

Microbial and Parasitological Quality of Pre-Harvest Vegetables Irrigated with Urban Wastewater and Water from Microdams in Mekelle and Southern Zones of Tigray, Region, Ethiopia

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Abstract: Currently wastewater vegetable irrigation in Ethiopia is growing due to water scarcity, population growth urbanization. Samples of pre-harvest leafy, fruit and root vegetables were collected using a random sampling technique from small scale farms of wastewater drainage canal in Mekelle and Southern Zone of Tigray Region in triplicates during dry season (November-May). Survey result revealed that different types of vegetable (Swiss chard, lettuce pepper, carrot, tomato, beef root, onion and cabbage) were produced using waste water. It was found that total of 146 household heads with ages ranging from 25-65 years were directly involved in urban and peri-urban irrigated vegetable production in Mekelle, Alamata and Maychew. Out of the 55 vegetable samples, the highest microbial load (14.53×10^6 , 5.93×10^6 , 5.53×10^6 and 3.87×10^6 cfu/ml) on Cabbage, Swiss chard, Lettuce and Tomato, respectively) at Mekelle followed by Maychew with load of (6.98×10^6 , 6.3×10^6 , 4.86×10^6 and 4.2×10^6 cfu/ml) and with the least microbial load of (5.78×10^6 , 4.79×10^6 , 2.3×10^6 and 3.64×10^6 cfu/ml) on Cabbage, Swiss chard, Lettuce and Tomato) at Alamata. Based on morphological, cultural and biochemical test wide range of bacteria (*Pseudomonas aeruginosa*, *Salmonella spp.*, *Shigella spp.*, *Escherichia coli*, *Campylobacter spp.*, *Listeria monocytogenes*, *Clostridium botulinum* and *Bacillus cereus* and intestinal parasites such as *Ascaris*, *Entamoeba spp.*, *Strongyloides stercoralis* and *Giardia spp.*) were detected. The analysis result revealed that all the sampled vegetables of the study area are contaminated with bacteria and intestinal parasites. Vegetables cultivated with such wastewater may be considered as unsafe for consumption.

Key words: Alamata · Maychew · Mekelle · Microdams · Vegetables · Southern Zone

INTRODUCTION

Many farmers of developing countries grow crops, especially vegetables, in urban and peri urban environments using wastewater, which is often heavily loaded with disease-causing organisms [1, 2]. The scarcity of water in dry climate and urbanization has driven farmers in Tigray region to use untreated wastewater for growing vegetables. This situation can seriously harm the health of the farmers, the traders who handle crops and the people who consume such vegetables. Bacteria such *Salmonella*, *Shigella*, *Escherichia coli* and *Campylobacter*

reside in the intestinal tracts of animals, including humans are more likely to contaminate raw vegetables through contact with faeces, sewage, untreated irrigation water or surface water [3-6]. Untreated wastewater flowing from urban areas are a source of microbial, parasitic pathogens that cause serious health problems to human beings [7-9]. The resistant nature of the aforementioned pathogens to extreme temperatures, desiccation, natural irradiation and commonly used disinfectants coupled with poor sanitation and inadequate personal hygiene contribute to the outbreak of vegetable-borne infections in developing countries [10-12]. In addition, life under

immunocompromised situation, mainly due to HIV/AIDS, exposes some percentage of the population across the world to opportunistic and emerging vegetable-borne pathogens [13]. It is reported that the factors responsible for microbial and parasitological contamination of vegetables are the use of sewage, use of animal and human fecal materials as fertilizers [10, 14]. Nevertheless, there remain many gaps in our knowledge in practice of checking microbial and parasitological contaminants involved. This work was designed to analyze microbial and parasitological contamination of vegetables grown in urban wastewater and dam water irrigated farms in Tigray region, Ethiopia.

MATERIALS AND METHODS

Description of Study Area: Mekelle is the capital city of Tigray Region located 780 km north of Addis Ababa, the capital city of Ethiopia. Its geographic location is 13°32' N and 39°33' E. It has an average altitude of 2200 meters above sea level with a mean minimum, mean maximum and mean average monthly temperatures of 8.7, 26.8 and 17.6°C, respectively [15]. Amount of rainfall is variable in Mekelle; on average about 600 mm more than 70% of it falls between July and August, followed by long dry season [15]. Southern Zone of Tigray Region is located in northern Ethiopia (longitude 39°5'-39°8' and latitude 12°3'-13°7'). In this area, agriculture is mixed crop and livestock farming. The climate is characterized by bimodal

rainfall. About 70–80% of the rain falls in the Kiremt season (June–September). The mean minimum and maximum temperature range is from 8 °C to 30 °C, with small annual variations [16].

Alamata is located 600km North of Addis Ababa. It is the most Southern Woreda of the Tigray Region and borders with Amhara Region from the South and West Afar Region from the East. Altitude in the area ranges from 1178 to 3148 meters above sea level (masl) and 75% of the Woreda is lowland (1500 masl or below) and only 25% is found in intermediate highlands and highlands falling between 1500 and 3148 masl. Many types of vegetables are grown in Alamata because of the favorable climate and easy access to surface and ground water sources. The Woreda is among the potentially best vegetable production areas in Tigray region. The subject of the study had been selected based on the number of farmers that use waste water for irrigation to produce vegetable for their house hold and other purpose such as commercial purpose.

Field surveys were conducted in farmers' fields in the aforementioned three locations of Tigray Region. Samples of pre-harvest vegetables were collected from small scale farms, located along the wastewater drainage canal in Mekelle and urban areas of Southern Zone of Tigray Region and farms irrigated with water from micro dams in the later areas using sterile zipped plastic bags in triplicates during the long dry season (November-May).



Fig. 1: Vegetable sampling

Table 1: Meteorological data of sampling locations

Locations	Altitude(m)	Latitude	Longitude
Maychew	2349-2350	012°.47'.337-012°.47'.461"N	039°.33'.105- 039°.33'.646"E
Alamata	1495-1535	012°.23'.273- 012°.23'.424"	N039°.33'.396- 039°.34'.999"E
Mekelle1	995-2085	013°.30'.053- 013°.30'.984"N	039°.27'.848- 039°.27'.885"E

Field survey and sample collection

The collected vegetable samples were transported to the Microbiology and Parasitology Laboratory of the Department of Biology, Mekelle University and stored at 4°C until analysis for microbes and parasites. Structured, semi-structured and open-ended questionnaires were used to gather demographic information of farmers, type of vegetable grown, source of water for irrigation and practice of fertilizer application in the farms.

Isolation and Identification of Microbial Pathogens: A 50 g of each vegetable sample was weighed and rinsed in 250 ml beaker containing 100 ml of normal saline and aliquot was diluted to 10^{-1} - 10^{-4} . From each of the dilutions 0.1 ml inoculated on nutrient agar medium and incubated at 37°C for 24 h for isolation of bacterial pathogens. The plates were examined for colony characters; colonies were subcultured on Mac-Conkey agar for isolation of Coliforms. Isolates obtained were identified based on morphological, cultural and biochemical tests.

Isolation Helminth Eggs and Protozoan Cysts: In the laboratory, a sample of 100g of each fresh vegetable was chopped into small pieces and put into a clean beaker containing physiological saline solution (0.85% NaCl), enough to wash the sample vegetable. After removing fragments of the vegetable sample from the washing saline using clean forceps, it was kept overnight for sedimentation, then top layer of the washing saline were discarded and 5 ml of the sediment was centrifuged at 2000 rotations per minute. After discarding the supernatant, the residue will be mounted on slides, stained in Lugol's iodine solution and examined using light microscopy to screen the samples for infective stages of intestinal parasites such as helminth eggs, larvae and protozoan cysts [17, 18].

Survey on Risk Factors: Observational check list and interview questionnaire were used to assess risk factors for biological (parasitological and microbial) contamination of water source of irrigation add irrigated vegetables. Structured, semi-structured and open-ended questionnaires were used to gather demographic information of farmers, type of vegetable grown, source of water for irrigation and practice of fertilizer application in the farms.

RESULTS

Demographic Characteristics Respondents: In general, the survey analysis showed that total of 146 household heads with ages ranging from 25-65 years were directly involved in urban and peri-urban irrigated vegetable production in Mekelle, Alamata and Maychew. Majority of the farmers were between 36 and 45 years of age and represented 43.7%, of farmers in Mekelle 41.8% in Alamata and 48.8% in Maychew. Irrigated vegetable production in the three locations is dominated by male (90.9, 83 and 80.6%, Alamata, Mekelle and Maychew respectively).

Types of Vegetable Grown: Different type of vegetables (Swiss chard, Lettuce, Beet root, Carrot, Pepper, Cabbage, Tomato and Onion were grown in the study locations (Table 3). However, the frequently cultivated ones were Cabbage, Swiss chard & lettuce depending on their house hold use and commercial purpose. Mostly the irrigation process mainly activated in the dry season. Vegetable production using wastewater provides employment for farmers and sellers of such crops.

Sources, Mode of Irrigation and Practice Fertilizer Used: The longer dry season in the region influence farmers to depend on irrigation as best option for crop cultivation.

Table 2: Demographic characteristics of respondents engaged in vegetable cultivation in Mekelle, Alamata and Maychew.

Parameter	Options	Percentage of respondents		
		Mekelle (N=60)	Alamata (N=55)	Maychew (N=31)
Age	25-35	10(16.7%)	14(25.45%)	10(32.3%)
	36-45	28(46.7%)	23(41.8%)	13(48.4%)
	46-55	14(23.3%)	12(21.8%)	6(19.4%)
	56-65	8(13.3)	6(10.9%)	2(6.45%)
Sex	Male	50(83%)	50(90.9%)	25(80.6%)
	Female	10(16.7%)	5(9.1%)	5(16.2%)
Educational status	Illiterate	36(60%)	26(47.3%)	10(32.25%)
	High school	8(13.3%)	12(21.82%)	9(29%)
	Elementary	4(6.7%)	4(7.3%)	4(12.9%)
	College	12(20%)	13(23.6%)	8(25.8%)
Occupational status	Farmers	36(60%)	32(58.2%)	12(38.7%)
	Government worker	20(33.3%)	10(18.2%)	8(25.8%)
	Merchant	4(6.7%)	13(23.6%)	11(35.5%)

Table 3: Vegetable cultivation in Mekelle, Alamata and Maychew

Crop grown	Percentage of respondents		
	Mekelle(N=60)	Alamata (N=55)	Maychew (N=31)
Cabbage	40(66.7%)	50(91%)	30(96.8%)
Swiss chard	50(83.3%)	55(100%)	15(48.4%)
Lettus	45(75%)	42(76.4%)	12(38.7%)
Carrot	21(35%)	18(32.7%)	2(6.45%)
Tomato	30(50%)	53(96.4%)	8(25.8%)
Pepper	15(25%)	20(36.4%)	4(7.3%)
Onion	19(31.7%)	48(87.3%)	5(16.1%)
Beetroot	10(16.7%)	8(14.5%)	6(19.4%)

Table 4: Water sources, mode of irrigation and practice fertilizer used in Mekelle, Alamata and Maychew

Parameter	Options	Percentage of respondents		
		Mekelle (N=60)	Alamata (N=55)	Maychew (N=31)
Source of water for irrigation	Rain water	0	0	0
	Surface water	40(66.7%)	38(69.1%)	20 (64.5%)
	Household waste water	8(13.3%)	3(5.45%)	4(12.9%)
	Microdams	12(20%)	14()	7()
Mode of irrigation	Drip irrigation using tubes	10()	40()	5()
	Irrigation by spray jerycan	29()	3()	22
	Canal irrigation	50()	52	30
	Both spray jerican and canal irrigation.	25	55	
Type of fertilizer used	Chemical	35	40	26
	House hold manure	25	15	5

Table 5: Diversity of bacterial population from waste water irrigated vegetables

Location	Bacterial distribution of different vegetables				
	Cabbage	Swiss chard	Lettuce	Tomato	Total 50
Mekelle	14.53x10 ⁶	5.93x10 ⁶	5.53x10 ⁶	3.87x10 ⁶	7.46x10 ⁶
Alamata	5.78x10 ⁶	4.79x10 ⁶	2.3x10 ⁶	3.64x10 ⁶	4.14x10 ⁶
Maychew	6.98x10 ⁶	6.3x10 ⁶	4.86x10 ⁶	4.2x10 ⁶	5.6X10 ⁶

Majority of farmers use surface of water, followed by water from microdams for irrigation. Result in Table 4 indicated that, canal irrigation is dominant mode of irrigation. Most of the farmers use chemical fertilizer followed by house hold manure such as composed manure and raw manure to facilitate vegetable growth. The frequency of application either chemical fertilizer or manure type, the majority of the farmers apply once/ months, 50% and some farmers apply the fertilizers where vegetable needs its possibility. The season of frequent application of fertilizers in vegetable production were dry season as well as farmers respondent that there were no rain fall that they must use waste water such as surface water and water stored in micro dam.

Microbiological Examination: The microbial loads of the vegetables samples are presented on Table 3, showed that the highest microbial load (14.53x10⁶ 5.93x10⁶ 5.53x10⁶ and

3.87x10⁶cfu/ml) on Cabbage, Swiss chard, Lettuce and Tomato, respectively) at Mekelle followed by Maychew with load of (6.98x10⁶, 6.3x10⁶, 4.86x10⁶ and 4.2x10⁶cfu/ml) and with the least microbial load of (5.78x10⁶, 4.79x10⁶, 2.3x10⁶ and 3.64x10⁶cfu/ml on Cabbage, Swisschard, Lettuce and Tomato) at Alamata. In the growing field, contamination with bacteria comes directly from faecal material and indirectly from: poor personal hygiene practices of workers, contaminated water applied directly to the vegetables, untreated manure used as fertilizer, human and animal faeces, including bird droppings in the growing fields and use of dirty harvest equipment, containers storage facilities. Microbes in waste water irrigated vegetables were several times higher than the WHO safety thresholds. Previous studies carried out in urban and peri-urban areas revealed that most of the surface water bodies used for irrigation is heavily polluted and not appropriate for crop irrigation [19-20].

Table 6: Parasitological contamination of vegetables with helminth eggs, larvae and Protozoan cysts in Mekelle, Maychew and Alamata southern zone of Tigray

Parasite	No. Vegetables (%) positive					
	Swiss chard =15	Cabbage=11	Onion =11	Lettuce=7	Tomato=11	Total =55
Ascaris	3 (20%)	2(18%)	1(9%)	0	0	6(10.9%)
Entamoeba spp	3 (20%)	4(36.4%)	0(0%)	5(71.43%)	4(36.4%)	16(29.01)
Strongloides stercoralis	2 (13%)	1(9%)	1(9%)	0	1(9%)	5(9.01%)
Giardia spp	1 (7%)	1(9%)	0(0%)	0	0	2(3.6%)
Total	9(60%)	8(46%)	2(18%)	5(71.43%)	5(45.45%)	29(52.73)

Based on morphological, cultural and biochemical test wide range of bacteria *Pseudomonas aeruginosa*, *Salmonella spp.*, *Shigella spp.*, *Escherichia coli*, *Campylobacter spp.*, *Listeria monocytogenes*, *Clostridium botulinum* and *Bacillus cereus* were detected. Use of waste water for irrigation and organic fertilizers (sewage sludge, animal manure, compost) may be source of potential risk for contamination of vegetables. They could be transferred from water to the vegetables during harvest (due to the lack of sanitary facilities to workers or dirty storage capacity) or post harvest treatment (handling, storage and transportation). Therefore, to minimize the risk of infection associated with raw vegetables, potential sources of contamination from the environment should be identified and specific measures and interventions to prevent and/or minimize the risk of contamination should be considered and correctly implemented.

The results revealed the prevalence of intestinal parasites such as *Ascaris*, *Entamoeba spp*, *Strongloides stercoralis* and *Giardia spp.* on all vegetables across the three locations. The highest rate of contamination was reported in lettuce (71.43%) followed by Swiss chard (60%) and Cabbage (46%) but the lowest in onion (18%), respectively. The use of wastewater can also affect the farm workers since significant *Ascaris* and hookworm infections have been reported by sewage farmers in India [21].

DISCUSSION

Studies on crop contamination with pathogenic microorganisms (bacteria, helminthes, protozoa viruses) where wastewater or partially treated wastewater is used have been reported in several studies carried out in different parts of the world [21, 22]. However; there remain many gaps in our knowledge in practice of checking microbial and parasitological contamination of vegetables grown in urban wastewater and dam water irrigated farms in Tigray region, Ethiopia. Out of the 55 vegetable samples wide range of bacteria (*Pseudomonas*

aeruginosa, *Salmonella spp.*, *Shigella spp.*, *Escherichia coli*, *Campylobacter spp.*, *Listeria monocytogenes*, *Clostridium botulinum* and *Bacillus cereus*) intestinal parasites such as *Ascaris*, *Entamoeba spp*, *Strongloides stercoralis* and *Giardia spp* were detected. This study also revealed that even at the farm level, wastewater is only one of several sources of crop contamination, although it can be the major one. Besides irrigation water, other identified contamination sources in the farm are immature manure as well as the previously contaminated soil. The consumption of vegetables irrigated with waste water as raw plays an important role in the transmission of microbial contaminations [23]. Bacterial numbers recorded in this study were still above the limit of 10^3 to 10^5 coliforms 100 g-1 wet weight of vegetables usually eaten raw when compared with previous findings from these farms [21]. This is might be due to use of faeces of domestic animals, human excreta, market and household waste which released into vegetable farms are often not protected and easily receive pollutants from the surrounding farm environment [24]. Another hand, the type of irrigation technique and the general morphology of vegetable expose much of its surface area to the irrigation water and soil particles from the splashes and this could account for the high contamination [25]. 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 [26-29].

CONCLUSIONS

- ✓ The study showed that some of the agronomic practices used by urban vegetable producers in the study areas could be sources of both microbial and parasitological contamination; and potentially put farmers, sellers consumers at risk. There is therefore the need for local authorities, researchers and other stakeholders to develop measures to optimize the benefits and minimize the risks associated with wastewater irrigated vegetable production.

- ✓ Vegetables grown in the studied locations were contaminated irrespective of the irrigation water used. Wastewater and use immature manure as fertilizer were identified as the main source of both microbial and parasitological contamination.
- ✓ The study has confirmed that irrigation water from different source used for vegetable production in the study areas are highly polluted with faecal coliform counts often above the recommended standard of 1×10^3 per 100 ml form unrestricted irrigation.
- ✓ Significantly higher pathogen contamination on vegetables occurs in the rainy season than in the dry season, despite usually lower irrigation frequency in the rainy than in the dry season.
- ✓ The farm was identified as the main source of faecal coliform and helminth eggs contamination
- ✓ The study showed that both animals and/or humans could be sources of faecal bacteria contamination of vegetables produced.
- ✓ Most of the faecal coliform isolates identified may be opportunistic pathogens capable of causing diseases/infection.
- ✓ To safeguard consumer's health, proper handling of wastewater irrigated vegetables is essential to prevent or reduce pathogen contamination at all entry points.

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