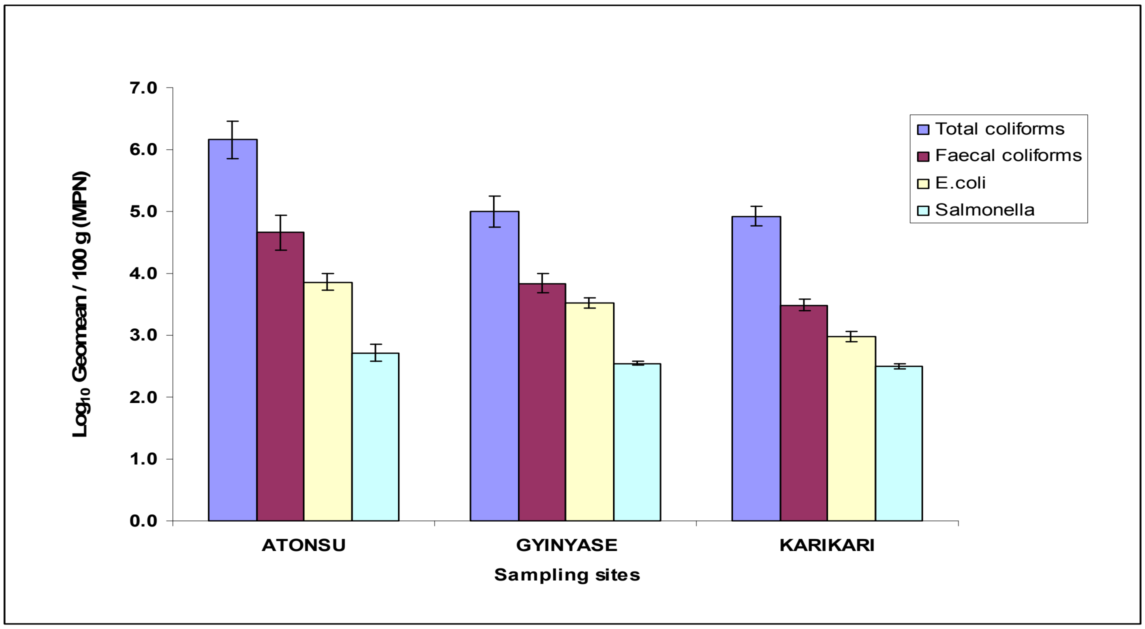

Figure 6: Summary of microbial quality of lettuce leaves from the three farms (n=36)

However, there were generally very few numbers of mean *Enterococci* counts on the lettuce leaves from all the three farms and the contamination which ranged from 0.68×10^0 - 2.05×10^0. Atonsu farm recorded the highest value whilst and lowest was at Gyinyase farm. By conversion, it means that about 7 - 21 faecal *enterococci* numbers (i.e. 0.007 – 0.021 per 1000ml) from the supernatant raw water samples which were collected into sterilized bottles for the analysis by using distilled water to rinse the contaminated lettuce from the three farms were very much on the lower counts and could not be plotted on the bar chart (Figure 6)

Generally, for all the bacteriological strains identified and counted from all the lettuce leaves, the differences in mean numbers recorded for *Salmonella* and faecal *enterococcus* between the three lettuce farms were not significant ($P < 0.111$ and $P < 0.182$) respectively at $P < 0.05$ level of probability. However, for total colifoms, faecal colifoms and *Escherichia coli* the differences in mean contamination loads on the lettuce leaves were generally significant for total colifoms ($P < 0.001$), but, much highly significant for faecal colifoms ($P < 0.000$) and *E. coli* ($P < 0.000$) respectively from the Atonsu, Gyinyase and Karikari farms.

1.5 Discussion

The study shows that wastewater irrigated vegetable farming practices increase bacterial loading on the vegetables, to levels that were similar to previous findings of Amponsah-Doku *et al.,* in 2006 at the Kwame Nkrumah University of Science and Technology, Kumasi, on bacterial contamination levels on cabbage and lettuce from these same sites. Bacterial numbers recorded in this study were still above the ICMSF (1998) limit of $10^3$to 10^5 coliforms 100 g^{-1}wet weight of vegetables usually eaten raw when compared with previous findings from these farms. Upon monitoring past trends, Amoah *et al.,* (2005), and Amponsah-Doku *et al.,* (2010), quite recently reported high bacterial numbers counted on lettuce leaves produced on farms within the Kumasi Metropolis as being comparatively similar to the previous 3-6 years published data of Keraita *et al*., in 2003a, and Cornish *et al*., 1999. These early researchers pointed several scientific reasons:

Firstly, the level of bacterial loading on the lettuce leaves on the farms may result from the high levels of indicator bacterial numbers in the farm irrigation water. The results of this study was much similar to that of Amponash Doku *et al.,* (2010) which agreed with the reports of earlier workers (Mensah *et al*., 2001; Keraita *et al*., 2003b; Amoah *et al*., 2005 and Obiri-Danso *et al*., 2005) that the bacterial quality of urban rivers and waterholes in Kumasi is low and most of these are the main sources of irrigation water for vegetable production. Besides, the water bodies in the Kumasi metropolis are often contaminated by diffused or non-point and point sources such as run-off from agricultural lands and abattoirs and outfall pipes of breweries and wood processing industries. It has further been established that inputs from the faeces of wild birds and domestic animals, human excreta, market and household waste which end up in the rivers and shallow wells on the vegetable farms are often not protected and easily receive pollutants from the surrounding farm environment (Drechsel *et al.,* 2000; Amoah *et al*., 2005; Obiri-Danso *et al*., 2002).

Secondly, the use of overhead irrigation technique and the general morphology of lettuce leaves expose much of its surface area to the irrigation water and soil particles from the splashes and this could account for the high contamination (Amoah *et al*., 2005).

Thirdly, the use of poultry manure by about 75% of vegetable farmers as the main nutrient source for the lettuce and for the management of soil fertility contributes high numbers of faecal coliforms (Westcot, 1997). Animal manure is a well-known source of food borne pathogenic bacteria and its inappropriate use in vegetable crops contributes a risk to consumer health (Lau and Ingham, 2001; Locking *et al.,* 2001; Riordan *et al.,* 2001; Wachtel *et al.,* 2002; Zschock *et al.,* 2000). Drechsel *et al*., (2002) reported that fresh poultry litter samples used for vegetable production in Kumasi had high faecal coliform counts ranging from 3.6×10^4 to 1.1×10^7 and could be a possible source of lettuce contamination especially where farmers broadcast manure on standing crops. Similarly, the report of Amponsah-Doku *et al.,* in 2010 further confirmed that thermotolerant coliforms on lettuce produced under similar conditions in the same Metropolis varied from 2.3×10^3 to 9.3×10^8 on farm, 6.0×10^1 to 2.3×10^8 on market, and 2.30 x 10^6 to 2.40 x 10^9 at street food vendor site. In this recent study in 2010/2011, the higher loads of faecal colifom counts which similarly ranged from 4.93×10^4 to 6.17×10^4 could not prove any significant improvement on any of the environmental conditions and farming practices, hence, accounting for the high level of lettuce contamination in the three Major Peri Urban vegetable harvesting sites namely - Atonsu, Gyinyase and Kakari, located within the Kumasi Metropolis.

Generally, enterococci numbers were found to be lower compared to the thermotolerant coliforms. This is because unlike coliforms, enterococci are more sensitive to variations in environmental conditions and are easily knocked-off by sunlight and temperature (Obiri-Danso *et al.,* 2001).

Conclusion

Bacterial numbers in all the three production sites exceeded both the WHO (1989) and ICMSF (1998) recommended levels making it risky for consumption in the raw state. This implies that even though the use of wastewater in vegetable production guarantees a secured livelihood for urban farmers, without any wastewater treatment, it poses serious health risks to farmers, sellers and consumers and care must be taken to educate the public on how to improve upon vegetable product handling and consumption due to the numerous associated health risk factors.

Recommendations

We strongly recommend that apart from treating waste water before its use to irrigate lettuce and other vegetables like carrots, green pepper and cabbage since these are eaten raw, alternative sources like pipe borne water can be used to irrigate these vegetables. The use of poultry droppings can be converted to compost and vegetable farmers must be educated on the proper way of applying it to the soil. We further, recommend that there should be public food hygiene and safety education on the consumption of lettuce. The general public should be made aware to use vinegar or salt solution to wash any lettuce prior to consumption. Comparative studies should further be conducted to ascertain the bacterial and trace metal contamination levels in the waste water which is used to cultivate these vegetables for public patronage.

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