International Journal of Bioprocess & Biotechnological Advancements
IJBBA, 4(1): 127-143
www.scitcentral.com

ISSN: 2380-0259

Original Research Article: Open Access

The Effect of Irrigation Water the Microbial Quality and Safety of Vegetables: The Case of "Bisnet" River at Gondar Town

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Received December 04, 2018; Accepted December 21, 2018; Published December 30, 2018

ABSTRACT

Vegetables are an extraordinary source of nutrients, micronutrients and vitamins for humans and are thus vital for health and wellbeing. Continued use of untreated waste water and manure as fertilizers for the production of vegetables is a major contributing factor to contamination. Microbial spoilage and contaminating pathogens pose a serious problem in food safety. Fresh produce can incorporate pathogenic microorganisms through the process of irrigation, harvesting, postharvest processing and distribution. The objective of this study was to assess the effect of irrigation water on the microbial quality and safety of in Gondar town. Fifty four vegetable yields and eighteen water (a total of seventy two) samples were collected from six farming sites for analysis. The water and vegetable quality was analyzed for total and fecal coliforms. Aerobic mesophilic, total coliforms, fecal coliforms counts were determined using standard methods. Additionally, 36 observation frequencies were used. Mean aerobic mesophilic count, total coliforms, fecal coliforms of vegetable yields were (4.93 to 4.90); (720.00 to 524.44) and (321.4 to 244.3) among the six sites, respectively. *Escherichia coli*, Enterobacter species, Proteous species, Klebsella species, Pseudomonas species and Penicillum species was isolated from vegetables. The water and vegetables samples showing high microbial load. The observational check list reveled that, the majority of growers did not practice sanitary cultivation and the farming environment also found unhygienic. One way ANOVA was used for comparing coliform counts among the six sites. There was statistically a significance difference in coliform counts of vegetable yields among sites ($p<0.05$). Most of the water samples were found to be contaminated. Prevention of contamination of fresh produce from both pre-harvest and post-harvest sources especially irrigation water still remains the only effective way to protect the public and to reduce the bacterial load.

Keywords: Coliform, Contamination, Irrigation water, Raw vegetables, Total and fecal coliform

INTRODUCTION

Vegetables are an extraordinary source of nutrients, micronutrients and vitamins for humans and are thus vital for health and wellbeing. Well balanced diets, rich in fruits and vegetables, are especially valuable for their ability to prevent vitamin C and vitamin A deficiencies and are reported to reduce the risk of several diseases [1]. Fresh produce can incorporate pathogenic microorganisms through the process of irrigation, harvesting, post-harvest processing and distribution. Most microorganisms use irrigation water and/or soil as a vehicle of transport [2,3]. In several African cities, between 50 and 90% of the vegetable consumed are produced within or close to the city [4]. The consumption of fresh vegetables has been increasing as consumers strive to eat healthy diets and the availability of these produce, up till recently considered as seasonal, has been extended over the whole year [5].

The consumption of "four range" vegetables, a term that refers to packaged, cleaned, possibly chopped and mixed vegetables ready to be seasoned and eaten, have gained popularity among consumers [6]. Fresh vegetables normally carry natural non-pathogenic epiphytic microorganisms, but during growth, harvest, transportation and further handling the produce can be contaminated with pathogens from animal and human sources [5]. As most of these produces are eaten without further processing, their microbial content may represent a risk factor for the consumer's health and therefore a food safety problem [4].

Irrigation water is an important alternative source of water for irrigation. However, apart from plant nutrients, it may contain various potentially toxic elements and organic matter

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Citation: Nega R & Sahile S. (2018) The Effect of Irrigation Water the Microbial Quality and Safety of Vegetables: The Case of "Bisnet" River at Gondar Town. Int. J. Biopro. Biotechnol. Advance, 4(1): 127-143.

with highly harmful effects on human and animal health [7]. Wastewater contains relatively high amounts of sodium, which can be accumulated in the soil during irrigation with this wastewater and display toxic effects on the plants [8]. In Africa, more than one-third of the population already lives in urban cities, and over the next 25 years starts from 1998, the rapid urbanization of Africa without a corresponding increase food production could lead to food insecurity in the cities [9]. According to Stine et al. [8], the factors that affect the transfer of pathogens from contaminated irrigation water to fresh produce are the type of vegetables, the irrigation method and the number days between the last irrigation event and harvest. The largest portion of freshwater in Africa is used for agricultural purposes and thus the use of grey water for irrigation may lead to municipal sources being available for other purposes [10]. This will additionally lead to the reduction in cost. However, the problem is that systems are not in place in South Africa to assure that this type of water is of sound microbial quality. For this to be a success, all involved parties should be properly educated [11].

Globally, over 70% of freshwater consumption is devoted to agricultural activities [6]. The declining productivity of commercial farms has led international policy networks to recommend the promotion of urban and peri-urban (UPA) agriculture as an escape from food crisis situations [12]. However, many households in poor areas lack access to fertilizers and have a limited supply of fresh water [13]. Wastewater treatment and reuse at the individual level can provide a combined solution to these problems by supplying the water and nutrients needed for household food production [6]. Indeed, this strategy is already in use by millions of farmers worldwide and it is estimated that 10% of the world's population consumes foods irrigated with wastewater [12]. Wastewater treatment and reuse for irrigation may well hold the key to easing demand on limited freshwater reserves while improving the food production capacity of households and farms [13]. However, this practice is known to have adverse public health and environmental effects, especially because untreated wastewater or polluted water has high levels of pathogenic organisms [2].

Urbanization in the developing world is proving to be one of the greatest challenges of the 21st century with annual growth rate of 5.8% in Sub-Saharan Africa [14]. The rapid rate of urbanization and the consequential rise in surface water pollution by wastewater discharge, combined with the scarcity of freshwater for irrigation in most cities, especially in arid areas, has led to a renewed interest in wastewater irrigation since the 1950's [1]. Numerous disease outbreaks are linked to contaminated fruit and vegetables [15]. Urbanization in the developing world is proving to be one of the greatest challenges of the 21st century 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 [16].

There have been some outbreaks of diseases like typhoid in Santiago, Chil and helminthes infections in Egypt and Jerusalem that have been associated with vegetable contamination from wastewater irrigation [4]. The frequency of outbreaks epidemiologically associated with raw vegetables have increased in some industrialized countries as a result of change in dietary habits and increased import of food [5]. In developing countries, foodborne illnesses caused by contaminated vegetables are frequent and in some areas they cause a large proportion of illness [17]. However, due to lack of foodborne disease investigation and surveillance in most of these countries, most outbreaks go undetected and the scientific literature reports only on very few outbreaks [2]. Untreated water is most likely to transmit several microorganisms, which may include pathogenic strains of Escherichia coli, Salmonella, Listeria, protozoa and viruses [15]. Studies in different countries indicate that the use of untreated water for irrigation of vegetables is the practice most related to fresh produce safety issues [18]. Contamination usually occurs before, during or after irrigation. In addition, the inhalation of aerosols from wastewater can affect farmers and surrounding communities [15].

The microbial safety of fresh vegetables is of global concern. Microorganisms that can cause illness or disease, collectively known as pathogens, are usually associated with human or animal fecal matter present in irrigation water and surface water sources [19]. Irrigation water contaminated with pathogens has often been blamed for outbreaks of foodborne illness. Efforts to minimize the microbial contamination of fresh vegetables are essential and timely. However the current microbial contaminations of leafy vegetables at Gondar town are not known. So, the aim of this study is to assess the microbial quality and safety of leafy vegetables cultivated using irrigation water on "Bisnet" River in Gondar town.

Significance of the problem

It is expected that the result of this study will provide necessary information on the hygienic situation of a number of water samples and leafy vegetables taken from Bisnet River in Gondar town, and some potential risk factors associated with it. It will provide a framework to assess the on-going vulnerability of the supply to contamination and the major health problems that may make future contamination likely. It will also help for awareness creation in the health and sanitary workers of the town to minimize the microbial contamination of fresh vegetables.

General objective

- The main objective of this study is to assess the effect of irrigation water on microbial quality and safety of

vegetable yields, in Gondar town, the case of Bisnet River.

Specific objective

- To assess the bacteriological water quality of irrigation water sources used for cultivation of cabbage, lettuce and spinach.
- To enumerate aerobic mesophilic count, fecal coliform and total coliform bacteria of leafy vegetables and irrigation water.
- To isolate and characterized the potential pathogens from the vegetables.
- To assess the safety practices around the farm sites and the irrigation water.

MATERIAL AND METHODS

Description of the study area

The study area is found in Gondar town, Amhara Regional State, Northern West Ethiopia. It is located 739 km far from Addis Ababa. Geographically Gondar is bounded by 12°36' N and 37°28' E longitude and it has a narrow range of altitude, i.e., 2133 m above sea level. Based on the Central Statistical Agency of Ethiopia (2012), Gondar has a total population of 308,257. The range of average monthly temperature is 19.5°C and the warmest average max/high temperature is 29°C (84°F) in March and May. The coolest average min/low temperature is 10°C (50°F) in December and January. The mean relative humidity for an average is recorded as 55.7% and on a monthly basis it ranges from 40% in January and February to 79% in July (http://www.gondar.climatemaps.com).

Bisnet River is crossing Gondar town and used for irrigation during the dry season of the year. Irrigation activities occur during months between October and May. Most of the growers are women, men and youth. Urban local growers use the river water for cultivating vegetables like lettuce, spinach, cabbage and tomato. Bisnet River crossing the town is most commonly used for disposal of sewage, animal and human feces from the community and sludge from factories and clinics. Therefore, the exposure of the irrigation water to different sources of pathogens and unsafe farm handling practices permits for microbial contamination of raw fresh produces such as lettuce, cabbage and spinach that are eaten raw or unprocessed resulting in foodborne disease **(Figure 1)**.

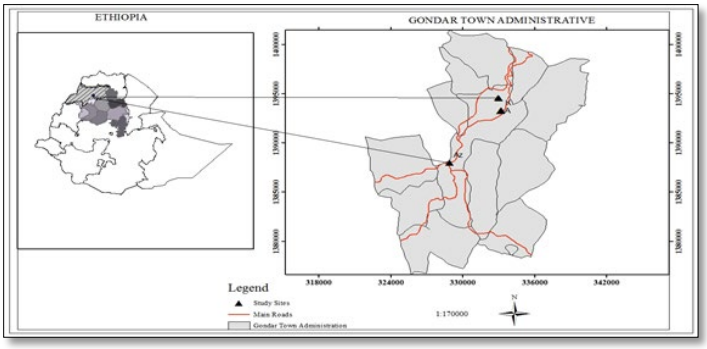

Figure 1. Location of rivers in Gondar town.

Source: Map from Gondar administrator

Design of the study

A cross sectional and experimental based study was conducted in Gondar town from October to December, 2013 to assess bacteriological quality of leafy vegetables like cabbage (*Brassica oleracea* L), spinach (*Spinacea oleracea*) and lettuce (*Lactuca sativa* L.) cultivated in six farming sites in connection with irrigating water. Additionally, sanitary practices around the farm and irrigation water were assessed using observational check list.

Vegetables and irrigation water sample collection

A total of 54 samples (9 from each farming site) comprising three types of fresh vegetables (cabbage, spinach and lettuce) and 18 water sample (3 from each farming sites), together a total of 72 samples were collected using simple random sampling technique from six farm lands on "Bisnet" vegetable farms around Gondar from October 2013-December 2013. Leafy vegetable samples in the six farming sites and the corresponding irrigation water (W) were coded as site 1 (Lettuce) (L1, L2, L3, L4, L5, L6,L7, L8, L9 and W1, W2, W3), site 2 (Lettuce) (L1, L2, L3, L4, L5, L6, L7, L8, L9 and W1, W2, W3), site 3 (Cabbage) (C1, C2, C3, C4, C5, C6, C7, C8, C9 and W1, W2, W3), site 4 (Cabbage) (C1, C2, C3, C4, C5, C6, C7, C8, C9 and W1, W2, W3), site 5 (Spinach) (S1, S2, S3, S4, S5, S6, S7, S8, S9 and W1, W2, W3) and site 6 (Spinach) (S1, S2, S3, S4, S5, S6, S7, S8, S9 and W1, W2, W3). All the collected vegetable samples were from cultivated and ready for harvest.

200 g of all the three leafy vegetable samples were collected in sterile polyethylene bags and each sample bags for each vegetable transported to Gondar University Department of Biology microbiology laboratory for analysis. The samples

were cooled during transportation with ice box to keep the normal condition of the micro flora of vegetables. At the same time, two hundred ml of each water sample was collected from "Bisnet" vegetable farms around six vegetable farm stations in sterile plastic bottles. The samples were analyzed for total coliform count and fecal coliform count according to the procedure described by FSSAI [20]. Each of irrigation water samples was taken from the source where the farmers drew. Samples were collected from 8:00 AM to 9:00 AM and analyzed within eight hours after collection.

Sample preparation

25 g of each leafy vegetable sample was aseptically removed from using a sterile forceps and vigorously shaken in 225 ml of bacteriological peptone water (Oxoid, England). Serial dilutions were prepared using 9 ml sterile normal saline as diluents to enumerate, isolate and characterize bacteria groups from samples [21]. Serial dilutions of samples were made up to 10^{-2} for total and fecal coliforms.

Similarly, from each eighteen (200 ml) water samples of plastic bottles 1 ml was taken and dropped to a test tube containing 9 ml sterile normal saline and thoroughly mixed to get 10^{-1} dilution. From 10^{-1} dilution, 1 ml was transferred to 9 ml of sterile normal saline in another test tube and thoroughly mixed to get 10^{-2} dilution. In such a way, serial dilutions of irrigation water samples were made up to 10^{-4} for total and fecal coliforms [20].

Bacteriological analysis of samples

Enumeration of aerobic mesophilic bacteria of vegetables (spinach, cabbage and lettuce): For aerobic mesophilic count (AMC), plate count agar (PCA) (Oxoid, England) was used as a culture medium [21]. Zero point one ml of samples was taken aseptically from the most diluted one and spread plated in plates. The plates were then incubated at 37°C for 24 h. After growth of organisms, plates with colonies between 30-300 were counted using colony counter. The colonies were counted and reported as CFU/g of spinach, cabbage and lettuce.

Total and fecal coliform population estimations in irrigated vegetables: Total coliforms and fecal coliforms were determined using the most probable number (MPN) method as recommended by APHA-AWWA-WEF [20] and Downes and Ito [22]. For the MPN method one ml of the sample was diluted up to a factor of 10-2 were made. 10 ml, 1 ml and 0.1 ml of the samples were inoculated in to triplicate tubes containing lactose broth (International diagnostics groups PIC, Lancashier, UK) and were incubated at 37°C for total coliforms and 44°C for fecal coliforms for 24-48 h. The tubes were examined for gas production. Tubes with gas formations at the end of the incubation periods were planted in to brilliant green bile broth (BGBB) and incubated at 44°C. Those tubes which formed a gas as a result of incubation process were evaluated according to the MPN table and results of a test were reported as MPN per ml of sample [23]. The inoculums were transferred into a slant media and characterized by biochemical tests such as Indole test, catalase test, Simmone citrate test, methyl red test; VP test and TSI test [21].

Enumeration of total and fecal coliforms from irrigation water: Irrigation water samples were serially diluted before inoculation and incubated at 37°C for total coliform and 44°C for fecal coliforms for 24 to 48 h. Positive tubes (acid or gas production or both) were selected and the numbers of coliforms were obtained from MPN (Most Probable Number) index [20].

Isolation and identification of microbes: The presence of microbes was confirmed by using the following standard method. 1 ml of the sample was diluted in nine ml of lactose broth and incubated at 37°C for 24 h. The sample was examined for gas formation. One loop of culture from lactose positive broth was streak on L-EMB agar (HiMedia, India). The culture was incubated at 44.5°C for 18-24 h. Dark center colonies with metallic sheen were considered as indicative of *E. coli*. The colonies were further confirmed by Indole test, MRVP test, TSI test, catalase test and Simmone citrate test.

The presence of other pathogens like Pseudomonas species, Klebsiealla species, Enterobacter species and Proteus species was characterized and identified by biochemical test. Biochemical test was done using Indole test, MRVP broth, triple sugar iron (TSI) agar (Oxoid), simmone citrate (SCA) agar (Oxoid). Colonies were picked from PCA (plate count agar), MacConkey Agar, Salmonella Shigella agar (Oxoid, England) and incubated in to tryptone soy agar slant. After incubation for about 24 h, a loop full of inoculums was taken for biochemical tests such as Indole test, MRVP test, TSI test, catalase test and simmone citrate test respectively. Thus, colonies were purified and tested biochemically [21].

For identification of fungi Potato Dextrose Agar (PDA) (Don whitely eqp. Pvt. Ltd., India) was used. One ml of the vegetable sample was serially diluted in peptone water. Zero point one ml from each dilution (10^{-1} to 10^{-2}) of the serially diluted sample was placed on PDA agar. The plates were incubated at 21°C for 5-7 days. The identification of the isolates fungi was done according to the microscopic methods. A drops of lacto phenol cotton blue stain (Don whitely eqp. Pvt. Ltd., India) was placed on a clean slid and with the aid of mounted needle, a small portion of the mycelium from the fungal cultures was removed and placed in the drop of the stain. The mycelium was spread very well on the slid and a cover slip was gently lowered on it. The slid was examined under the microscope. The observation was done at low and high power objectives of the microscope [24]. Morphological characters of the hyphae were observed and recorded.

Assessment of the sanitary condition of the farm land and the irrigation water: An Observation check list was used for assessment of the sanitary condition of the farm land and the irrigation water. The assessments were done on (a total of 36 observational frequencies) in the six farms three at the time of sample collection and other three in accidental time.

DATA ANALYSIS

The data collected from all the experiments and field study were subjected to the analysis of SPSS-20 computer software. Average values were used and all the countable dilution were used to calculate the average number of colonies in terms of colony forming unit per gram (cfu/g) or (cfu/ml) for aerobic mesophilic plate count, most probable number per gram (MPN/g) or MPN/ml for the statistical estimation of coliform. $P<0.05$ was taken as statistically significant association.

RESULTS AND DISCUSSION

Aerobic mesophilic count of leafy vegetables

The finding of this result showed, higher mean counts of aerobic mesophilic count was recorded in site one (4.9 log 10 cfu/g) whereas lowest mean count was recorded in site 5 (4.5 log 10 cfu/g). Analysis of variance (ANOVA) showed that aerobic plate count was significantly different ($p<0.05$) among the various study sites.

The high aerobic mesophilic bacterial count might be due to pollution by humans, animals or irrigation water just on the nearby area [25]. The difference in AMC among the study sites may be due to variation cultivation practices of the growers, different pre-harvest handling practices and hygienic of the farming environment. The presence of aerobic organisms in the result reflects the exposure of the sample to any contamination and in general, the existence of favorable conditions for multiplication of microorganisms [26]. A study carried out in Turkey by Aycicek et al. [27], revealed that the aerobic coliform counts were up to 7.4 and 6.9 cfu/g. similarly, the study carried out in Iran by Mohammad et al. [28] were ranged from 4.1 log cfu/g to 8.3 log cfu/g in mixed fresh-cut salads and from 4.3 log cfu/g to 8.3 log cfu/g in mixed green leaves vegetables. The more related finding to this study was a study conducted by Nguz et al. [29] and Johnston et al. [30] reported the means of aerobic mesophilic count ranged from 4.5 to 6.2 log cfu/g on fresh produces and 5.4 log cfu/g to 8.9 log cfu/g in mixed salads **(Table 1)**.

Table 1. Aerobic mesophilic count of vegetables (cabbage, spinach and lettuce) at Bisnet River.

Farming site	No. of samples	Aerobic mesophilic count (log 10 cfu/g)
1	9	4.93 ± 0.026
2	9	4.90 ± 0.028
3	9	4.79 ± 0.056
4	9	4.70 ± 0.055
5	9	4.52 ± 0.064
6	9	4.53 ± 0.079

Note: values are mean ± standard error
Note: Site 1 and 2=spinach; site 3 and 4=cabbage; site 5 and 6=lettuce

Total coliforms count of leafy vegetables

Total coliform count and *E. coli* are used as indicator of the hygienic quality parameter of food. The presence of total coliform is an indicator for enteric pathogens [31]. The present study of total mean total coliform levels of all the three vegetables was ranged from 79.4 to 720.0 in site 3 and site 5, respectively and it exceeds the International Commission on Microbiological Specifications for Food According to ICMSF the acceptable upper limit of total coliforms is 100 MPN/g. Analysis of variance (ANOVA) showed that total coliform count was significantly different ($p<0.05$) among the various study sites.

The World Health Organization [32] has recommended that vegetables to be eaten should be irrigated only with biologically treated effluent that has been disinfected to achieve a coliform level of not more than 100/100 ml in 80% of the samples. The result showed major content of bacterial burden; these results correlate with the probability of the analyzed vegetables to be in contact with the source of contamination during growth: soil, organic fertilizers and irrigation water [33].

A study carried out by Nguz et al. [29] 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. For organic lettuce produced in Spain it was reported that more than 75% of produce samples analyzed exhibited MPN values for presumptive *E. coli* of <30/100 g [34]. Department of Health [35] recommends that raw fruits and vegetables should have

total coliform levels not exceeding 200/g. According to the Department of Water affairs and Forestry, water quality is considered as a determinant of the microbial quality of the final vegetable product **(Table 2)** [36].

Table 2. Total coliform count of leafy vegetables (cabbage, spinach and lettuce) at "Bisnet River".

Farming site	No. of samples	Total coliform MPN/100 g
1	9	720.00 ± 0.384
2	9	524.44 ± 0.055
3	9	79.44 ± 0.907
4	9	91.11 ± 0.641
5	9	99.89 ± 0.476
6	9	103.89 ± 0.571

Note: values are mean ± standard error
Note: Site 1 and 2=spinach; site 3 and 4=cabbage; site 5 and 6=lettuce

Fecal coliform count of leafy vegetables

According to Nguz et al. [29], fecal coliform counts are efficient indicators of sanitization, but the presence of fecal coliforms does not necessarily indicate the presence of a pathogen. The total mean fecal coliform value of all the three vegetable samples was ranges from 321.4 to 39.8 in site 1 and site 3, respectively. Analysis of variance (ANOVA) showed that fecal coliform count was significantly different ($p<0.05$) among the various stations sampled.

The mean fecal coliform levels of all the three vegetables was between the International Commission on Microbiological Specifications for Food [37] recommended level of 10^3 fecal coliform/g fresh weights but the *E. coli* loads detected on all vegetables meant that the river water was unsafe to be used for irrigation. These results correlate with the probability of the analyzed vegetables to be in contact with the source of contamination during growth: soil, organic fertilizers and irrigation water [33,38]. The US-Environmental Protection Agency [39] guidelines have recommended for irrigation of vegetables likely to be eaten uncooked, no detectable fecal coliforms/100 ml are allowed (compared to <1000 FC/100 ml for WHO) **(Table 3)**.

Table 3. Fecal coliform count of leafy vegetables (cabbage, spinach and lettuce) at "Bisnet River".

Farming site	No. of samples	Fecal coliform MPN/100 g
1	9	321.44 ± 0.47
2	9	244.33 ± 0.03
3	9	39.89 ± 0.81
4	9	51.44 ± 0.06
5	9	44.11 ± 0.35
6	9	52.44 ± 0.99

Note: values are mean ± standard error
Note: Site 1 and 2=spinach; site 3 and 4=cabbage; site 5 and 6=lettuce

Total coliforms count of irrigation water sample

The values of total coliform count for irrigation water ranges from 43 MPN/100 ml to 1100 MPN/100 ml. The highest TCC (1100 MPN/100 ml) was recorded in farming site 1, 2, 3 and 4. The lowest TCC (43 MPN/100 ml) was recorded in farming site 6. Analysis of variance (ANOVA) showed that total coliform count was significantly different ($p<0.05$) among the various study sites **(Table 4)**.

Table 4. Total coliform count of irrigation water samples from Bisnet River.

Farming site	No. of samples	Total coliform MPN/100 ml
1	3	886.67 ±0.33
2	3	477.67 ± 0.04
3	3	783.33 ± 0.66
4	3	764.33 ± 0.66
5	3	165.00 ± 0.00
6	3	395.33 ± 0.33

Note: values are mean ± standard error
Note: Site 1 and 2=spinach; site 3 and 4=cabbage; site 5 and 6=lettuce

The California (USA) State Health Department adopted a bacterial standard for unrestricted wastewater irrigation of <2.2 total coliforms/100 ml which was close to the existing drinking water standard. Many countries followed this lead and adopted the same criteria with little or no adaptation to local constraints or to the level of technology available to meet this standard [40]. Duffy et al. [41] showed that irrigation water is the leading pre-harvest and post-harvest source of contamination of produce. Total coliform counts can be considered as a hygiene indicator, especially for fecal contamination. The presence indicates that pathogens might be present due to fecal contamination by human, animal or irrigation water [42].

Total coliform counts also revealed that the water samples had the total counts value (<1100 MPN/ml) shows contamination of the river water **(Table 5)** and the water samples from the sites met the international standards for the guideline limit for fecal coliform bacteria in unrestricted irrigation of vegetables likely to be eaten raw: 10^3 to 10^5 [13].

Table 5. Total coliform (MPN/100 ml) of water used for irrigation for cultivation of leafy vegetables.

Sample	Sample site	TCCMPN/ml
1	1	1100
2	1	460
3	1	1100
4	2	93
5	2	240
6	2	1100
7	3	1100
8	3	150
9	3	1100
10	4	1100
11	4	93
12	4	1100
13	5	210
14	5	75
15	5	210
16	6	43
17	6	43
18	6	43

Note: Farm area 1(1) Farm area 2(2) Farm area 3(3) Farm area 4(4) Farm area 5(5) Farm area 6(6)
**TCC: Total Coliform Count*

Fecal coliform count of irrigation water sample

The present study demonstrated that mean fecal coliform counts of irrigation water range from (65.33 to 386.67 MPN/ml) for site 6 and site 1, respectively **(Table 6)**. The presence of fecal coliform bacteria in aquatic environments indicates that the water has been contaminated with the fecal material of man or other animals. At the time this occurred, the source water may have been contaminated by pathogens or disease producing bacteria or viruses which can also exist in fecal material [43]. Previous studies in Accra show fecal coliform population of irrigation water sources ranging between 4.8×10^3 and 2.8×10^6 100 ml^{-1} [44,45] which exceed the WHO recommended level of 1×10^3 100 ml^{-1} for unrestricted irrigation. WHO recommended that <1000 fecal coliforms/100 ml must be in reclaimed water before it can be used for irrigation [46].

Table 6. Fecal coliform count of irrigation water samples from Bisnet River.

Farm sites	No. of samples	Fecal coliform MPN/ml
1	3	386.67 ± 0.33
2	3	198.67 ± 0.46
3	3	337.67 ± 0.33
4	3	247.67 ± 0.43
5	3	114.33 ± 0.66
6	3	65.33 ± 0.33

Note: values are mean ± standard error
Note: Site 1 and 2=spinach; site 3 and 4=cabbage; site 5 and 6=lettuce

The values of fecal coliform count for water ranges from 23 MPN/ml to 460 MPN/ml. The highest FCC (460 MPN/ml) was recorded in farming site 1, 2, 3 and 4. The lowest FCC (23 MPN/ml) was recorded in farming site 6. Analysis of variance (ANOVA) showed that fecal coliform count was significantly different ($p<0.05$) among the various study sites.

The presence of fecal coliform is an index of the bacteriological quality of water [47]. The US-Environmental Protection Agency (US-EPA) in their 1992 guidelines has recommended the use of much stricter standards for wastewater use in the USA, than those recommended by the WHO. The main guideline is that fecal coliforms should not exceed 14 MPN/100 ml in any sample, which in practice means not detectable **(Table 7)**.

Isolated pathogens in the growing field

A total of eighteen samples were tested to isolate pathogens from the three leafy vegetables among the six sites are shown in **Table 8**. Five bacteria and one fungus were isolated: *Escherichia coli*, Klebsiella species, Proteus species, Enterobacter species, Pseudomonas species and fungus (Penicillum species).

Normally, *E. coli* does not cause disease although some strains frequently cause diarrhea in travelers [48] and it is the most common cause of urinary tract infections. Johannessen et al. [49] reported that E. coli is more specifically associated with fecal contamination and is a more appropriate indicator organism of fecal contamination of fresh produce. The common microorganisms isolated from vegetable samples include *E. coli*, Pseudomonas, *Enterobacter cloacae*, *Salmonella arizonae* [50]. Members of the genera Penicillium, Aspergillus, Sclerotinia, Botrytis and Rhizopus are commonly involved in this process. The majorities of bacteria found on the surface of plants are usually Gram-negative and belong either to the Pseudomonas group or to the Enterobacteriaceae [51]. Many of these organisms are normally non-pathogenic for humans. Most of the reported outbreaks of gastrointestinal disease linked to the fresh produce have been associated with bacterial contamination, particularly with members of the Enterobacteriaceae family [52]. The presence of *Klebsiella pneumoniae* was also not surprising, since it has been recorded in the stream and drain water sources. The bacterial species are known to cause high fever, chills, flu-like symptoms and pneumonias well as gastrointestinal symptoms in humans (http://www.sproutnet.com/Reports/klebsielle.htm). The presence of indicator bacteria in three leafy vegetables can be indicator of poor farming, use of waste irrigation water and insanitary activity of growers that lead to contamination of the produce.

Table 7. Fecal coliform (MPN/100 ml) of water used for irrigation for cultivation of leafy vegetables from Bisnet River.

Sample	Sample site	FCCMPN/ml
1	1	460
2	1	240
3	1	460
4	2	43
5	2	93
6	2	460
7	3	460
8	3	93
9	3	460
10	4	460
11	4	43
12	4	240
13	5	150
14	5	43
15	5	150
16	6	23
17	6	23
18	6	150
Ave. Mean		233.4

Note: Farm area 1(1) Farm area 2(2) Farm area 3(3) Farm area 4(4) Farm area 5(5) Farm area 6(6)
**FCC: Fecal Coliform Count*

Table 8. Number of isolated pathogens from the samples among the six farming sites (n=18) from Bisnet River.

Pathogens	Number	Percent
Escherichia coli	18	100
Klebsiella species	8	44.4
Proteus species	3	16.7
Enterobacter species	15	83.3
Pseudomonas species	4	22.2
Penicillum species	5	27.8

Assessments of sanitary practices around the farm and irrigation water

Growers farming practices, cares being taken during growing and the farm land, irrigation water and the surrounding environment have significant role for contamination of the product. In the present study in Bisnet vegetable farm 24 (66.6%) of farming sites in the production field stored manure near the vegetable farm **(Table 9)**. 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 [53]. In developing countries, continued use of manure as fertilizers for the production of vegetables is a major contributing factor to contamination that causes numerous foodborne disease outbreaks [49]. For this problem, 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 [54].

In the study field, 12 (33.3%) of the fields are separated from growing fields with a fence **(Table 9)**. The dangerous microorganisms in animal feces can survive for a long time up to several months. The risk of fecal contamination increases with the number of animals entering the field, the number of times they enter the field, and the length of time

they remain in the field. Droppings from wild birds can contaminate fruits and vegetables and cause human illness [55].

Table 9. Assessments of the hygienic sanitary practices around farm land, around Bisnet River.

Characteristics	Frequency	Percent
Are manure stored near to vegetable production fields?		
Yes	24	66.7
No	12	33.3
Are animals separated from the growing fields with a fence?		
Yes	12	33.3
No	24	66.7
Is the house hold wastes/trashes removed around the growing fields?		
Yes	18	50
No	18	50
Is the fecal waste well-aged or properly treated?		
Yes	0	0
No	36	100
Was the visible dirt and debris on the vegetables removed in the growing field?		
Yes	6	16.7
No	30	83.3

From the farm assessment 18 (50%) of the farming sites removed the house hold wastes/trashes around the growing fields **(Table 9)**. The common sources of irrigation water used in South Africa are large reservoirs, farm dams, rivers, ground water, municipal supplies and industrial effluent [56].

Observations result shows that (0%) none of the farming sites fecal waste is not well-aged or properly treated **(Table 9)**. Properly treated fecal waste (manure and human excreta) is an effective and safe fertilizer [55]. Dangerous microorganisms in human and animal fecal waste can survive for long period of time and contaminate fruits and vegetables. Fecal waste must be treated to kill the microorganisms [57].

Additionally 6 (16.7%) of the field the visible dirt and debris on the vegetables is not removed in the growing field. Lack of cleanliness in the area increases the risk of contamination of fruit and vegetables [57]. Microorganisms and dirt from the dirty fruits can all get drawn into the interior of the commodity. The reduction of pathogens on produce is important to reduce food borne illness, to decrease spoilage and to improve appearance and nutritive value [58] **(Table 10)**.

Table 10. Assessments of the hygienic sanitary practices on the river irrigation water.

Characteristics	Frequency	Percent
Are there fences or other means to keep poultry or cattle from defecating in water sources?		
Yes	12	33.3
No	24	66.7
Is the latrine located downhill or away from water sources?		
Yes	12	33.3
No	24	66.7
Is the family applying control measures when using contaminated water?		
Yes	0	0
No	36	100
Are there any town waste disposal sites along the river flow sides?		
Yes	30	83.3
No	6	16.7

In the present day, 12 (33.3%) of the irrigation water of the farming sites, has fences to keep poultry or cattle from defecating in water sources. Dirty storage facilities and the presence of rodents, birds and insects may increase the risk of contamination with food borne pathogens [59]. Possible corrective actions may include fencing to prevent large animal contact, appropriate well casing and head maintenance and placement of wells, filtering water, not stirring the sediment when drawing water, building settling or holding ponds and water treatment facilities. Settling or holding ponds that are used for subsequent irrigation may be microbiologically safe but may attract animals or in other ways increase the microbial risks associated with water for irrigating crops [60].

The sanitary survey also revealed that 12 (33.3%) of the irrigation water latrine was located downhill or away from water sources. Locating latrine downhill or away from the growing field prevent contamination during heavy rain fall or natural flooding (FAO (CAC/RPC, 2003).

Result from the observation check list showed that (0%) or none of the family was applying control measures when using contaminated water. Animal and human fecal waste can contaminate water with dangerous microorganisms, with surface waters especially prone to contamination. Measures need to be put in place to prevent such contamination passing to fruits and vegetables and impacting on human health [57]. If the quality of water is poor, unknown or cannot be controlled, crop contamination is minimized by applying control measures. Properly treated fecal waste is a good source of nutrients for fruits and vegetables. However, fecal waste must be properly treated to kill dangerous microorganisms. Helping participants understand the control measures needed to treat fecal waste is important for maintaining the safety of the fruits and vegetables (FAO (CAC/RPC, 2003).

From the observation it has been also discovered that 30 (83.3%) shows there was town waste disposal sites along the river flow sides. Industrial wastes, atmospheric deposition from crowded cities and other domestic wastes are among the major sources of heavy metals in the surface water, ground water and soils [61,62] stated that waste water irrigation led to the accumulation of heavy metals in soil and consequently into the vegetables, and also found out that the metal accumulation in vegetables grown in the vicinity of industrial sites represents a potential risk for public health **(Table 11)**.

Table 11. Correlation matrix of Bacterial count of vegetables and water with sanitary survey of farm sites.

	AMC	TCV	FCV	TCW	FCW	ASSWA	ASSVEG
AMC	1						
TCV	0.370**	1					
FCV	0.408**	0.991**	1				
TCW	0.283**	0.616**	0.637**	1			
FCW	0.258**	0.517*	0.557*	0.946**	1		
ASSWA	-0.031	-0.274	-0.279	0.412	0.431	1	
ASSVEG	-0.250	0.175	0.128	-0.430	-0.431	-0.466	1

Key: AMC: Aerobic Mesophilic Count; TCV: Total Coliform Vegetables; FCV: Fecal Coliform Vegetables; TCW: Total Coliform Water; FCW: Fecal Coliform Water; ASSWA: Assessment Water; ASSVEG: Assessment Vegetables
***. Correlation is significant at the 0.01 level (2-tailed)*
**. Correlation is significant at the 0.05 level (2-tailed)*

A correlation among bacterial count of vegetables, water (AMC, TCV, FCV, TCW and FCW) and sanitary survey of farming sites (ASSVEG and ASSWA) is shown on **Table 11**. A positive relationship existed between AMC and TCV ($r=0.370$, $p<0.001$) and AMC and FCV ($r=0.408$, $p<0.001$). TCV exhibited significant positive correlation with FCV ($r=0.991$, $p<0.001$), TCW ($r=0.616$, $p<0.001$) and FCW ($r=0.517$, $p<0.0001$). FCV exhibited significant positive correlation with TCW ($r=0.637$, $p<0.001$) and FCW ($r=0.557$, $p<0.0001$). TCW also exhibited significant positive correlation with FCW($r=0.946$, $p<0.001$). Positive correlation means that high scores on one are associated with high scores on the other, and that low scores on one are associated with low scores on the other [63-66].

CONCLUSION AND RECOMMENDATION

Conclusion

The study revealed that there was bacterial contamination of fresh leafy vegetables (lettuce, cabbage and spinach) grown in Gondar Bisnet river vegetable farms. Spinach farm site 1 and 2 (4.93, 4.90); (720.00, 524.44) and (321.4, 244.3) was found to be the most contaminated vegetable by aerobic mesophilic bacterial count, total coliform count and fecal coliform count respectively. This might be due to the fact that spinach have wider leaf surface for contact with wastewater, soil and dust. In contrast lettuce (farm site 5 and 6) was the least in aerobic mesophilic bacterial count, total coliform count and fecal coliform count. Therefore, great attention should be paid in using contaminated water for production of vegetables for the public health perspective. The presence of coliforms is an indication of fecal contamination that comes from river water used for irrigation. We tested the vegetables samples for the presence of Salmonella and they were not detected [67-73].

The presence of low level coliforms may be because of pathogens survived on leaf surfaces for a shorter time than in the soil as they are less well protected from the harsh effects of sunlight and desiccation and also the temperature of the environment. The results obtained have demonstrated that the microbiological quality of fresh leafy salad vegetables grow in Bisnet River is acceptable [74,75]. Escherichia coli, Klebsiella species, Proteus species, Enterobacter species, Pseudomonas species and fungi (Penicillum species) were isolated from leafy vegetables. The safety measures also shows high burden of water and vegetable farm contamination using observational check lists and concluded that manure, animal and human feces, from house hold wastes and factories, clinical wastes etc. might be the sources of contaminations. Analysis of variance has shown there was significant difference ($p<0.05$) between bacteriological quality and safety of the samples [76-82].

Recommendations

There is a need for improved surveillance systems on food-borne pathogens, on food products and on outbreaks.

Water used in the irrigation vegetables should be of a quality that does not introduce microorganisms at a level that might cause harm to the consumer [83-88].

Further studies are required on the microbiological status and survival of various pathogens in/on raw vegetables and the most efficient decontamination procedures [89-96].

There should be public food hygiene and safety education on the consumption of leafy vegetables [97-101].

Local authorities should provide alternative means facilities for growers for safer cultivation of fresh produces [102-111].

To reduce the bacterial load on the produces and diseases risks of diseases to consumers need to wash the produces using potable water [112-117].

Acknowledgement

Firstly, my special gratitude goes to my heavenly Father for giving me the capabilities, courage and perseverance for this study.

My special gratitude and appreciation goes to my advisor Dr. Samuel Sahile for his expert guidance and support during the course of my study and unreserved encouragements and advice in conduction of this thesis. Following him, I thank Miss. Atsede Muleta who shared and supported me her expertise and professional guidance in laboratory activity.

I would like to express my heartfelt gratitude for the peoples behind my success, my parents, my mother Wudinesh Sintayehu, my father Nega Mengistu and my brother Biniam Nega. My thanks also goes to my friend Askal Molla, Tigist Malede, her mother and also all her families for their endless encouragement, love, support and motivation throughout my studies.

I would like to express my gratitude to University of Gondar for giving me this opportunity.

My two "lab partners", Hellen and Habtamu for their love, support and assistance and for great memories that I will always cherish. Finally, I thank my fellow post-graduate students as well as the staff of the Department of Biology for their friendship, support and assistance and I also thanks to my friends and colleagues in Walya School.

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