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Assessment of bacteriological contaminants of some vegetables irrigated with Awash River water in selected farms around Adama town, Ethiopia

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Food safety issues are of growing concern to consumers globally because of the risk associated with consumption of foods contaminated with pathogens in irrigated vegetables. The study was conducted to assess the extent of bacterial contamination of vegetables due to irrigation with polluted Awash River water. Three leafy vegetable samples, namely, cabbage (*Brassica oleracea L. var. capitata*), lettuce (*Lactuca sativa L. longifolia*) and spinach (*Spinacea oleracea*) from both farms were examined for bacterial contaminants. The results show that spinach was found to be the most heavily contaminated vegetable in both farms by aerobic bacteria. The aerobic mesophilic bacterial count on this vegetable was 2.2×10^8 and 2.0×10^8 CFU/g, for spinach sampled from Melka Hida and Wonji Gefersa vegetable farms, respectively. The highest total coliform count (6.6×10^6) was also recorded from lettuce in Melka Hida vegetable farm. The mean fecal coliform values of all the three vegetable samples exceed the International Commission on Microbiological Specifications for Foods (ICMSF) recommended level. The highest faecal coliform count (5.7×10^5) was recorded in cabbage sampled from Wonji Gefersa. The high microbial contamination rates associated with these vegetable samples indicated poor water quality for irrigation employed in the overall production of vegetables in the study area.

Key words: Indicator bacteria, pathogen, vegetables.

INTRODUCTION

Food safety is a major public health concern worldwide. During the last decades, the increasing demand on food safety has stimulated research regarding the risks associated with consumption of food stuffs contaminated with pathogenic microorganism. Several studies have revealed that contamination of vegetables with pathogens poses a threat for consumers (D'Mello, 2003; Zandstra and De Kryger, 2007). Vegetables are produced in significant quantities both in urban and pre urban areas.

The United Nations Development Program estimated in 1996 that more than 800 million people were engaged in urban agriculture globally. Of these people, about 200 million practice market-oriented farming on open spaces, often using poor quality irrigation water. As urban populations in developing countries increase, and residents seek better living standards, larger amounts of freshwater

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are consumed by domestic, commercial and industrial sectors, which generate greater volumes of wastewater (Qadir et al., 2007a; Asano et al., 2007).

In addition to these, waste and sewage water are still considered as the richest sources of nutrients for plants. In many cities and towns, the sewage water is sold and it is a good source of income to municipalities (Scott et al., 2004). However, the situation is changed now with the establishment of industries in suburban areas where the wastewater is mixed with industrial effluents and big culverts are coming out from the cities. The polluted water is still used for growing vegetables in the nearby areas of the cities without knowing their adverse impacts on the life of consumers (Qadir, 1999). Furthermore farmers, consumers, and some government agencies in many countries are not fully aware of the potential impacts of irrigation with wastewater (Qadir et al., 2007b; Amoah, 2009).

The access to clean water for irrigating vegetables is a major challenge. Consequently, urban and peri-urban vegetable farmers have no other choice than to use water from these highly polluted sources. This raises public health concerns due to possible crop contamination with pathogens where vegetables are eaten uncooked (Amoah et al., 2006). In developing countries, continued use of untreated wastewater and manure as fertilizers for the production of vegetables is a major contributing factor to contamination that causes numerous foodborne disease outbreaks (Johannessen et al., 2002; AdeOluwa, and Cofie, 2012).

Adama and Wonji Gefersa Towns are found in Adama *Woreda* of East Showa zone, Oromia Region, with a population of 260,600 and 23,510, respectively (CSAE, 2005). Awash River is the only important river in Adama *Woreda* used for irrigating about1132 ha of land. Awash River flows from central highlands through Ethiopia's major industrial and agro-industrial belt, taking in a whole burden of all types of raw effluent stands as one of Ethiopia's river streams in urban areas of developed rivers (Tesfamariam, 1989). Besides this, the expansion of new industries and disposal of industrial wastes to the Awash River basin is of great concern to the nation (Girma Taddese, 2001). Application in farming of such untreated waste can pose significant health hazard to those who have direct contact with it and eventually the public becomes affected through the food chain link (Furedy et al., 1999).

Information on the microbial safety of food items in Ethiopia is limited. Many farm households around Adama Town that are irrigating their farmlands with Awash River are not aware of the risks or the potential harmful environmental consequences. This may be attributed to illiteracy, lack of adequate information and exposure to poor sanitary conditions for most of their lives. Altogether, the situation puts the consumers at high risk of contracting diseases. The magnitude of microbial contaminants in vegetables grown on irrigated with Awash River is not known in the study area. Therefore, the study was designed to assess the levels of indicator bacteria on these vegetables irrigated with wastewater in Melka Hida and Wonji Gefersa farms around Adama Town.

MATERIALS AND METHODS

Description of the study area

This study was conducted at two wastewater irrigated vegetable growing farms, that is, Melka Hida and Wonji Gefersa that are found in Adama *Woreda*. Melka Hida is found in Adama Town Administrative Zone, Oromia Region, which is 99 km away from Addis Ababa and is located at latitude of 8° 33′ 0″ North and longitude 39° 16′ 12″ East. It has an elevation of 1620 m above sea level.

Wonji Gefersa is a town that is found in Adama *Woreda* of East Showa Zone, Oromia Region, nearby Adama Town, which is 107 km away from Addis Ababa and located at a latitude of 8° 26' 59'' North and longitude of 39° 16' 48'' East. It has an elevation of 1588 m above sea level and its temperature and annual rain fall is 23°C and 500-800 mm, respectively (Environmental Protection Authority, 2005). Awash River is the only important river in Adama *Woreda* used for irrigating around 1132 ha of land, which originates from the highlands of Dandi *Woreda* located west of Addis Ababa, Ethiopia, and flows along the rift valley into the Afar region, where it eventually terminates in a salty lake, Lake Abbe, found on the border with Djibout (Figure 1)

Study design

A cross sectional survey was conducted to assess the load of bacterial contamination on the main leafy vegetables [lettuce (*Lactuca sativa* L.), cabbage (*Brassica oleracea Linn*.), and spinach (*Spinacea oleracea*)] that were grown in Melka Hida and Wonji Gefersa vegetable farms irrigated with wastewater. The samples were regularly collected at three week interval during February 2012 and April 2012, and analyzed for aerobic mesophilic bacterial count, feacal coliform count and total coliforms.

Sample collection

A total of 72 samples comprising three types of fresh vegetables (cabbage, lettuce, spinach,) were collected from Melka Hida and Wonji Gefersa vegetable farms using a random sampling technique method. Recently, mature leaves of lettuce, cabbage and spinach were sampled at early maturity according to methods used by Fisseha (1998). All samples were collected aseptically in a sterilized universal container and plastic bags and transported to Haramaya University for laboratory processing. The samples were cooled during transportation using a cooler box to keep the normal conditions of the microflora of vegetables. The analysis began immediately after the sample arrival at laboratory.

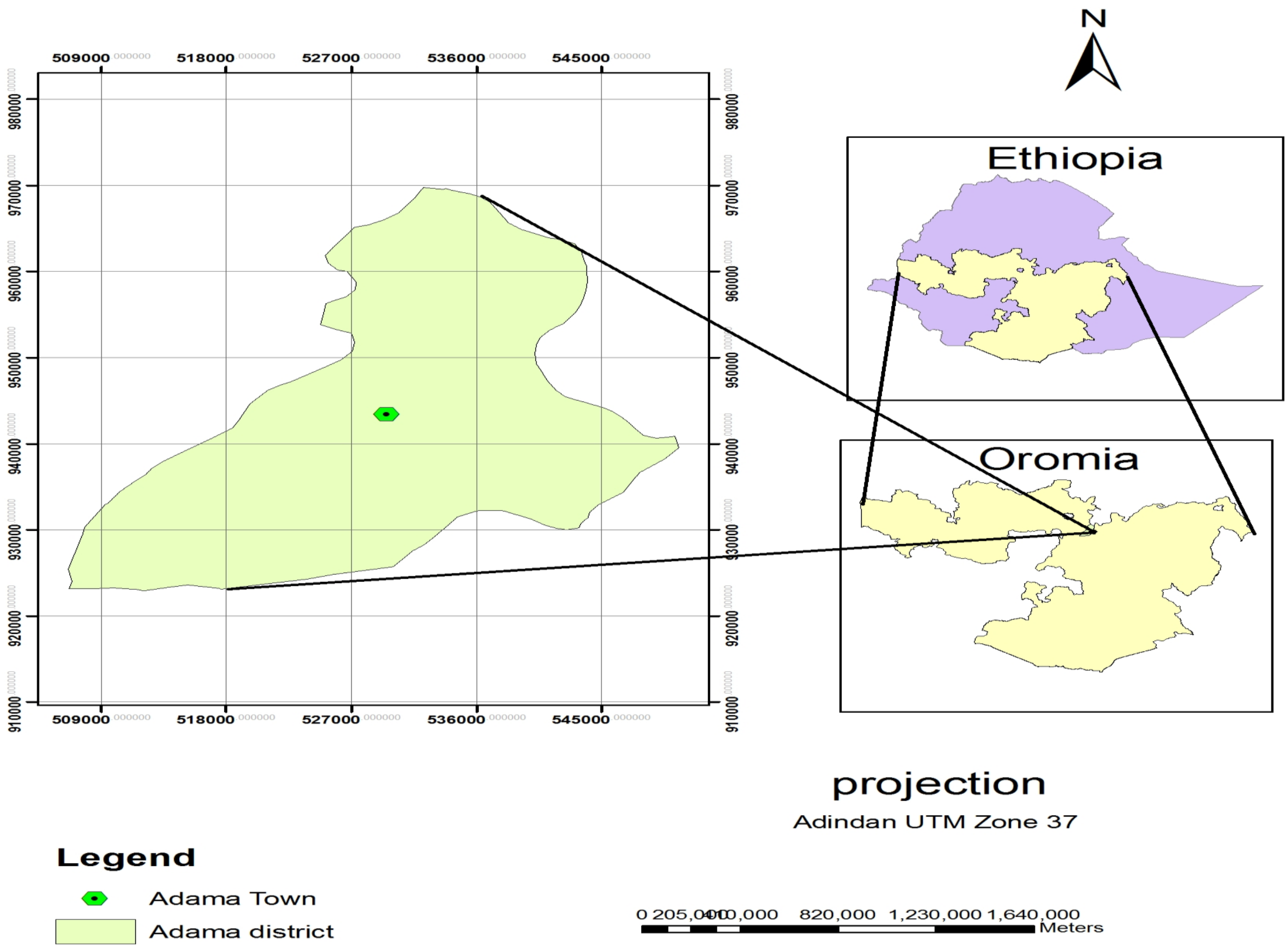

Figure 1. Location of the study area. Source: Aynalem Adugna (2010).

Microbiological analysis

For microbiological analysis, 25 g of leafy material was aseptically removed from each vegetable sample using a sterile scalpel and blended in 225 ml of sterile 0.1% (w/v) bacteriological peptone water for 1-3 min. The samples were homogenized using stomacher (Seward Stomacher, Model: 400 Circulator, Rate frequency: 50-60Hz). The homogenate was used as a source of microbial source for determining the aerobic mesophilic bacterial counts, total coliform counts and feacal coliform counts following the standard methods of APHA (1998).

Data analysis

In this study, all statistical analyses were computed using SAS software version 9.1 for microbiological analyses. The data were subjected to analysis of variance (ANOVA) to assess the effect of Vegetable type and site of production on the concentrations of microbial contaminants in the vegetables tested. As the level of microbial contamination might vary with sample collection site and vegetable type, ANOVA was used to test the existence of significant difference between means. In all statistical analyses, confidence level was held at 95% and $P<0.05$ (at 5% level of significance) was considered as significant.

RESULTS AND DISCUSSION

Bacteriological analysis of leafy vegetables

This study attempted to determine the percentage of vegetable contamination with aerobic mesophilic bacteria (AMB), coliform bacteria (TCC) and faecal coliforms (FC) as well as their microbial loads through aerobic mesophilic bacterial counts (AMB), total coliform counts (TCC) and fecal coliforms (FC). The highest percentage was obtained

Table 1. Percentage of positive samples of indicator bacteria in spinach, lettuce and cabbage samples from both farms.

Site	Indicator organism	No. of sample examined	Type of plant sample					
			Spinach		Lettuce		Cabbage	
			F	%	F	%	F	%
	AMB	12	12	100	12	100	12	100
Melka Hida	TCC	12	12	100	10	83.3	10	83.3
	FC	12	8	66.6	7	58.3	9	75
	AMB	12	12	100	12	100	12	100
Wonji Gefersa	TCC	12	12	100	11	91.6	10	83.3
	FC	12	9	75	8	66.6	7	58.3

F= Frequency of positive samples, AMB = aerobic mesophilic bacteria, TCC = total coliform count, FC = faecal coliforms.

Table 2. Mean number of indicator bacteria among the tested vegetable samples of Melka Hida and Wonji Gefersa.

No. of examined samples	Number of indicator organisms (CFU/g)	Site	Vegetable types			
			Cabbage	Lettuce	Spinach	Mean
12		MH	$9.3 \times 10^7 \pm 8.8^c$	$1.7 \times 10^8 \pm 8.8^b$	$2.2 \times 10^8 \pm 8.8^a$	$1.6 \times 10^8 \pm 5.1$
12	AMB	WG	$8.0 \times 10^7 \pm 8.8^c$	$1.6 \times 10^8 \pm 8.8^b$	$2.0 \times 10^8 \pm 0.8^a$	$1.5 \times 10^8 \pm 5.1$
		Mean	$8.7 \times 10^7 \pm 6.2$	$1.6 \times 10^8 \pm 6.2$	$2.1 \times 10^8 \pm 6.2$	$1.6 \times 10^8 \pm 3.1$
12		MH	$5.0 \times 10^6 \pm 0.8$	$6.6 \times 10^6 \pm 0.8$	$5.2 \times 10^6 \pm 0.8$	$5.6 \times 10^6 \pm 0.5^d$
12	TCC	WG	$4.1 \times 10^6 \pm 0.8$	$4.1 \times 10^6 \pm 0.8$	$3.1 \times 10^6 \pm 0.8$	$3.8 \times 10^6 \pm 0.5^e$
		Mean	$4.5 \times 10^6 \pm 0.6$	$5.4 \times 10^6 \pm 0.6$	$4.1 \times 10^6 \pm 0.6$	$3.7 \times 10^6 \pm 0.3$
12		MH	$5.2 \times 10^5 \pm 0.7^a$	$3.1 \times 10^5 \pm 0.7^b$	$3.7 \times 10^5 \pm 0.7^b$	$4.0 \times 10^5 \pm 0.4$
12	FC	WG	$5.7 \times 10^5 \pm 0.7^a$	$2.3 \times 10^5 \pm 0.7^b$	$2.2 \times 10^5 \pm 0.7^b$	$3.4 \times 10^5 \pm 0.4$
		Mean	$5.5 \times 10^5 \pm 0.5$	$2.7 \times 10^5 \pm 0.5$	$3.0 \times 10^5 \pm 0.5$	$3.7 \times 10^5 \pm 0.2$

$^{a\text{-}b\text{-}c}$ Means with different superscript letters along the row for the same parameter in the same site do significantly differ (P<0.05); $^{d\text{-}e}$ means with different superscript letters along the column for the same parameter do significantly differ (P<0.05).

for aerobic mesophilic bacteria (100%) as demonstrated by its occurrence in all vegetable samples analyzed. On the other hand, 100, 83.3, 83.3 and 100, 91.6, 83.3% of the samples of spinach, lettuce and cabbage collected from Melka Hida and Wonji Gefersa, respectively, were found contaminated with coliform bacteria. Similarly, fecal coliforms were found in 66.6, 58.3, 75, and 75, 66.6, 58.3% of spinach, lettuce and cabbage collected from Melka Hida and Wonji Gefersa farms, respectively (Table 1).

From the data in Table 1, it can be understood that there was an improper pre harvest handling of the vegetables in selected study area. The high percentage of vegetables contaminated with coliform bacteria and fecal coliforms may suggest high risk of acquiring infectious diseases through the consumption of these vegetables. The occurrence of such indicator microorganisms is an indication of the contamination of the vegetables with faecal matter derived from humans and other animals (Cornish et al., 1999).

The results of this investigation additionally showed that the vegetable samples collected from both farms (Melka Hida and Wonji Gefersa) were heavily contaminated by aerobic mesophilic bacterial counts ranging from $8.0 \times 10^7 \pm 8.8$ to $2.2 \times 10^8 \pm 8.8$ CFU/g. The mean values of aerobic mesophilic bacterial counts were in the order of spinach > lettuce > cabbage for both sites as shown in Table 2. The data further showed that all the bacterial counts recorded in this study exceeded the recommended levels by WHO and International Commission on Microbiological Specifications for Food (ICMSF) standards (10 to 10^2 coliforms g^{-1}, 10 faecal coliform g^{-1} and 4.9×10^6 aerobic count g^{-1}) wet weight vegetables.

The results of the analysis of variance for bacterial counts between locations (Melka Hida and Wonji Gefersa) and vegetables (spinach, lettuce and cabbage) are shown in Table 2. The data showed that there was a highly significant difference ($p<0.01$) in the average counts of AMB amongst the vegetable types, but there was no significant difference ($p>0.05$) between sites. On the other hand with respect to the mean TCCs, the ANOVA did not show significant difference ($p>0.05$) amongst vegetable types, but there was significant difference ($p<0.05$) between sites. However, as can be seen from the same tables, there was a significant difference between the two sites. Similarly, the results of the analysis of variance for FC counts showed that in both farms, there was a significant difference amongst vegetable types but not between sites.

The AMB for spinach, lettuce and cabbage were in the range of 2.0×10^8 - 2.2×10^8, 1.6×10^8 - 1.7×10^8 and 8×10^7 - 9.3×10^7 CFU/g, respectively. This high aerobic mesophilic bacterial count might be due to pollution by humans, animals or irrigation water. In agreement with this result, Thunberg et al. (2004), reported total viable count as 5.0×10^8, 4.0×10^8, 3.1×10^7, 2.5×10^7 and 2.0×10^6 CFU/ g for spinach samples collected from various farm sites. However, spinach collected from both farms (Melka Hida and Wonji Gefersa) accounts a very high aerobic mesophilic bacterial count in terms of CFU/g. The high aerobic mesophilic bacterial counts in spinach could be due to the wide surface area of vegetable leaves which is suitable for water contact and microbial contamination (Anonymus, 2002). The mean aerobic mesophilic bacterial counts of the lettuces in this study is 1.7×10^8 CFU/g. In agreement with this result, Viswanatha and Kaur (2001) from in India indicated that total aerobic plate count for cabbage and lettuce was found to be 2.8×10^6 - 1.2×10^8 and 1.3×10^7 - 2.3×10^7CFU/g, respectively.

The total coliform levels recorded under this study were high in all the three vegetable samples analyzed (Table 2). Total coliform levels ranged from 4.1×10^6 - 5.0×10^6 CFU/g for cabbage, 4.1×10^6 -6.6×10^6 CFU/g for lettuce and 3.1×10^6 -5.2×10^6 CFU/g for spinach at both sites. High total coliform counts (6.6×10^6 CFU/g) were observed on lettuce collected from Melka Hida as compared to the other two vegetable samples. Similar findings are reported by Nguz et al. (2005) in Zambia which found a range of total coliform counts on vegetable products between 1.6×10^2 and 7.9×10^5 CFU/g. According to Nguz et al. (2005), fecal coliform counts are efficient indicators of sanitization, but the presence of fecal coliforms does not necessarily indicate the presence of a pathogen. In this study, the fecal coliform counts of vegetable samples (cabbage, lettuce and spinach) collected from both sites ranged between 5.2×10^5 to 5.7×10^5, 2.3×10^5 to 3.1×10^5 and 2.2×10^5 to 3.7×10^5 CFU/g, respectively. The mean fecal coliform values of all the three vegetable samples exceed the ICMSF recommended level of 10 fecal coliform g^{-1} fresh weights. This may be due to Awash River used for irrigation of vegetables, which flows from central highlands through Ethiopia's major industrial and agro-industrial belt, taking in a whole burden of all types of raw effluent. In addition to this, application of organic manures is common practices of farmer for production of crops in that area.

However, sources of fecal coliform contamination of lettuce may include overhead irrigation of lettuce with already contaminated water, planting in contaminated soils and frequent application of poultry manure which was not well composted (Amoah et al., 2005). Of the three vegetables, cabbage shows significant difference ($p<0.05$) among crop types of both farms by contamination of fecal coliform counts. This shows cabbage was more contaminated by fecal coliforms than other leafy vegetables.

These results correlate with the probability of the vegetable samples to be more in contact with the source of contamination during growth (Heaton and Jones, 2008). Moreover, application of fresh poultry manure without sufficient drying used for vegetable production registers high fecal coliform counts (Drechsel et al., 2000). Generally, variation in the AMB, TCC and FC values of the present study and previous works may be either due to differences in the geographical location of the cultivation area, or due to the difference in contamination load at different sections of the drainage canal and different pre-harvest handling practices.

Conclusion

The study revealed that there was bacterial contamination of fresh leafy vegetables (lettuce, cabbage and spinach) grown in Melka Hida and Wonji Gefersa vegetable farms. Bacterial numbers recorded in this study range from 2.2×10^5 to 2.22×10^8CFU g^{-1} which is above the ICMSF (1998) limit of 10^3 to 10^5 coliforms $100g^{-1}$ wet weight of vegetables usually eaten raw. Spinach was found to be the most contaminated vegetable by aerobic mesophilic bacterial count (2.03×10^8 to 2.22×10^8, CFU/ g). This might be due to the fact that spinach have wide leaves surface in contact with wastewater, soil and dust. In contrast cabbage was the least in aerobic mesophilic bacterial count (8×10^7 to 9.3×10^7 CFU/g).

In this study, high total coliform counts (6.6×10^6 CFU/g) were observed on lettuce collected from Melka Hida among the three vegetable. Likewise, the fecal coliform counts of vegetable samples collected from both site ranged between 2.2×10^5 and 5.7×10^5 CFU/g. However, cabbage showed high fecal coliform counts among the three analyzed vegetable samples. Therefore, great attention should be paid in using contaminated water for production of vegetables for the public health perspective.

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