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Full Length Research Paper

Bacteriological quality and safety of green pepper and tomato irrigated with Abay River water in Bahir Dar town

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ABSTRACT

The objective of this study was to determine the bacteriological quality and safety of green pepper (*Capsicum annuum*) and tomato (*Lycopersicon esculentum*) grown along Abay river of Bahir Dar town, Ethiopia. The mean aerobic mesophilic counts from tomato and green pepper were 4.72 and 5.01 respectively. The mean total coliform counts from tomato was 234.9 MPN/g and green pepper 303.2 MPN/g. Similarly, the mean fecal coliform counts of tomato was 45.08 MPN/g and 58.1MPN/g from green pepper. The mean bacterial counts of green pepper were higher than tomato vegetables. Moreover, 5 (8.3%) Salmonella, 3 (5%) *Shigella spp.* and 6 (10%) *E. coli spp*. were isolated from tomato; and 6 (10%) Salmonella, 5 (8.3%) Shigella and 9 (15%) *E. coli spp*. were detected from green pepper samples. The mean total coliform counts from Bezawit were 1040 MPN/100ml and 711.67MPN/100ml Gudguad site. Similarly, the mean fecal coliform counts of Bezawit was 689.67MPN/100ml and 390.00MPN/100ml from Gudguad. In both sites, lack of awareness and poor irrigation practice promote the probability of vegetable contamination. Therefore, keeping the irrigation practice of irrigated vegetable and providing regular training for farmers are some of the practices to improve bacteriological quality, safety and shelf-life of vegetables and their products.

Keywords: Tomato, green pepper, Abay river water, Irrigation practice, Bacterial quality

INTRODUCTION

Vegetables are important sources of vitamins, minerals and antioxidants in human diets and also contribute large quantities of vitamin C, but the vitamin's high water solubility and sensitivity to heat makes it susceptible to loss during thermal preparation (Howard et al., 1999; Murcia et al., 2000). For this reason, consumption of minimally processed food or raw vegetables has been increased tremendously due to their nutritive values in human dietary (Amoah, 2008).

Tomato (*Lycopersicon esculentum*) and Green pepper (*Capsicum annuum*) vegetables are largely consumed in raw, without further processing in most parts of Ethiopia. Although fresh produce can become contaminated at any time in the agri-food chain, pre-harvest contamination is considered the most likely origin. One important source of pathogens found on fresh produce is from faecally contaminated irrigation water (De Roever, 1999). In many cases river water did not meet international faecal guidelines for safe irrigation with *Escherichia coli* concentrations exceeding 1000 cfu /100 ml. However many local growers resort to use this contaminated river water; due to lack of awareness of food safety, and lack of potable water used for irrigation of vegetables. Thus, the presence of these bacteria on these vegetables is dangerous for human consumption (Gemmell et al., 2013).

In Ethiopia, particularly in Bahir Dar town there is no information about the bacteriological quality of pre-harvest vegetables and irrigation water; and also no continuous assessment of vegetable safety has been developed in the irrigated site along Abay river. In addition, farming practices of vegetables and Abay river water used for irrigation purpose are poor. These factors may affect the bacteriological quality and safety of vegetables which cause a serious health risks to the consumers. Therefore, this study was undertaken to

evaluate the bacteriological quality and safety of green pepper and tomato grown along Abay river.

MATERIALS AND METHODS

Sampling Techniques

Vegetables: A cross-sectional study was carried out on a total of 60 (30 tomato and 30 green pepper) vegetable samples were collected from each purposively selected farm sites ('Bezawit' and 'Gudguad') of Bahir Dar town were taken from December 2014 to April 2015 for bacteriological analysis. From each selected sites, one kilogram of vegetable samples; 500 g of tomato and green pepper were purchased and collected aseptically before harvesting by using sterile surgical glove. The collected samples were then transported to Microbiology Laboratory in the icebox and then vegetable samples were analyzed within an hour of procurement. From the samples, physically damaged and spoiled vegetables were excluded in the study. Additional information on risk factors of farming and sanitation practices of vegetables were also taken from both sites by checklist at the same time.

Irrigation water: A total of 60 water samples, 30 from each (Bezawit and Gudguad) was collected from purposively selected sites. 200 ml of water sample were collected in sterile glass bottle from Abay river from the source where the farmers drew and also collected according to the Germen standard (DIN, 2010) between 7:00 am to 8:00 am. Water samples were kept in ice box and transported to the laboratory within an hour of collection to keep the normal conditions of the micro flora of samples. The analyses were begun with in a maximum of four hours after the sample arrival at Microbiology Laboratory of Bahir Dar University.

Sample Preparation for Bacteriological Analysis

Bacteriological analysis of vegetable samples: Twenty five gram of tomato and green pepper samples were aseptically weighted from 500 g of each collected samples and homogenized in 225 ml of sterile 0.1% (w/v) buffered peptone water for 3 minutes. Ten-fold serial dilutions (10-2 to 10-4) were prepared from homogenized samples using 9ml sterile saline solution blank as diluents. The homogenate was used to enumerate, isolate and characterize bacteria groups from vegetable samples (Kiiyukia, 2003).

Enumeration of aerobic mesophilic bacteria: One ml of homogenized serial diluted sample from 10-2 to 10-4 were pour plated on to plate count agar in triplicates, appropriately leveled Petri dishes. The plate were allowed to solidify and incubated at 37°C for 24 hours. After incubation the petridishes containing 30 to 300 colonies were selected and counted using colony counter and express as colony forming units per gram (FSSAI, 2012).

Enumeration of total and faecal coliforms

- Total coliforms: Three tube most-probable-number (MPN) method using lauryl tryptose broth was used for enumeration of total coliforms. One ml aliquots from each dilution (102,103 and 10-4) were aseptically transferred in to triplicate tubes containing sterile lauryl tryptose broth (Oxoid Ltd, Basingstoke, Hampshire, England) with inverted Durham's tubes. Incubation was done at 37°C for 24 hours. Tubes showing gas and growth were considered as presumptive positive for total coliforms. Then, a loop full of inoculum from all presumptive-positive lauryl tryptose broth tubes was inoculated into tubes which contained 10ml of Brilliant Green Lactose Bile broth and incubated at 37°C for 24 hours. Following incubation period, BGLB broth tubes were observed for gas formation in the Durham tubes. All positive BGLB broth tubes were considered positive for coliform confirmation. The number of coliforms was estimated from MPN table. For data analysis purposes, total coliform counts that were less than the detection limit (<3.0 MPN/g) were assigned 1.5 MPN/g, which is midway between absence of colonies and the detection limit (FDA/CFSAN, 2001).
- Faecal coliforms: The same procedure as of total coliforms was carried out for fecal coliforms, triplicate tubes containing Lauryl Tryptose broth (Blulux Laboratorie Ltd, India) with inverted durham tubes incubated at 44.5°C for 24 hours. Then, confirmatory test for fecal coliforms was done using MacConkey broth with inverted Durham tubes and incubated at 44.5°C for a maximu m of 24 hours. Confirmation was obtained by gas production. The result was reported as the most probable number (MPN) per gram of food (FDA/CFSAN, 2001).

Bacteriological analysis of Abay River water samples

- Enumeration of total and faecal coliforms: Fifteen culture tubes were used per sample; five tubes contained sterile 10 ml double strength and the remaining ten contained 10 ml single strength Lauryl tryptose broth (Blulux Laboratorie Ltd, India) with inverted Durham tubes. Each water sample was shaken vigorously several times before transferring into broth to obtain a homogenous dispersion of microorganisms. With a sterile pipette, 10 ml of the water sample was aseptically dispensed into each of the five culture tubes containing the double strength Laurel tryptose broth. One milliliter of the sample was then inoculated into each of the second five culture tubes and 0.1 ml inoculated into the remaining five

tubes all containing sterile single strength Laurel tryptose broth. The tubes were gently shock to distribute the sample uniformly throughout the medium and incubated at 37°C for 48 hours. After 48 hours of incubation, the cultures were observed for the presence of acid production (color change from reddish purple to yellow) or gas formation (displacement of medium from inverted Durham tube). Tubes showing gas and growth will be considered as positive for total coliforms. Finally results reported as MPN/100 ml using Most Probable Number method.

The same procedure as total coliforms was also used for faecal coliforms with Laurel tryptose broth (Blulux Laboratorie Ltd, India) and inverted Durham tubes. Then the tubes were incubated at 44.5°C for 24 hours. Tubes showing growth and gases were considered as positive for faecal coliforms. Finally results reported as MPN/100 ml using Most Probable Number method.

Isolation of pathogens from Green Pepper and Tomato

- Escherichia coli spp.: The procedure continued from each positive MacConkey broth used during faecal coliforms detection, loop full of inoculum was streaked on Eosin Methylene Blue (EMB) agar using a sterilized loop, and was incubated at 37°C for 24hrs. Escherichia coli colonies were differentiated by their characteristic green metallic sheen due to the fermentation of lactose. Potential positive plates were sub-cultured on nutrient agar plates and incubated at 37°C for 24 hrs. After incubation period, typical *E. coli* colony was streaked on Trypetic Soy Sgar (TSA) slant. The slant was incubated at 37°C under aerobic atmosphere for 24 hours. Finally the slant was maintained in 5°C for the purpose of biochemical characterization.
- Salmonella and Shigella spp.: In the vegetable samples, Salmonella and Shigella spp. may be present in low numbers in addition to other microorganisms, and they may be injured. To diminish the risk of obtaining false negative results, pre-enrichment (peptone water) and selective enrichment (Selenite Cysteine broth) were used. The homogenized samples from surface of each vegetables were incubated at 37°C for 24 hours for pre-enrichment. From each pre-enriched samples, one ml was transferred in to tubes containing 10 ml of Selenite Cysteine broth (HiMedia ltd, India) and thoroughly mixed for two minutes. Following mixing up, tubes were incubated at 37°C for 24 hours (FDA/CFSAN, 2001). A loop-full of inoculum was aseptically taken from each incubated Selenite Cysteine broth and streaked onto Xylose Lysine Deoxycholate agar (XLD) (Oxoid, England) for Salmonella and Shigella spp. MacConkey agar (Oxoid, England) was also used additionally for Shigella, which were then incubated at 37°C for 24 hours. Morphologically, pink colonies with or without black centers were assumed to be presumptive Salmonella and red or pink colonies were assumed to be presumptive Shigella on XLD agar but on MacConkey agar smooth colorless, opaque or transparent colonies were also assumed to be presumptive Shigella (FSSAI, 2012). The presumptive colonies from XLD agar were aseptically picked and streaked on to nutrient agar (Don Whitley, India) for purification purpose and incubated at 37°C for 24 hours. Pure colonies were also transferred aseptically in to Triptic Soya agar (TSA) (Don Whitley ltd, India) slants as stock cultures and stored in refrigerator at 4-5°C. The pure cultures were then subjected to biochemical tests like Citrate utilization test, Motility test, Indole test, Lactose fermentation and H_2S production test, Lysine Iron agar test and Urea hydrolysis test (Kiiyukia, 2003; FSSAI, 2012).

Data analysis: All the data were analyzed with SPSS version 20.0 for Windows software. The significance between the values was evaluated at 95% confidence level. Statistical significance was set at $P<0.05$. Statistical analysis of the data from the two vegetables was performed using T-test to test whether there is a statically significance difference between the two vegetables for microbial load. One way ANOVA was also used to compare mean bacterial count of vegetables from different locations. The results obtained for cfu/gm of juices were transformed into log values.

RESULTS AND DISCUSSION

Aerobic Mesophilic Counts

In the present study, the mean value of tomato was 4.72 log10 cfu/g and 5.01 log10 cfu/g was documented in green pepper samples (Table1). There were statistically significant differences between the mean AMC of Tomato fruit and green pepper surface ($p=0.002$). This could be due to tomato has a pH of below 4 where most bacteria inhibited, which may have contributed to the lower bacterial count. Hazard analysis and critical control point total quality management (HACCP-TQM) technical guide line set the microbial quality standard for raw foods where by the food containing <4, 4.0-6.7, 6.7-7.7 and >7.7logcfu/g aerobic plate count rated as good, average, poor and spoiled food, respectively (Berger et al., 2010).

Table 1. Mean and range of aerobic mesophillic bacteria (log10cfu/g) of irrigated tomato and green pepper with Abay river in Bahir Dar town, 2015.

Vegetable type	No. of samples	Mean ± SD	Minimum	Maximum	p- value
Tomato	60	4.72 ± 0.53	3.02	5.98	0.002
Green pepper	60	5.01 ± 0.47	3.5	6.37	

SD= Standard deviation

Based on the above criteria, all the samples in this study are categorized as good and average in its microbial quality; however, there is need to safeguard the health of the final consumers by proper washing of these products which are consumed in their raw forms.

The finding of the result showed that, higher mean counts of aerobic mesophilic bacteria was recorded in green pepper (mean value of 5.14log10 cfu/g) and (mean value of 4.85 MPN/g), whereas, least value of aerobic mesophilic counts was obtained in tomato (mean value of 4.74logcfu/g) and (mean value of 4.64logcfu/g) in Bezawit and Gudguad sites, respectively.

Even though mean variation between sites, there was no statistical significance difference of mean aerobic bacterial count in tomato (p=0.852). But statistical significance difference of mean aerobic count was obtained in green pepper (p=0.01) between sites (Figure 1).

Total Coliforms Counts

The present study demonstrated that total coliform counts of tomato was a mean value of 234.9 MPN/g and green pepper was mean of 303.2 MPN/g (Table 1). Even though mean variation between vegetables, there was no statistical significant deference in coliform counts between vegetable types (p=0.252) (Table 2).

Table 2. Mean and range of total and faecal coliform bacteria (log10cfu/g) of irrigated tomato and green pepper with Abay river in Bahir Dar town, 2015.

Vegetable type	No. of samples	Mean ± SD	Minimum	Maximum	p- value
Tomato	60	234.9 ± 290.2	3	1100	0.25
Green pepper	60	303.2 ± 356.0	6.1	1100	

SD= Standard deviation

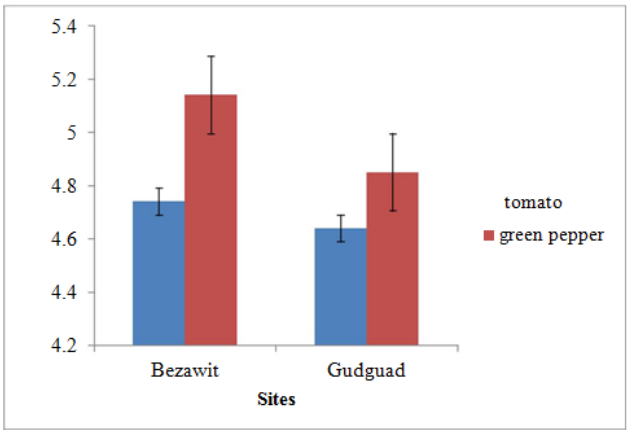

Figure 1. Aerobic mesophillic bacteria (log10 cfu/g) of irrigated tomato and green pepper with Abay River between sites in Bahir Dar town, 2015.

The finding of the study also revealed that 38 (31.7%), 69 (57.5 %) and 13 (10.8%) out of the total samples had level of coliform contamination categorized as good, acceptable and unsatisfactory, respectively. Five (8.3%) of tomato and 8 (13.3%) of green pepper samples were above the accepted limit (≥ 1000MPN/g) (Table 3).

The mean values of total coliform counts of tomato samples obtained from Bezawit and Gudguad sites

Table 3. Level of Coliform contamination categorized as good, acceptable and unsatisfactory.

Vegetable type	Good	Acceptable	Unsatisfactory
Tomato	22 (36.7%)	33 (55%)	5 (8.3%)
Green pepper	16 (26.7%)	36 (60%)	8 (13.3%)

were 273.5 and 196.3 MPN/g, respectively whereas mean total coliforms in green pepper from the two sites were 378.1 and 228.4 MPN/g, respectively

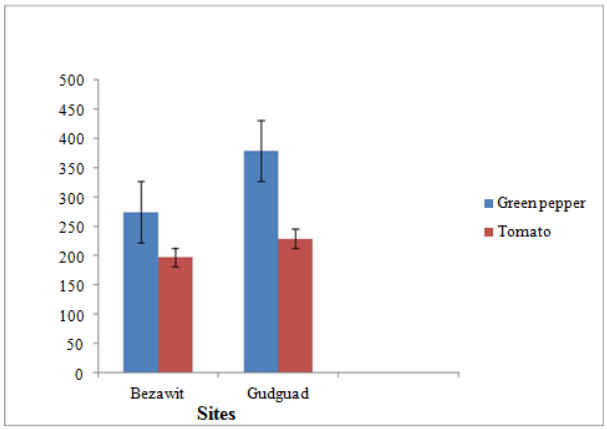

Figure 2. Total coliforms count (MPN/g) of irrigated tomato and green pepper between the two sites in Bahir Dar town, Ethiopia, 2015.

Table 4. Mean and range of fecal coliforms count (MPN/g) of irrigated tomato and green pepper in Bahir Dar town, 2015.

Vegetable type	No. of samples	Mean ± SD	Minimum	Maximum	p- value
Tomato	60	45.08 ± 37.69	3	160	0.1
Green pepper	60	58.10 ±47.35	3	240	

Table 5. The number and percentage of good and unsatisfactory level of fecal coliforms in irrigated tomato and green pepper in Bahir Dar town, Ethiopia, 2015.

Vegetable type	**Good No (%)**	**Acceptable No (%)**	**Unsatisfactory No (%)**
Tomato	13 (21.7%)	NA	47 (78.3%)
Green pepper	7 (11.7%)	NA	53 (88.3%)
NA= Not applicable			

This study also revealed that the percentage of tomato and green pepper contaminated by fecal coliforms were 13 (21.7%) and 7 (11.7%), categorized as good and the remaining 47 (78.3%) tomato and 53 (88.3%) green pepper categorized as unsatisfactory respectively.

Faecal Collforms Count

In the present study, the mean fecal coliforms count of tomato was 45.08 MPN/g and similarly green pepper was 58.1MPN/g. Although mean variation between vegetable items, there was no statistical significant differences in mean fecal coliforms count between vegetables (p=0.097).

NA= Not applicable

In which, the mean contamination of tomato by fecal coliforms was obtained 56.6MPN/g and 38.7MPN/g in Bezawit and Gudguad sites; whereas green pepper had the mean contamination of 70.87MPN/g and 51.76 MPN/g in Bezawit and gudguad respectively (Figure 2).

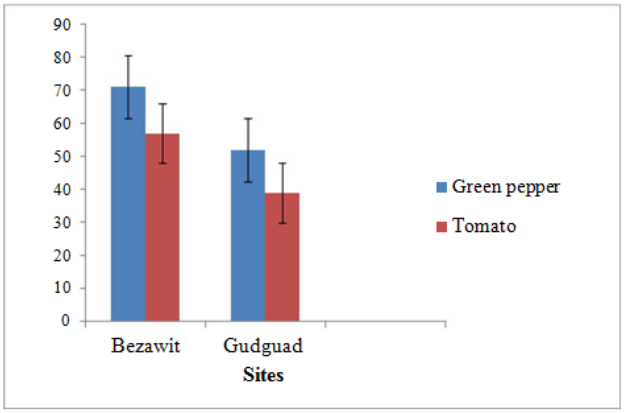

Figure 3. Fecal coliforms count (MPN/g) of irrigated tomato and green pepper between sites in Bahir Dar town, Ethiopia, 2015.

There were mean variation between study sites. However there was no statically significant difference in tomato (p=0.391) and green pepper (P=0.305) between sites (Table 4). Overall, the presence of faecal coliforms in food particularly in irrigated tomato and green pepper is indicative of recent contamination and there is a greater risk that pathogens may also be present (Table 5).

Pathogenic Bacteria in Tomato and Green Pepper

Total number of bacteria that were isolated from both vegetable samples in Bezawit and Gudguad site was demonstrated below (Table 6). From each 60 vegetable samples, Salmonella 5 (8.3%), Shigella 3 (5%) and *E. coli* 6 (10%); and Salmonella 6 (10%), Shigella 5 (8.3%) and *E. coli* 9 (15%) were detected from tomato and green pepper samples respectively.

Table 6. Frequency of occurrence of Salmonella, Shigella and E. coli isolates associated with irrigated tomato and green pepper along Abay river in Bahir Dar town, 2015.

Organisms	**Vegetables**	
	Tomato No (%)	**Green pepper No (%)**
Salmonella spp.	5 (8.3)	6(10)
Shigella spp.	3 (5)	5(8.3)
Escherichia coli	6 (10)	9(15)
Total	14 (23.3)	20 (33.3)

Total number of bacteria that were isolated from both vegetable samples in Bezawit and Gudguad site was demonstrated below (Figure 3).

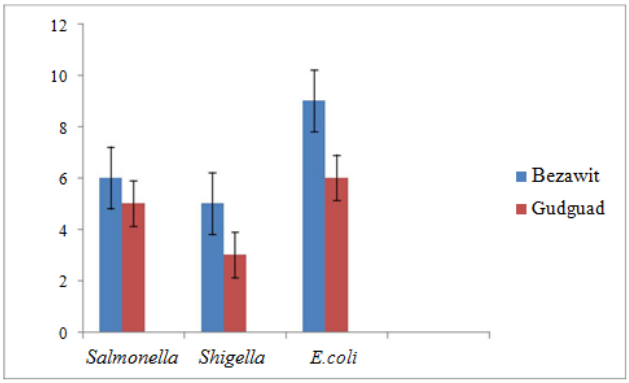

Figure 4. Isolated bacteria frequencies in irrigated tomato and green pepper samples between sites in Bahir Dar town, 2015.

Bacteriological Analysis of Irrigation Water

Total coliforms: The mean total coliform count of gudguad was 711. 67MPN/100ml and mean value of (1040 MPN/100 ml) was recorded in Bezawit site. The present study in total coliorm counts revealed that, 10 (33.3%) in Bezawit site and 4 (13.3%) of the samples in Gudguad sites were over in upper limit (≥ 1600MPN/100 ml) (Figure 4). There were statistically significant difference in mean total colifom counts of irrigation water (p=0.002) between sites (Table 7). The difference of aerobic mesophilic counts between sites may be due to the location of the two farming sites along the river flow, Gudguad site compared to Bezawit located on the river entry point to the town, which was less exposed to a chance of contamination of different residential, industrial and commercial effluents, whereas downstream was exposed to these wastes and effluents from the town.

Table 7. Total coliforms (MPN/100 ml) of Abay river water used for irrigation of vegetables in Bahir Dar, 2015.

Farming site	Mean count ±SD	Range MPN/100ml	p-value
Bezawit	1040.00 ± 617.05	350 - 1600	0.001
Gudguad	711.67 ± 592.05	170 - 1600	

Faecal Coliforms In Irrigation Water

In this study, the mean faecal coliform counts of irrigation water at Bezawit was 689.67MPN/100m and 390.00MPN/100 ml was observed at Gudguad site. There was statistically significance difference in irrigation water sample between the two sites (P=0.010) (Table 8). The difference among sites might be faecal indicator counts increased from upstream to downstream.

Table 8. Faecal coliforms (MPN/100ml) of Abay river water used for irrigation of vegetables in Bahir Dar, Ethiopia, 2015.

Farming site	No. of samples	Mean ± SD	Range MPN/ 100ml	p-value
Bezawit	30	689.67 ± 572.04	110- 1600	0.01
Gudguad	30	390 ± 226.04	90- 900	

The detection of pathogens like *Salmonella* and *Shigella spp*. in 25 gm of vegetable samples examined is regarded as potentially hazardous to consumers and is unacceptable for consumption (Berger et al., 2010). This also indicates the necessity for observing hygienic conditions during production and preparation. Because such type of contamination can occur from water, soil, waste and humans who can be carriers of pathogenic species like *Salmonella* and *Shigella* that eventually transfer these foodborne hazards to consumers (Brooks, 2014).

Assessments of Farmer Practices, the Farming Sites and the Irrigation Water

Assessments of farmer practices during irrigation: Among 60 farm workers 7 (21.9%), 4 (12.5%) and 12 (37.5%) of *Salmonella, Shigella spp.* and *E. coli* were isolated from those farmers who did not get formal education in the school; and 2 (14.3%), 2 (14.3%) and 1 (7.2%) of *Salmonella, Shigella* and *E. coli* were isolated from farmers who attend up to elementary schools respectively. Whereas 2 (20%) of Salmonella 1 (10%) of Shigella and 1 (10%) of *E. coli* were isolated from farm workers who complete their education and the remaining 1 (3.2%) of *E .coli* were isolated from certified growers. With regard to this, the present study found that educational status has significant association with the presence of *E. coli* in tomato and green pepper at farm level (p= 0.042) (Table 9). But *Salmonella* and *Shigalla spp* in this study did not have significant association with educational status in fresh vegetable.

From those farmers, who were not get training about good manufacturing practice, 8 (22.9%) of *Salmonella spp*, 6 (17.1%) of *Shigella* spp. and 12 (34.3%) of *E. coli* were isolated. As a result there were statistical significant associations with the presence of only *E. coli* with (p=0.42).

Table 9. Association between farming practice with isolation of Salmonella, Shigella and *E. coli* from tomato and green pepper along Abay river in Bahir Dar town, 2015.

Parameter	Frequency (%)	*Salmonella spp.*		*Shigella spp.*		*E. coli*	
		Positive No (%)	Negative No (%)	Positive No (%)	Negative No (%)	Positive No (%)	Negative No (%)
Educational status							
No schooling	32 (53.3)	7 (21.9)	25 (78.1)	4 (12.5)	28 (87.5)	12(37.5)	20 (62.5)
Elementary	14 (23.3)	2 (14.3)	12 (85.7)	2 (14.3)	12 (85.7)	1(7.2)	13(92.8)
High school	10(16.7)	2 (20)	8 (80)	1 (10)	9(90)	1 (10)	9 (90)
Graduated	4(6.7)	0	4 (100)	0	4 (100)	1	3 (75)
Special clothing							
Yes	9 (15)	3 (33.3)	6 (67.7)	2 (22)	7 (78)	3 (33.3)	6 (67.7)
No	51 (85)	8 (15.6)	43 (84.4)	6 (11.8)	45 (88.2)	12 (23.5)	39 (76.5)
Farming experience							
Yes	44(73.3)	7(15.9)	37(84.1)	5(11.4)	39(88.6)	10(22.7)	34(77.3)
No	16(26.7)	4(25)	12(75)	3(18.8)	13(81.2)	6(37.5)	10(62.5)
Training on irrigation							
Yes	25(41.7)	3 (12)	22 (88)	2 (8)	23 (92)	3 (12)	22 (88)
No	35(58.3)	8 (22.9)	27 (77.1)	6 (17.1)	29 (82.9)	12 (34.3)	23 (65.7)
Means to control contamination							
Yes	7 (11.7)	2 (28.6)	5 (71.4)	1 (14.2)	6 (85.8)	2 (28.6)	5 (71.4)
No	53(88.3)	9 (17)	44 (83)	7 (13.2)	46 (86.8)	14 (26.4)	39 (73.6)

Table 10. Association between sanitation practice of farms with isolation of Salmonella, Shigella and *E. coli* from tomato and green pepper along Abay river in Bahir Dar town, 2015.

Parameter	Frequency (%)	*Salmonella spp.*		*Shigella spp.*		*E. coli*	
		Positive	Negative	Positive	Negative	Positive	Negative
		No (%)	No (%)	No (%)	No (%)	No (%)	No (%)
Manure application							
Yes	32(53.3)	9 (28.1)	23(71.9)	4 (12.5)	28 (87.5)	12 (37.5)	20 (62.5)
No	28(46.7)	2 (7.1)	26(92.9)	5 (17.8)	23 (86.2)	3 (10.7)	25 (89.3)
Time of irrigation							
Morning	56(93.3)	11 (19.7)	45 (80.3)	7 (12.5)	49 (87.5)	13 (23.2)	43 (71.7)
Afternoon	4(6.7)	0	4 (100)	1 (25)	3 (75)	2(50)	2(50)
Type of irrigation production system							
Sole cropping	11(18.3)	2 (18.2)	7 (81.8)	2 (18.2)	9 (81.9)	4 (36.4)	7 (63.6)
Mixing	49(81.7)	9(18.4)	40(81.6)	6(12.2)	43 (87.8)	11 (22.4)	38 (77.6)
Hand contact with fresh produce							
Yes	60(100%)	11 (18.3)	49(81.7%)	8 (13.7%)	52 (86.3%)	15 (25%)	45 (75%)
No	000	000	000	000	000	000	000

Assessment of the sanitation practice of farms that is used for irrigation purpose: In the present study 9 (28.1%) of *Salmonella spp.* 3 (10.8%) of *Shigella* and 12 (37.5%) of *E. coli* were isolated from those farmers used manure as a fertilizer and the remaining 2 (7.1%) of *Salmonella* 5 (17.3%) of *Shigella* and 3 (10.3%) of and *E. coli* were isolated from farmers that were not used manure as a fertilizer. As a result, Manure application had statistically significant association with the presence of Salmonella and *E. coli* in tomato and green pepper samples grown along Abay river with (P= 0.036) and (p= 0.017), respectively (Table 10).

General characteristics of attributes for the quality of irrigation water: In this study, 8 (29.7.6%) of *Salmonella*, 7 (26%) of *Shigella* and 14 (51.8%) of *E. coli* were isolated from tomato and green pepper irrigated with water sources had a chance of contamination of feces and urine. With regarded to this the present study found that contamination of feces and urine with irrigation water has statistically significant association with the presence of *Salmonella* (p=0.04), *Shigella* (p=0.02) and *E. coli* (p= 0.001) in tomato and green pepper samples grown along Abay river (Table 11).

Regarding to the system of irrigation, 7 (36%) of Shigella and 11 (57.9%) of *E. coli* were isolated from those vegetable grown with surface irrigation system, whereas 1 (4.3%) of *Shigella spp*. and 2 (8.6%) of *E. coli* were isolated from those used other than surface irrigation system (Table 10). There was significant association between the presences of *Shigella spp*. (p=(0.03) and *E. coli* (p=0.00) with the system of irrigation of tomato and green pepper grown along Abay river (Table 11).

Table 11. Association between sanitation of irrigation water with isolation of Salmonella, Shigella and *E. coli* from tomato and green pepper along Abay river in Bahir Dar town, 2015.

Parameter	Frequency (%)	*Salmonella spp.*		*Shigella spp.*		*E. coli*	
		Positive	Negative	Positive	Negative	Positive	Negative
		No (%)	No (%)	No (%)	No (%)	No (%)	No (%)
Conatmination with feces							
Yes	27 (71.7)	8 (17.6)	18 (82.4)	7 (13.7)	20 (86.3)	13 (25.5)	14 (74.5)
No	33 (28.3)	3 (22)	30 (88)	1 (11)	21 (89)	2 (22.2)	29 (77.8)
Waste disposal to river sites							
Yes	38 (63.3)	6 (15.8)	32 (84.2)	5 (13.2)	33 (86.8)	9 (23.7)	29(76.3)
No	22 (36.7)	5 (22.7)	16 (81.3)	3 (13.6)	19 (86.4)	6 (27.3)	16 (72.7)
System of irrigation							
Surface	19 (31.6)	5 (26.3)	13 (74.7)	7 (36.8)	12 (63.2)	11 (57.9)	8 (42.1)
On the Soil	41 (68.4)	6 (14.6)	35 (85.4)	1 (2.4)	39 (97.6)	4 (21.1)	37(88.9)
Access of animal Irrigation water							
Yes	14 (23.3)	4 (28.6)	10 (72.4)	3 (21.4)	11 (78.6)	6 (42.9)	8 (57.1)
No	46 (68.3)	7 (15.2)	39 (84.8)	5 (10.9)	41 (89.1)	10 (21.7)	36 (78.3)

CONCLUSION

The finding revealed that all the samples were contaminated with one or more indicator bacteria (aerobic mesophilic bacteria, total and faecal coliforms). The majority of tomato and green pepper samples were at satisfactory level in case of aerobic mesophilic bacteria and total coliforms, but almost all tomato and green pepper samples were contaminated with faecal coliforms exceeded level for the maximum rang allowable. The use of polluted irrigation water and fresh animal manure may account for the high levels of fecal coliform contamination recorded in most of the analysed vegetable samples. In addition to this, *E. coli*, Salmonella and Shigella were also isolated from irrigated samples. So, farm owners that produce vegetables should be aware that preventative measures through food safety control strategies is important. Education level may not have relationships for the level of pathogens in the produce. Further study should be recommended after harvest at market level together with isolation of other pathogens.

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