Evaluation of the Effect of Wastewater Irrigation on the Microbiological Quality of Vegetables in Northeast Ethiopia: Implication for Food-Borne Infection and Intoxications

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Source: Environmental Health Insights, 16(1)

Published By: SAGE Publishing

URL: https://doi.org/10.1177/11786302221127856

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Environmental Health Insights
Volume 16: 1–7

DOI: 10.1177/11786302221127856
SAGE

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ABSTRACT

BACKGROUND: Cultivation of vegetables using untreated wastewater is a common phenomenon in many drought-prone areas of the world. Consumption of such microbiologically unsafe vegetable increase the risk of food-borne diseases. As a result, evaluating the effect of wastewater irrigation on the microbiological quality of vegetables would be beneficial to consumers' health.

METHOD: A total of 192 vegetable samples (lettuce, cabbage, carrot, and tomato) and 64 irrigation water samples were collected and analyzed for total bacteria and coliform count using a standardized protocol over a 4 month period. One-way analysis of variance and Pearson correlation coefficient was used to analyze the data. Statistical significance was defined at a *P*-value of less than 0.05.

RESULT: Carrots were the least polluted vegetable, according to the study, while cabbage had the highest contamination levels. The mean bacterial count among the vegetables differs significantly ($P < 0.05$). The fecal coliform counts of the irrigation wastewater exceed the international wastewater irrigation standards. The mean microbial count between vegetables and water samples showed a significant positive relationship ($P < 0.05$). All of the analyzed vegetable samples were of borderline microbial quality for fecal coliform bacteria.

CONCLUSION: Irrigation wastewater has a low microbiological quality, which significantly contributes to the contamination of vegetables grown on it.

RECOMMENDATION: Measures should be taken to improve the microbial quality of wastewater as well as the quality of vegetables grown in order to protect consumers' health from food-borne diseases.

KEYWORDS: Microbial quality, vegetables, irrigation water, food-borne infection, food-borne intoxications, northeast Ethiopia

RECEIVED: July 2, 2022. **ACCEPTED:** September 5, 2022.

TYPE: Original Research

FUNDING: The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by Wollo University. The sponsoring organization had no role in the study design, sample collection, analysis, or interpretation of the data, in drafting the manuscript, or in the decision to submit this version of the manuscript.

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Introduction

Vegetables play a key role in maintaining the health of consumers because they are low in calories and fat, free of cholesterol, rich in carbohydrates and fiber. For this reason, consumption of vegetables helps to lower the risk of various chronic illnesses.[1-6] In most cases, people prefer to consume vegetables while they are raw so as not to lose heat-sensitive nutrients.[7] This increases the risk of food-borne illnesses if the vegetable is of poor microbial quality. For instance, salad vegetables are high in vitamins and minerals and are often eaten raw, without being washed or peeled, increasing the risk of food-borne illness.[2]

Cultivating vegetables using irrigation wastewater is becoming increasingly popular in many parts of the world, especially in those areas where drought is common.[1,8-12] Increasing pollution and eutrophication of rivers, streams, springs, and other water reservoirs because of anthropogenic activities are a challenge around the world, especially in Africa, particularly in Ethiopia, where there are no restrictions to limit the discharge of untreated wastewater into rivers, streams, and other water bodies.[1] Ethiopia's existing pollution legislation is ineffective and poorly applied, making it difficult to protect water bodies and other environmental entities.[13]

Apart from plant nutrients, irrigation wastewater may also contain a variety of pathogenic microorganisms.[9,11] The use of untreated wastewater for irrigation may transfer these pathogenic microorganisms to the vegetables during either farming, transporting, or even consumption due to poor handling practice, unclean hygienic condition of the preparation area, or the use of contaminated water to rinse vegetables.[4,8,10]

Because of pathogenic microorganisms being transferred from irrigation wastewater to the crops, various food-borne pathogens have been identified from the vegetable samples. Salmonella and *Vibrio cholera*, for example, was found in lettuce and cabbage samples, which are common causes of diarrhea. *Streptococcus faecalis, Salmonella Typhi*, Shigella, and Pseudomonas were found in salad vegetables.[4] In several studies, protozoan parasites such as Amoeboid, Giardia, and Cryptosporidium have been reported as the primary causes of parasitic diarrhea. *Entamoeba coli*, *Giardia lamblia*, and *Blastocystis hominis* were also identified from lettuce vegetable samples.[14] Salmonella spp. Shigella spp. and Shiga toxigenic *E. coli (STEC), Listeria monocytogenes, S. aureus*, and Campylobacter spp. were also found in vegetable samples.[15-17]

Clostridium botulinum was also found in a carrot sample in the United States.[18] *Staphyloccous Aureus*, Campylobacter, Salmonella, Shigella species, *A. lumbricoide* eggs, *E. histolytica* and *G. lamblia* cysts were also reported in Harar, Fiche, Nekemte, Mekelle, and Adama Town, Ehiopia.[2,12,19-21] As a result, eating such microbiologically hazardous vegetables has been linked to a variety of food-borne illnesses that affect many people in both developed and developing countries, with the majority of cases reported in developing countries due to a lack of food hygiene regulations.[22,23] Researchers found that raw vegetable intake was responsible for around 75% of *E. coli* O157 illnesses.[24]

Hundreds of edible vegetable varieties are eaten in Ethiopia, which is one of the lowest-income countries in the world and in Sub-Saharan Africa. The most common vegetables in the country are lettuce, carrots, onions, cabbage, and tomatoes.[25]

Due to the regular incidences of drought and the lack of clean water for irrigation, urban and semi-urban vegetable farmers used contaminated water to cultivate vegetables near rivers, disregarding the negative effects on public health.[11,26] This leads to the occurrence of food-borne illnesses when vegetables are eaten uncooked.[15,21] Many farmers in Kombolcha town use irrigation water from the Borkena River, one of the region's largest rivers, to grow vegetables.[26]

However, it is a widespread practice to dump untreated wastewater into water bodies in many locations along the Ethiopian river, including Borkena, Akaki river, and other water bodies.[27,28] Human activities such as washing clothes and bodies, fishing, defecating, and animal watering all have a significant impact on Borkena river water quality. Farmers that live close to this river cultivate vegetables using wastewater that is extensively contaminated with disease-causing bacteria, similar to many farmers in developing nations. Additionally, they washed vegetables in this river water before selling them at the local markets, thereby reducing the hygienic standard of the product. However, there is limited study conducted to determine the microbiological quality of irrigation wastewater and its impact on the quality of vegetables grown. As a result, this research aimed to evaluate the effect of wastewater irrigation on the microbiological quality of vegetables in Northeast Ethiopia.

Materials and Methods

Study area and setting

The study was conducted in Kombolcha town, Northeast Ethiopia, which is 367 km away from Addis Ababa. It has a total population of 102 244 of whom 51 124 are men.[29] The town is the main industrial zone for the Amhara regional state area as well as the country. In the town, there are over 10 heavy factories, as well as numerous medium and small-scale factories. As a result, the town attracts a large number of people from both rural and urban areas, especially children and young people looking for work. Borkena is the town's largest river. Thousands of people depend on the Borkena River's water, which is essential for their survival. Swimming, animal irrigation, washing clothing, bodies, and cars, discharging wastes, defecation, and other human activities affect river water quality. In towns like Kombolcha, Harbu, Dawa Chefa, and Kemissie, however, the rivers are used for irrigation, drinking, and everyday household needs. The most common crops grown with river water are lettuce, tomatoes, cabbage, and carrots.[30] (Figure 1)

Study design and period

A laboratory-based cross-sectional study design was used to determine the microbial quality of lettuce, carrots, cabbage, and tomatoes. The study was conducted from March 2021 to June 2021.

Sample size and sampling procedure

For 4 months (March 2021-June 2021), 8 vegetable samples 2 each of lettuce (Lactuca Sativa), cabbage (Brassica Oleracea), carrot (Daucus Carrot), and tomato (Solanum Lycopersicum) were collected once a week from the agricultural area where farmers used Borkena river irrigation water. Similar to this, 4 samples of vegetables one each of lettuce, cabbage, carrots, and tomatoes were gathered once a week for 4 consecutive months (March 2021-June 2021) from the farmland where the farmers employed irrigation water from protected water sources.

For 4 months, 2 irrigation water samples were taken once a week from the point where river water enters the irrigation system. In addition, for 4 months, 2 water samples were gathered once a week from agricultural lands where vegetables were produced using protected water sources. Hence, 192 vegetable samples (128 treatment and 64 control) and 64 irrigation water samples (32 treatment and 32 control) were collected for this study. These vegetables were chosen because they are the most widely grown and are usually consumed raw or with minimal heat treatment.[6]

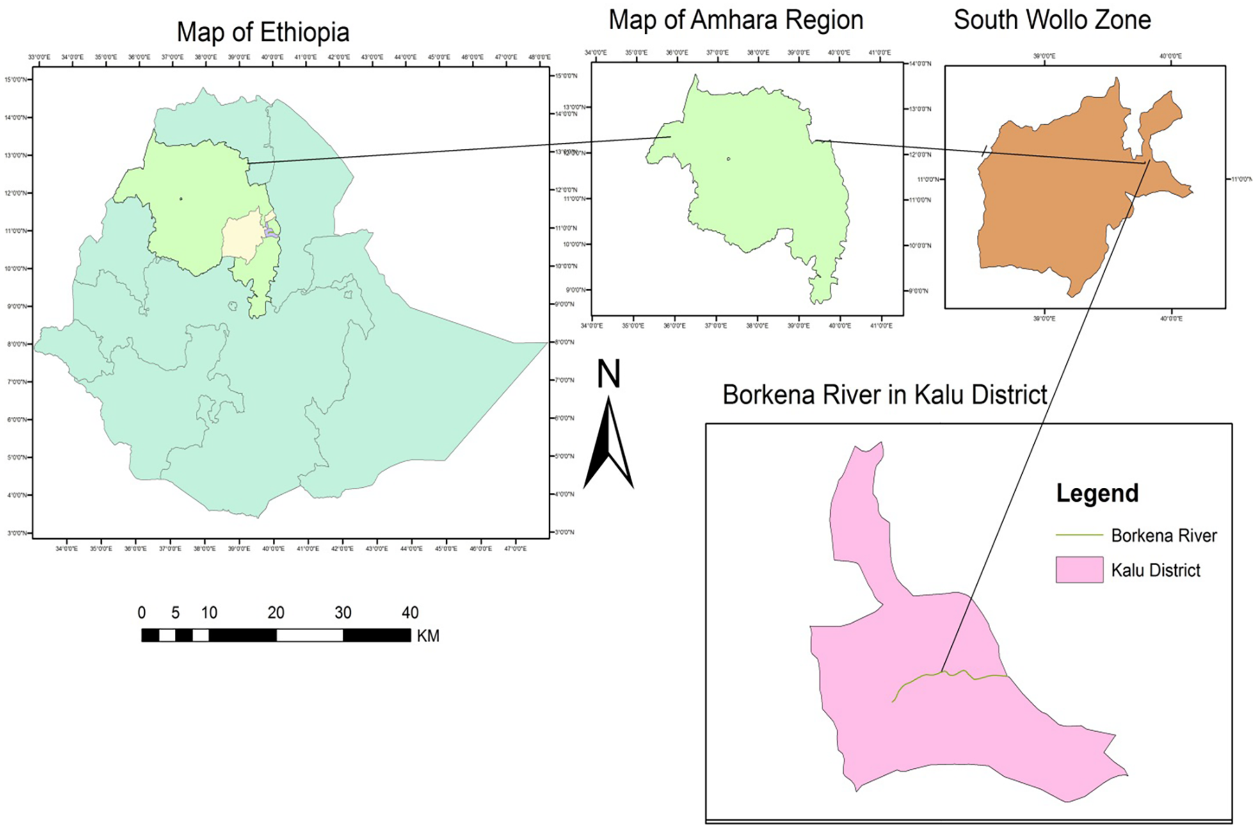

Figure 1. Study area map.

Sample collection

An edible portion of 50 g of each vegetable sample was purchased using sterile polyethylene zip bags. Irrigation water samples of each 100 mL was also collected aseptically using a sterile screw-capped plastic sample bottle. Then the sample bag and bottles were labeled and kept under the icebox and transported immediately to the laboratory of the Environmental Health Department at Wollo University for analysis within 2 to 4 hours of collection.[6,31,32]

Sample analysis

Upon arrival at the laboratory, vegetable samples were washed thoroughly with tap water for the removals of soil particles and further washed with distilled water. The samples were aseptically chopped into small sizes using seizures.[9] Twenty-five grams of each vegetable sample was weighted and added to 100 mL of sterile saline solution for 2 minutes. A serial dilution of the vegetable and river water samples was made by transferring 1 mL of suspension and adding it to the tube containing 9 mL of sterilized peptone water.[31,33] Then, the Total Bacterial Count (TBC) and Total Coliform (TC) were determined by spreading 0.1 mL of suspension and spreading it onto sterile Petri dishes containing sterile Plate Count Agar (Oxoid, UK) and MacConkey agar (Oxoid, UK), respectively. The plates were incubated at 37℃ for 24 hours. Fecal Coliform (FC) was determined by spreading a 0.1 mL dilution onto the plate containing MacConkey agar plate and incubating it at 44℃ for 24 hours. After incubation, the pink to red colonies from 37℃ and 44℃ incubated plates were identified as TC and FC bacteria, respectively. The colonies were counted using a digital colony counter and the colony count per gram of the vegetable and per mL of water samples was determined.[32,34-36]

Four to five colonies were inoculated into tubes containing 2% Brilliant Green Bile Broth as a confirmatory test for coliform bacteria (Oxoid, UK). The gas production as indicated by the rising of Durham tubes after 48 hours of incubation at 35°C was used to assess the presence of coliform bacteria in a vegetable and water sample.[37]

Operational definition

Raw Vegetable: Vegetables comprising lettuce, cabbage, carrots and tomatoes cultivated by using wastewater or other water sources.

Microbial quality of vegetables: It can be evaluated by the presence of total bacterial count and coliform bacterial count in vegetables as defined by the Center for Food Safety (CFS) guidelines.[38]

Microbial quality of the raw vegetables due to TBC could be:

Satisfactory: if the count of TBC in the raw vegetable is $<10^5$ CFU/g (<5 log CFU/g)

Table 1. Microbiological quality of lettuce, cabbage, carrot, and tomato irrigated with river water in Kombolcha Town, Northeast, Ethiopia.

SAMPLE TYPE	MICROBIAL COUNTS IN A SAMPLE (MEAN LOG CFU/G ± STD)								
	TBC			TC			FC		
	TREATMENT	CONTROL	*P*-VALUE	TREATMENT	CONTROL	*P*-VALUE	TREATMENT	CONTROL	*P*-VALUE
Lettuce	4.5 ± 0.4	3.8 ± 0.04	0.001*	3.9 ± 0.2	3.7 ± 0.2	0.002*	3.5 ± 0.4	3.3 ± 0.1	0.336
Cabbage	4.6 ± 0.5	3.9 ± 0.1		3.7 ± 0.2	3.4 ± 0.1		3.3 ± 0.2	3.0 ± 0.3	
Carrot	4.1 ± 0.2	3.8 ± 0.1		3.7 ± 0.2	3.5 ± 0.1		3.5 ± 0.2	3.0 ± 0.3	
Tomato	4.2 ± 0.3	3.8 ± 0.1		3.9 ± 0.2	3.4 ± 0.1		3.5 ± 0.2	3.1 ± 0.1	

Abbreviations: FC, fecal coliform; TBC, total bacterial count; TC, total coliform
*Mean bacterial count significantly differs at $P < 0.05$.

Borderline: if the count ranges from 10^5 to $\leqslant 10^7$ CFU/gram (5-7 log CFU/g]
Unsatisfactory: if the count in the raw vegetables exceeds 10^7 CFU/g (>7 log CFU/g)
Microbial quality of raw vegetables due to coliform bacteria was evaluated as:
Satisfactory: if the count of coliform bacteria in raw vegetable is $<10^2$ CFU/g (<2 log CFU/g)
Borderline: if the count ranged from 10^2 to 10^4 CFU/g (2-4 log CFU/g]
Unsatisfactory: if the coliform count exceeds 10^4 CFU/g (>4 log CFU/g)

Data quality assurance and statistical analysis

All the used media and reagents used were analytical grade. Sample analyses were carried out in triplicate with their control. All media and reagents used were up to date, and the microbiological analysis was carried out inside a level II biosafety cabinet (BDK, Germany). The used culture media and materials were sterilized using an autoclave (Astell, England). The adequacy of sterilization was also assured using the sterilization indicator. The microbial counts of the samples were recorded on each day of counting, and the values were transformed into log values. The mean and standard deviations for each bacterial count were computed. An Analysis of Variance (ANOVA) was used to compare the mean microbial count in lettuce, cabbage, carrot, and tomato samples. A Pearson correlation coefficient was calculated to determine whether the microbial quality of river water influences the microbial quality of vegetables grown on it. A *P*-value of less than 0.05 was considered statistically significant.

Results

Microbiological quality of vegetables

The mean Total Bacterial Count (TBC) of lettuce, cabbage, carrot, and tomato irrigated with wastewater was 4.5 ± 0.4, 4.6 ± 0.5, 4.1 ± 0.2, and 4.2 ± 0.3 log CFU/g, respectively, whereas the mean TBC of lettuce, carrot, cabbage, and tomato irrigated with the control sample was 3.8 ± 0.04, 3.9 ± 0.1, 3.8 ± 0.1, and 3.8 ± 0.1 log CFU/g, respectively. The results obtained from ANOVA revealed that the mean TBC and Total Coliform (TC) of lettuce, cabbage, carrot, and tomato samples showed a statistically significant difference ($P < 0.05$) among the 4 vegetable samples analyzed. However, the mean Fecal Coliform (FC) did not show a statistically significant difference ($P > 0.05$) (Table 1).

Evaluation of microbial quality of vegetable samples

Among 32 lettuce samples analyzed, about three-fourths, 24 (75%) of them were of satisfactory microbial quality, whereas the remaining 8 (25%) of them were in the borderline microbial quality based on TBC. All of the analyzed carrot and tomato samples were satisfactory microbial quality for TBC. All of the analyzed vegetable samples were of borderline microbial quality for FC. Overall, 106 (82.8%) of the analyzed vegetable samples were of satisfactory microbial quality for TBC (Table 2).

Microbiological quality of irrigation water samples

The mean TBC, TC, and FC of the treatment water samples were 4.2 ± 0.2, 3.9 ± 0.2, and 3.7 ± 0.3 log CFU/mL, respectively. The mean TBC, TC, and FC of the control water sample were 3.9 ± 0.1, 3.8 ± 0.1, and 3.5 ± 0.1 log CFU/mL, respectively. Based on the TBC, TC, and FCC, there is a significant difference ($P < 0.05$) in the contamination level between treatment and control water samples, showing that the treatment water samples were the most contaminated (Table 3).

Correlation between microbiological quality of irrigation wastewater and irrigated vegetables

According to the Pearson correlation coefficient, the mean TBC of the vegetables and river water samples showed a fair

Table 2. Classification of microbial quality of vegetables irrigated with Borkena river water in Kombolcha town, Northeast, Ethiopia.

VEGETABLE TYPE	MICROBIAL QUALITY LEVEL (ACCORDING TO CFS, 2014)				
	TOTAL BACTERIAL COUNT		TOTAL COLIFORM BACTERIA		FECAL COLIFORM
	SATISFACTORY	BORDERLINE	BORDERLINE	UNSATISFACTORY	BORDERLINE
Lettuce	24 (75%)	8 (25%)	26 (81.3%)	6 (18.7%)	32 (100%)
Cabbage	18. (56.3%	14 (43.7%)	30 (93.7%)	2.0 (6.3%)	32 (100%)
Carrot	32 (100%)	0%	30 (93.7%)	2.0 (6.3%)	32 (100%)
Tomato	32 (100%)	0%	26 (81.3%)	6 (18.7%)	32 (100%)
Overall	106 (82.8%)	22.0 (17.2%)	112 (87.5%)	16 (12.5%)	128 (100%)

Table 3. Microbial quality of water used to cultivate vegetable in Kombolcha Town, Northeast, Ethiopia.

GROUP OF BACTERIA ISOLATED	MEAN BACTERIAL COUNT (LOG CFU/ML)		*P*-VALUE
	TREATMENT	CONTROL	
Total bacterial count	4.2 ± 0.2	3.9 ± 0.1	<0.001
Total coliform	3.9 ± 0.2	3.8 ± 0.1	0.012
Fecal coliform	3.7 ± 0.3	3.5 ± 0.1	0.005

Table 4. Correlation study between vegetable and irrigation water sample in Kombolcha town, Northeast, Ethiopia.

GROUP OF BACTERIA ISOLATED	PEARSON CORRELATION COEFFICIENTS (R)	*P*-VALUE
TBC of vegetables and river water	0.322	0.009*
TC of vegetables and river water	0.582	<0.001*
FC of vegetables and river water	0.538	<0.001*

Abbreviations: FC, fecal coliform; TBC, total bacterial count; TC,total coliform.
*Correlation is significant at $P < 0.05$.

and positive relationship ($P < 0.05$; $r = 0.329$) whereas the mean TC and FC showed a good and positive relationship ($P < 0.05$ and $r = 0.582$ and 0.538, respectively) (Table 4).

Discussion

This study provides firsthand information on the microbial quality of irrigation water and its relationship to the quality of vegetables grown in it. Because the presence of a microbiological hazard can pose a serious public health risk, continuous regulatory monitoring is essential for preventing food-borne pathogen outbreaks and thus protecting public health. One approach to checking the microbiological hazard in suspected food samples is to examine the quality of food potentially contaminated with various sources of contaminants.[39]

In this study, the mean TBC of lettuce samples was 4.5 log CFU/g. Higher results (7.0, 8.2, 7.7, and 6.7, log CFU/g) were reported by Akoachere et al,[7] Zerihun et al,[9] Alamnie et al,[12] and Alemayehu et al,[19] respectively. Slightly higher result (4.75 log CFU/g) TBC was also reported in lettuce samples irrigated with Akaki river water in Addis Ababa, the capital city of Ethiopia.[13] The mean TBC of the cabbage sample in this study was found to be 4.6 log CFU/g. Higher values, though (7.2, 7.5, and 7.4 log CFU/g, respectively) were noted in the Ethiopian towns of Harer, Mekelle, and Fiche, respectively.[2,12,19] The mean TBC of the cabbage sample in Nekemte Town, Ethiopia, was discovered to be 4.2 log CFU/g, which is a lower result.[20] Vegetables can become infected with pathogens directly from feces or indirectly by touch with human or animal faces, poor worker personal hygiene habits, contaminated water applied directly to the vegetables for irrigation or washing purposes, and the use of untreated manure as fertilizer.[19]

Coliforms and fecal coliforms are usually indicators, and their presence indicates the presence of harmful organisms.[40] In this investigation, the lettuce sample's mean TC and FC were 3.9 and 3.5 log CFU/g, respectively. All of the vegetable samples tested positive for fecal coliform in this investigation. Akoachere et al[7] also reported that fecal coliforms were found in all of the analyzed vegetable samples.

Microbiological guidelines for ready-to-eat food state that food with TBC less than 105 CFU/mL (<5 log CFU/mL is of satisfactory microbial quality.[38] In the present study, out of the total lettuce samples analyzed, 24 (75%) of them were of satisfactory microbial quality for TBC, indicating that the vegetable can be consumed without adverse health effects. However, it does not necessarily imply that the vegetable is completely safe to eat, as pathogenic bacteria in low numbers might be dangerous to people's health. Therefore, adequate precautionary measures should be taken to safeguard the health of vegetable consumers from various food-borne diseases. This finding is better than the one reported by Abakari et al[5], who reported that only 16.7% of the lettuce sample was of satisfactory microbial quality.

Out of the total cabbage samples analyzed 14 (43.7%) of them were of borderline microbial quality for TBC. It indicates that test results that are neither unsatisfactory nor satisfactory are at the upper limits of acceptability, indicating the possibility of public health concerns and unacceptable risks if adequate prevention measures are not applied.[38]

The mean TBC of the irrigation water sample in the present study was 4.2 log CFU/mL. Higher results (5.5 and 7.1 log CFU/mL) were reported in Ibadan, Nigeria,[41] and Hawassa, Ethiopia[9] respectively. The TC level of 3.9 log CFU/mL in the present study is slightly lower than the study reported by Akinde et al,[41] where the mean TC in the irrigation water sample was found to be 4.1 log CFU/mL. Zavadil[11] also reported higher TC and FC (6.2 and 5.4 log CFU/mL, respectively) values from the irrigation wastewater. It's conceivable that vegetables grown with this effluent are unfit for human consumption.[19] Lower levels of total coliform bacteria in irrigation water samples could mean that the water is of good quality and can be used to grow high-quality vegetables. The mean FC of the irrigation water sample in this study was 3.7 log CFU/mL which exceeds the recommended microbiological quality guidelines for wastewater use in agriculture.[42]

In addition, all of the analyzed irrigation water samples were positive for fecal coliform. This is not surprising given that washing bodies and clothes and animal watering and discharging wastewater into the river is a common phenomenon among Kombolcha residents. In the present study, the microbial quality of irrigation water significantly contributes to the contamination of vegetables cultivated. This suggests that the presence of these organisms on vegetables could be attributable to feces-contaminated irrigation water, exposing consumers to risk.[19] As a result, we observed that freshly grown vegetables might contain pathogenic microbes, posing a risk of food-borne disease to consumers.[13] In our study, the nearby villages discharge their waste directly into the river where farmers once grew vegetables, contaminating the vegetables grown. Consuming these infected vegetables could therefore expose one to microbiological risks that could result in a number of infectious ailments, including gastroenteritis, diarrhea, and typhoid and paratyphoid fevers. Therefore, eating it could provide a serious danger of infection or be a source of food poisoning, which could result in a public health issue

Goja et al[40] confirmed that the microorganisms present in vegetables are a direct reflection of the sanitary quality of the irrigation water. Muinde and Kuria[43] also agreed that wastewater from the bathroom, kitchen, and laundry used in the cultivation of these vegetables is the most common source of bacterial contamination. In addition, Akinde et al[41] indicated that microbial contamination of vegetables occurs throughout the life cycle, from cultivation to consumption, including the irrigation water sources.[3,42]

Conclusion

Although satisfactory microbiological quality does not always imply complete safety, more than three-quarters of the examined vegetable samples had adequate microbiological quality. Since the amount of FC exceeds the recommended microbial quality guideline for agricultural use, irrigation water is regarded to have poor microbiological quality. Microbial quality of irrigation water significantly contributes to the deterioration of the quality of vegetables grown on it.

Recommendation

Steps should be done to enhance both the quality of the vegetables farmed and the microbiological quality of irrigation water. Avoiding activities like washing, swimming, watering animals, and flushing waste into rivers will help achieve this. A capacity-building program for irrigation farmland owners, farmers, and stakeholders who are directly involved in the vegetable value chain should also be put in place as a long-term goal. Vegetables should be carefully washed by consumers before consumption. As the study focuses on isolating hygiene indicator bacteria using conventional cultural method, future researchers are advised to conduct additional research on the confirmation of isolates using PCR technique, quantification of specific food-borne pathogenic microorganism in vegetable sample, and antimicrobial testing of the food-borne pathogen.

Acknowledgements

Firstly, the authors would like to thank Wollo University for covering the cost of the study. The authors would also like to acknowledge the research assistant for collecting and analyzing samples. The authors are grateful to the irrigation farmland owners and farmers.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Availability of Data

All the data in the manuscript can be obtained from the corresponding authors upon request.

Ethical Consideration

All of the methods employed in this investigation were compliant with the Helsinki Declaration of 1975, as revised in 2000, and the committee responsible for human experimentation's ethical guidelines. Ethical clearance was obtained from the ethical review board of the College of Medicine and Health Sciences, Wollo University.

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