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Assessment of Microbial Quality of Vegetables Irrigated with Polluted Waters in Dar es Salaam City, Tanzania

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Abstract This study was undertaken to assess microbial quality of fresh vegetables irrigated with polluted waters from Msimbazi River in Dar es Salaam City. Samples of river water were taken from seven sampling stations along Msimbazi River stretching 20.4 km from Pugu to Jangwani and its tributary (Ubungo River) at Mabibo. Vegetable samples of 5 different species were taken from two different vegetable gardens in different locations along Msimbazi river valley and its tributary (Ubungo river), and from six markets including one super market. The examination of samples was carried out in accordance with the standard methods. Results revealed that water from the Msimbazi River contains high concentration of salts and pathogenic organisms. Fecal coliforms were found in all river water samples with a maximum count of $2.1x10^6$ cfu/100ml. Pollution of the river was increasing downstream and varies seasonally with wet season having poorer water quality than dry season. Vegetables from all the markets including super market were highly contaminated with fecal coliforms of up to 10^8 Cfu/kg-wet vegetables. Pathogenic organisms were detected on vegetables and all river water samples examined had *Ascaris Lumbricoides* except for the sample collected at Pugu station. Fresh vegetables were contaminated with pathogenic organisms such as *Escherichia coli, Citrobacter ssp, Proteus ssp*, *Klebsiella ssp*, *Salmonella spp* and *Basillus spp*. Vegetable samples collected from the markets were more contaminated than those collected from the gardens. High number of fecal coliforms in the markets was due to poor handling of these vegetable by contaminated hands and sprinkling of vegetables with polluted water to keep vegetables moist. It was concluded that water used for irrigating vegetables along river Msimbazi was highly polluted and vegetables from gardens and markets are not safe for consumption.

Keywords Faecal Pollution, Microbial Quality, Vegetables, Msimbazi River

1. Introduction

Worldwide, agriculture is under a strong pressure to produce more food for the ever increasing population [1, 2]. The rapid rate of urbanization in developing countries has led to serious concerns on household food security and also increased demand for water supply for both drinking and agricultural activities [3, 4]. Urban agriculture including vegetable farming, offers an important role in the economic, social, and dietary life of many urbanites with a vital economic role of acting as a source of income for producers and distributors [2]. Fruits and vegetables in particular are important sources of essential trace elements for communities especially in developing countries as they can be consumed at a relatively low price [5].

Owing to rapid population growth associated with urban development in relation to increased socio-economic activities, and environmental challenges like climate change, pollution and land degradation has reduced the availability and quality of water sources [1]. As a result of water scarcity, there is high potential of using untreated wastewater as well as exploiting undeveloped water sources [6, 7]. Since urbanization leads to an increase in wastewater production, wastewater has become a potential source of water for agricultural activities [8]. Because the use of potable water for irrigation is very expensive [9], the alternative option remains the use of surface water from polluted rivers [10], which poses potential human health risks due to the presence of pathogenic organisms from infected livestock or human hosts [4, 7]. As a result, there is a potential risk for the health of consumers of crops irrigated with contaminated water, especially fresh vegetables, which may at times be consumed without cooking when eaten as salad [11].

Several studies have shown that raw vegetables may harbor potential food-borne pathogens [11, 12]. In particular, tomatoes, cantaloupes, and sprouts have been linked to outbreaks of salmonellosis [13]. Some scholars have also reported outbreak of illnesses caused by Escherichia coli O157:H7, which is associated with lettuce,

and radish sprouts [14]. In other studies coleslaw, cabbage, potatoes, radishes, bean sprouts, and cucumbers contaminated with Listeria *monocytogenes* have been linked to disease outbreaks and salad vegetables also may be contaminated with Campylobacter [15, 16]. Examples of disease outbreaks linked with consumption of raw vegetables include *E. coli* O157: H7 in Montana and Connecticut in U.S.A and *S. Sonnei* in Sweden and other European countries, Hepatitis A infections in Kentucky in U.S.A. and Sweden, which were associated with the consumption of lettuce or green salad. In accordance with Raicevic *et al.* [11], *Salmonella* infection in 1990, 1993 and 1999 in United States was due to consumption of tomatoes, and *Salmonella* spp., *E. coli* 0157: H7, *B. cereus*, *L. Monocytogenes*, *Y. Enterocolitica*, and *Shigella* spp in Japan were associated with radish sprouts that affected 10,000 people.

In Tanzania, urban agriculture is characterized by unsafe practices and high risk of chemical pollution and contamination of pathogens, which puts the farmers and crop users at a very high rate of infections [17]. Most of vegetables cultivated for commercial purposes are grown near polluted water bodies particularly along the rivers because river water is cheap and reliable. However, rivers flowing through towns and cities in Tanzania such as Karanga, Njoro and Rau in Moshi; Mirongo in Mwanza and Themi in Arusha, are known to be highly polluted [4].

The demand for vegetables is high in major cities and towns in Tanzania, particularly in Dar es Salaam with over 4.3 million people. To meet such high demands of vegetables, many farmers use polluted surface water from rivers within the city for irrigation of vegetables because of inadequate clean water supplies [4, 7]. Unfortunately, river waters are polluted by informal settlements located near these rivers systems, which lack adequate sanitation and are therefore prone to microbial contamination [8, 18]. For instance, in Dar es Salaam city, most of the vegetables are grown along rivers like Msimbazi, Mlalakua, Tegeta and Kizinga, which are recipients of wastewater from industries, commercial areas and residential areas [7, 19]. Therefore fresh vegetables grown in these areas have the potential of being contaminated with human pathogens due to the use of untreated wastewater for irrigation. This represents an important route for transmission of pathogenic organisms [20] into the soil once water is used for irrigation [21]. The study aimed at assessing the microbial contamination of fresh vegetables along River Msimbazi and its tributaries**.**

2. Materials and Methods

Description of Study Area and Sampling Sites

Dar es Salaam city, which covers an area of about 1,393 km^2, is located between latitude 6°20' S to 7°30' S, and longitude 39°00' E to 39°30' E [22]. The city has population of 4,364,541 and annual growth rate of 5.6%, which is the highest rate of growth in the country in accordance with 2012 Population and Housing Census-PHC [23]. About 70% of the city's population live in unplanned settlements, which are characterized by lack of basic urban infrastructure services including water supply system, proper sanitation facilities, access roads, drainage and proper waste management systems. Samples were collected from six stations along Msimbazi River at Pugu at Banguro bridge (Station S1), Gongo la Mboto at Ulongoni bridge (Station S2), Vingunguti bridge (Station S3), Tabata Matumbi at Mandela Expressway bridge (Station S4), Kigogo Sambusa at Kawawa road culvert (Station S5) and Morogoro road at Jangwani bridge (Station S6) and Ubungo river at Bonde la Mchicha (Station S7). The surroundings of the sampling stations have different characteristics ranging from high density residential areas, to low density communities (Table 1). Some of the stations are surrounded by industries, farm lands, garages, waste stabilization ponds and abattoir.

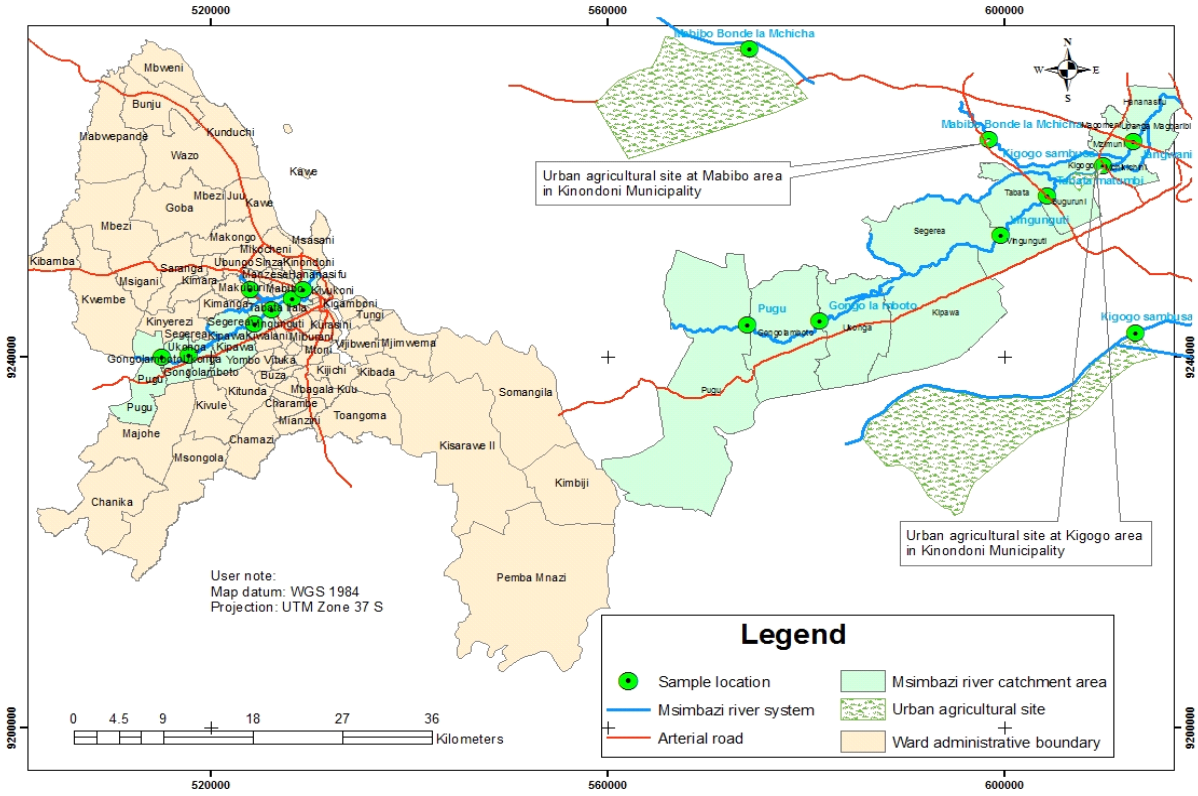

Figure 1. Map of Dar es Salaam showing the location of sampling station

Table 1. Sampling stations with coordinates

Location	River/ Station	Code	GPS Coordinates		Activity/ Establishment
			Northing	Easting	
Pugu at Banguro Bridge	Msimbazi Main	S1 (River)	0515133	9240055	Residential area (low density)
Gongo la Mboto at Ulongoni Bridge	Msimbazi Main	S2 (River)	0517801	9240189	Residential area
Vingunguti Bridge	Msimbazi Main	S3 (River)	0524454	9243573	High density residential area, Wastewater ponds, Industries
Tabata Matumbi at Mandela Bridge	Msimbazi Main	S4 (River)	0526166	9245117	Residential, Industrial area
Kigogo Sambusa at Kawawa road Culvert	Msimbazi Main	S5 (River)	0528256	9246285	Residential area
		Farm	0528230	9246252	
Morogoro road at Jangwani Bridge	Msimbazi Main	S6 (River)	0529345	9247274	Residential area (high density)
Mabibo at Bonde la Mchicha	Tributary	River (R3)	0523990	9247339	Residential area, Garage, Petrol station
		Farm (F3)	0524019	9247344	

Sampling Procedures and Preparation of Samples

Fresh vegetable samples were collected once a week for four months from the selected stations along the river during dry and wet seasons (Fig. 1). Samples were collected from Kigogo Sambusa farm (Vf1), Mabibo Bonde la Mchicha farm (Vf2) Kigogo Market (Vm1), Mabibo Market (Vm2), Buguruni Market (Vm3), Kariakoo Market (Vm4) Mwenge Market (Vm5) and from one large super market at Mlimani city. Water samples were collected using 250 ml sterilized glass bottles. Sample was collected from 7 stations (Table 1) using sampling techniques and protocols in accordance with the Standards methods for the examination of water and wastewater samples [24]. Samples were stored in cool boxes during transportation and were tested immediately upon arrival (within 4 hrs). Samples were prepared in accordance with the standard procedures and incubated for 24 hrs for fecal coliform. Water samples were analyzed for microbial parameters, which includes fecal coliforms and nematodes including *Dracunculus medinesis, Enterobius vermicularis, Ascaris lumbricoides* and *Trichuris trichiura*.

Samples of fresh vegetables of African Spinach leaves (*Amaranthus spp*), Pumpkin leaves (*Curcubita moschata*), Sweat potato leaves (*Ipomea batata*), Chinese cabbage leaves (*Brassica chinensis*) and Kale leaves (*Brassica oleracea*) were collected; just before harvesting, fresh from the farm and from markets where they were picked randomly from the vendors. Vegetable samples were put in labeled sterile polythene bags, and transported in a cool box to the laboratory where they were analyzed immediately. Vegetable samples were examined for fecal coliforms and helminthes eggs including *Dracunculus medinesis, Enterobius vermicularis, Ascaris lumbricoides* and *Trichuris trichiura.* Vegetables were also examined for the presence of *Escherichia coli*, *Citrobacter spp, Proteus spp, Krebsiella spp, Salmonella spp,* and *Bacillus spp*.

Examination of Water Samples

- Procedure for pour plate count for fecal coliform in irrigation water

Water samples were analyzed quantitatively for fecal coliform using standard plate count method in accordance with the Standard methods for the examination of water and wastewater samples [24]. Sterilized MF/C agar base for fecal coliforms was melted in a hot plate by immersing the 125 ml Erlenmeyer flask in the autoclave operated at 121^{o}C for 15 minutes. The agar was then allowed to cool to about 45^{o}C and held in a water-bath at temperature of 43^{o}C-45^{o}C. Then water sample was shaken vigorously and 1 ml of well shaken sample was aseptically transferred with a sterile pipette to a sterile Petri dish. About 10 ml of melted agar medium cooled to about 43^{o}C was then poured and mixed by rotating and tilting Petri dishes. The medium was then allowed to harden and Petri dish contents were incubated for 24 hours at 45^{o}C, after which colonies of bacteria were counted using an illuminated colony counter.

- Procedure for Helminthes eggs examination in irrigation water

About 200 ml of wastewater sample was collected and allowed to sediment in a beaker for 2 hours. About 90% of the supernatant was then removed using syringe and sediments were transferred to two centrifuge tubes of 10 ml capacity each and centrifuged at 3000 rpm for 15 minutes. The supernatant thereafter was removed and all sediments transferred to one tube and centrifuged at 1000 g for 15 minutes. The pellet was then transferred in an equal volume of acetoacetic buffer to make a total volume of 4 ml and an equivalent volume of ethyl acetate was then added and mixed using vortex equipment. The solution was then centrifuged at 1000 g for 15 min where three distinct layers were observed, the bottom layer contained non fatty material and the eggs, the middle layer contained the buffer and top layer contained fatty and other materials which formed a thick duck plug. The volume of the bottom most layer, which contains helminthes eggs, was recorded and the rest of the supernatant layers were poured off. The pellet was then re-suspended into five volumes of zinc sulphate solution, and the solution mixed by vortex. An aliquot of 1.5 ml was then transferred to McMaster slide and left for 5 min before examination; two slides one at a time was thereafter placed and viewed from the microscope at 10 xs for eggs count. Shapes of eggs as viewed from the microscope were compared to Plates I-XVII in the Ayres and Mara [25] that show a number of eggs of the human parasitic helminthes most frequently encountered in wastewater and other source samples to identify the type of helminthes available.

Microbial Examination of Pathogens of Vegetables

(a) Procedure for plate count for fecal coliform in vegetables

Approximately 20 g of vegetables was weighed and soaked into 180 ml sterilized buffered Peptone Water with NaCl for 5 to 10 minutes, which is recommended as a diluents for carrying microbial tests. The liquid content was then shaken vigorously before sample was extracted and diluted with appropriate sterilized dilution water before determination of fecal coliform density. The examination of fecal coliforms was carried out using pour plate technique in accordance with the Standard methods for the examination of water and wastewater samples [24].

(b) Procedure for determination of bacteria organisms in vegetables

About 1 g of the sample was immersed into 5 ml of nutrient broth and left for 30 minutes. The diluents was then inoculated on Blood Agar (BA) and MacConkey Agar by using a wire loop, which was then streak on the Petri dish and incubated at 37^{o}C for 24 hours. The identification of bacterial colonies was done by Macro morphology method, which was later followed by microscopic observation of different bacterial cells (micro morphology) using Grams stain technique to determine whether bacteria are Gram-Negative or Gram-Positive. Further tests such as Triple Sugar Iron (TSI) and Indole, Methyl red, Voges Proskeur and Citrate (IMVIC) were done for confirmation of the type of organisms.

(c) Procedure for determination of Helminthes eggs in vegetables

Vegetable samples were immersed immediately in sterilized water inside a sterile container and left for approximately 6–7 minutes in order to allow mud and other solids to settle in the bottom of the container. Then, it was gently collected and was put in a plastic basket. Each vegetable sample was eluted by vigorous agitation for 30 min and allowed to sediment in a beaker for two hours, ninety percent of the supernatant was removed using siphonic action (syringe) and sediments were transferred to two centrifuge tubes of 10 ml capacity each and

centrifuged at 1000 g (3000 rpm) in 15 minutes. The supernatant was then removed and all sediments were transferred to one tube and centrifuged at 1000g for 15 minutes. The pellet was then transferred in an equal volume of acetoacetic buffer to make a total volume of 4ml, which was followed by the addition of 4mL of ethyl acetate. The contents were then mixed using vortex equipment and the solution was thereafter centrifuged at 1000g for 15 minutes.

Statistical Analysis

Statistical analysis was done using excel window 2007 and GIS-ArcMap Version 9.2. Graphs and Charts were generated to establish characteristics of water quality and vegetables at different selected sampling stations. Spearman's Correlation (rs) analysis was applied to quantitatively analyze and confirm the relationship among the water quality characteristics of selected rivers and the microbial quality of irrigated vegetables.

3. Results and Discussions

Assessment of the Microbial Characteristic of Irrigation Water

Table 2 shows the results of the microbial quality of irrigation water from different sites along Msimbazi River. The results indicate that *Ascaris lumbricoides* eggs were detected in all sites except at the most upstream sampling station (station 1 -Pugu at Banguro Bridge). All other nematodes (*Dracunculus medinesis, Enterobius vermicularis* and *Trichuris trichiura*) were not detected in the river water. The presence of *Ascaris lumbricoides* eggs in the river, which is the most common intestinal parasite affecting human beings [26, 27], might be due to its prevalence worldwide particularly in tropical areas [27] and their ability to resist various external environmental conditions including chemicals [1]. They have direct life cycle and their ova are found in raw or insufficiently treated sewage, fertilizer and soil that may simply contaminate water bodies especially when erosion occurs. Also they may contaminate crops grown in soil or fertilizes that has receives non-lethal treatment [28, 29].

The examination of fecal coliforms shows that there was a difference in the fecal coliform counts of water between the sites in the study area (Fig. 2). Station 1 (Pugu at Banguro Bridge), Station 3 (Vingunguti), Station 4 (Tabata Matumbi) and Site 5 (Kigogo Sambusa) had higher fecal coliform counts than Station 2 (Gongo la Mboto) and Station 6 (Jangwani). Higher level of coliform counts at Pugu might be due to non point source of pollution including open defecation, which is later taken by water into the river. The variation of coliform counts at Vingunguti, Tabata Matumbi and Kigogo Sambusa is influenced by the coliform dynamics in the stream and the bacteriological quality of point's sources, which contribute to the pollution to the sites. However, levels of fecal coliform counts observed were greater than 1,000 cfu/100 ml in all the sampling sites, suggesting that none of the water samples from the sites met the guideline limit for fecal coliform bacteria in unrestricted irrigation of crops likely to be eaten raw in accordance with international standards [28]. Thus water from the river is unsuitable for human consumption and for irrigation of vegetables and salad crops without prior treatment [30].

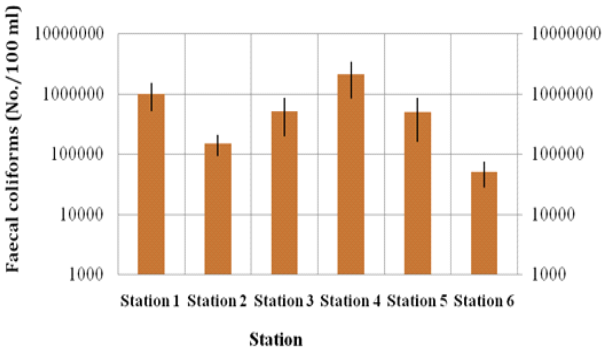

Figure 2. Fecal coliform counts of irrigation water along Msimbazi River

Table 2. Microbial characteristic of irrigation water from the river

Parameter	Sampling Station					
	S1	S2	S3	S4	S5	S6
Fecal coliform (No/100ml)	$1.0x10^6$	$1.5x10^5$	$5.2x10^5$	$2.1x10^6$	$5.0x10^5$	$5.0x10^4$
Ascaris lumbricoides	Not Detected	Detected	Detected	Detected	Detected	Detected
Dracunculus medinesis	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
Enterobius vermicularis	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected
Trichuris trichiura	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected	Not Detected

Figure 4 shows that among the organisms present on vegetables, counting the percentage variations indicates that contamination by *Escherichia coli* was about 61% where, *Citrobacter spp* 18%, *Proteus spp* 12%, *Klebsiella spp* 3%, *Salmonella spp* 3% and *Bacillus spp* 3%. High percentage of *E. coli* might be due to irrigating vegetables using water containing untreated sewage or manure, and contaminated wash water [44]. E. coli O157:H7 is known to be very resistant unless is destroyed by thorough cooking or pasteurization. It can survive for extended periods of time in water and soil, under frozen and refrigerated temperatures, and in dry conditions as well. It also can adapt to acidic conditions [45] and they are widely distributed in air, dusts and soils [46]. Some researchers have shown that the use of contaminated water is the source of pathogens from harvested produce [47]. However, leafy vegetables have the greatest risk of infection from manure application to soil. At the farm vegetable contamination can be due to contact with cattle, sheep, birds, insects and squirrels feces.

At the market, contaminated surfaces, including human hands and places like tables where vegetables are placed are major source of bacterial contamination that often come in contact with produce and represent potential points of cross-contamination throughout packing, processing, and preparing produce for consumption [42, 44]. Though many pathogens can cause health problems with fresh produce, *Escherichia coli* is of special concern since very few of their cells are enough to cause illness, and the illness can progress quickly to cause severe consequences in susceptible people, like children and the elderly. One group of *Escherichia coli,* which includes O157:H7, produces a powerful toxin that damages the lining of the small intestine, which can cause bloody diarrhea. One can develop an *Escherichia coli* infection when this strain of bacteria is ingested [48].

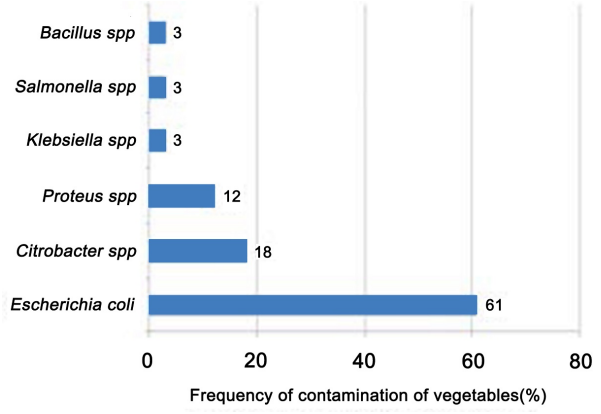

Figure 4. Variations of various microorganisms on fresh vegetables

Figure 5 shows variation of contamination of different types of vegetables whereby African spinach leaves (*Amaranthus* ssp.) was found to be highly contaminated by 29%, followed by pumpkin leaves (*curcubita moschata*) by 24% and Chinese cabbage leaves (*Brassica chinensis*), 23% and Kale leaves (*Brassica Oleracea*) 3%. High incidence of microorganisms in African spinach leaves might be due to general morphology of its leaves that expose much of its surface area to the contaminated water [20].

Figure 5. Percentage variation of vegetables types' contaminated

4. Conclusions

Based on the findings of the study, the following conclusions can be drawn.

- Water from Msimbazi River is not suitable for irrigation because it contains some pathogenic bacteria, Helminthes eggs and high concentration of salts. Pollution of the river is increasing downstream and is greatly contributed by land use. The quality of the river is affected by seasonal variations whereby there is much pollution during wet season than dry season.
- Vegetables from all the markets including super market are highly contaminated with fecal coliforms of up to 10^8 Cfu/kg-wet and helminthes eggs especially *Ascaris lumbricoides*. Vegetables that are sold at the markets are highly contaminated as compared to those at the farms. High number of fecal coliforms in markets is due to poor handling of these vegetable mostly by contaminated hands and sprinkling of vegetables with polluted water to keep vegetables moist.
- Fresh vegetables may potentially cause illnesses since they were all found contaminated with disease causing organisms such as *Escherichia coli, Citrobacter ssp, Proteus ssp*, *Klebsiella ssp*, *Salmonella spp* and *Basillus spp*.

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