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Microbial Quality of Fresh Lettuce Irrigated with Untreated Waste Water Around Aksum University, Central Zone of Tigrai Region.

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Abstract

Vegetables are essential components of the human diet and there is considerable evidence of the health and nutritional benefits related with the consumption of fresh vegetables. However, they can cause health problem if contaminated by pathogenic bacteria. The present study focused on the microbial quality of vegetables irrigated with untreated waste water. Data were analyzed using percentage and tables. The result of the study shows that 100% of the lettuce samples and 75% of the water samples were positive for salmonella spp. On the other hand, higher numbers of coliforms from lettuce were counted ranging from 3.207×10^3 - 4.063×10^5cfu/ml. Many people consume and sold lettuce in nearby towns and cause major health problems. Therefore, the present study can be used as an alarm for the concerned bodies to take measure.

Key words: Coliform, Lettuce, Salmonella spp., Waste water

1. Introduction

Fresh fruit and vegetables are important components of the human diet and there is considerable evidence of the health and nutritional benefits associated with their consumption. In the USA, Canada, New Zealand and several European Union states, public health institutions have run campaigns recommending the daily consumption of at least five daily servings of fruit and vegetables. As well as a significant rise in the consumption of fresh produce for health benefits, there have also been significant changes in lifestyles and major shifts in consumption trends (Vose et al., 2010).

Vegetable salad is a very common food accompaniment in many countries. The vegetables that usually make up this method include tomatoes, cucumber, carrots, green chili, cabbage and lettuce. They are sold in almost every market, and can be seen hawked around by traders. Fruits and vegetables have been identified as significant sources of pathogens and chemical contaminants (Uzeh et al., 2009).

Vegetables have been associated with outbreaks of food borne disease in many countries. Organisms involved include bacteria, viruses and parasites. Raw vegetables can harbour many microorganisms, which may be dispersed over the plant or appear as microcolonies fixed in the plant tissue (Szabo and Coventry, 2001). The majority of microorganisms associated with raw vegetables are gram negative organisms tend to dominate the microbial population. Vegetables are highly exposed to microbial contamination through contact with soil, dust and water and by handling at harvest or during postharvest processing. They therefore collect a different type of microorganisms including plant and human pathogens (Nguyen and Carlin, 1994).

Differences in microbial profiles of various vegetables result largely from unrelated factors such as resident microflora in the soil, application of nonresident micro flora via animal manures, sewage or irrigation water, transportation and handling by individual retailers (Ray and Bhunia, 2007). Use of untreated waste water and manure as fertilizers for the production of fruits and vegetables is the main causal factor to contamination (Olayemi, 1997). Tomato, Cucumber, Carrot, Green chili, Lemon, Coriander leaf, Pepper mint and Beet root are some of the vegetables that are normally consumed raw in order to obtain their valuable nutrients in best form and their traditional use in best form and their traditional use in preparing salads is familiar throughout the world in the same manner.

Commercial and small-scale farmers generally irrigate their produce with water from nearby rivers, streams, ponds and dams most of which do not meet the required standard for irrigation (Westcot, 1997). The microbiological quality of irrigation water is critical because water contaminated with animal or human wastes can introduce pathogens during pre-harvesting and post-harvest (FDA, 2005). Furthermore, Palese et al. (2009) reported that contaminated irrigation water and surface run-off water might be major source of pathogenic microorganisms that contaminate fruits and vegetables in fields. Food safety issues are of growing concern to consumers globally because of the risk associated with consumption of foods contaminated with pathogens in irrigated vegetables. In the study area farmers were commonly cultivated vegetables irrigated with waste water drained from different cafeterias. Therefore, the objective of the current study is to assess the quality of lettuce irrigated with waste water around Aksum University.

2. Materials and methods

2.1. Sample size and sampling techniques

The study was conducted for about 3 months from April to June 2014. A total of 8 samples of lettuce and waste water were collected randomly at different times. The raw lettuce was collected by using sterile glove and placed in sterile polyethylene plastic bags while the waste water samples were placed in sterile bottles. Samples were taken to the laboratory and processed immediately within 2 hours.

2.2. Isolation techniques of salmonella spp.

A 25 gram of lettuce sample were weighed and immersed in 250 ml beaker containing 100 ml of sterile distilled water. From the rinsed water a serial dilutions were prepared. Media were prepared according to the manufacturer's guide. Finally, a 0.1 ml of each dilution was spread plated on SS-agar and incubated at 37^0c for 24 hours. Finally, colonies with black centers were confirmed by using, triple sugar iron agar test, motility test and colors of the colony (Khan et al., 1992).

2.3. Coliform count using MacConkey agar

The enumeration of coliforms was done using MacConkey agar following the methods recommended by (Roberts and Greenwood, 2003). Medias were prepared according to the manufacturer's guide. A 25 gram of lettuce sample were weighed and immersed in 250 ml beaker containing 100 ml of sterile distilled water. From the rinsed water a serial dilution were prepared. Volumes of 1 ml of appropriate dilutions were pour plated to MacConkey agar and plates were incubated at 37^0C for 24 hours. Finally, colonies between 30-300 were counted.

3. Result and discussion

Table 1. Isolation of Salmonella spp. from lettuce and Waste Water

Sample source	Number of samples	Salmonella species	
		Positive	Negative
Lettuce	4	4(100%)	-
Waste water	4	3(75%)	1 (25%)

According to the above Table, 75% of the lettuce samples were positive for salmonella pp. The current result agreed with the works of (Melloul et al. (2001) where they detect Salmonella spp. in 80% of waste water samples. Only 25% of the water samples were free of salmonella. Our study also shows that 100% of Salmonella spp. were detected in raw lettuce samples. This is in line with the results of Itohan et al. (2011) where they isolate Salmonella spp. in 20% of raw lettuce samples. According to PHLS (2000) guide lines in 25 gram of raw vegetables Salmonella spp. should not be detected. However, in most of the samples salmonella spp. were detected. The existence of salmonella spp. in lettuce is due to the use of waste water for irrigation purpose. As it was observed from the preliminary survey, farmers use waste water for irrigation purpose to cultivate vegetables. This can result in major health problem of the society if care is not taken by the consumers. Salmonella spp. were isolated from waste water, indicating that waste water is the major source of contamination for lettuce in the agricultural field. The source of Salmonella to the waste water could be the student cafeterias where utensils used for the preparation of food were washed every day and the left over of the food may be discharged to the river. According to WHO (2002) effect of microbiological hazards such as Salmonella on food safety is now a major public health concern worldwide. However, our result shows that all of the samples were positive for salmonella spp.

Table 2. Coliform count from lettuce sample

Sample source (lettuce)	Coliform count (Cfu/ml)
Sample 1	4.063×10^5
Sample 2	5.031×10^3
Sample 3	3.207×10^3
Sample 4	4.873×10^3

Table 2 shows coliform count ranging from 3.207×10^3- 4.063×10^5. Our current study is similar with the works of Cobbina et al. (2013) where they reported coliform counts of 3.7 ± 0.5 cfu/g in lettuce samples. According to PHLS (2000) guide line the accepted value for coliform count in fruits and fresh vegetables should be $<10^4$ cfu/ml. However, our current result exceeds this value. This indicates that the lettuce which is cultivated by the farmers is poor in quality. The higher number of coliforms indicates that pathogenic bacteria may exist in the lettuce.

Table 3. coliform count from waste water

Sample source (waste water)	coliform count (Cfu/ml)
Sample 1	2.54×10^5
Sample 2	1.555×10^5
Sample 3	3.754×10^4
Sample 4	3.163×10^4

Table 3, indicates that the waste water which is used for irrigation purpose contains higher number of coliforms. The number of colony forming units (cfu/ml) of the sample ranges from 3.163×10^4-2.54×10^5. Farmers use this water for irrigation purpose without any treatment. As a result, freshly consumed fruits and vegetables may be easily contaminated by pathogenic bacteria. The higher numbers of coliforms in food samples indicate that the food may contain pathogenic bacteria. This was confirmed by detecting salmonella spp. from both lettuce and waste water. The existence of Salmonella spp. in the water samples may be from left over foods that come from student cafeteria.

4. Conclusion

In conclusion, the high coliform load in lettuce samples could serve as an indicator for the need to promote awareness about the possible health hazards that could be due to poor handling of these vegetables such as lettuce. On the other hand, the detection of Salmonella spp. in the lettuce samples could cause a major risk in the society. Therefore, the need for regulatory bodies to ensure that microbiological standards are established and practiced by farmers and marketers for the handling and distribution of vegetables is required.

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