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IRRIGATION WATER AS POSSIBLE SOURCE OF FOOD BORNE PATHOGENS IN RAW VEGETABLES

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ABSTRACT

The effects of different sources of water on the microbial quality of raw leafy vegetables were studied. Two vegetables, lettuce (*Lactuca sativa*) and fluted pumpkin (*Telfairia occidentalis*) were planted in a sterile soil in the screen house, irrigated with rain, sewage-polluted stream, tap and well waters, for 60 days and harvested. Coliform and bacterial loads were counted on vegetable leaf surfaces and in the different water samples. The total bacterial counts of vegetable leaf surfaces ranged from 5.46$\log_{10}$ cfu/g to 6.11$\log_{10}$ cfu/g while coliform counts ranged from 0.00 to 5.43$\log_{10}$ cfu/g. The total bacterial counts of the irrigation water samples ranged from 5.60$\log_{10}$ cfu/ml to 6.12$\log_{10}$cfu/ml while coliform counts ranged from 0.00 to 5.48$\log_{10}$cfu/ml. Pathogenic bacteria observed in the samples were *Escherichia coli, Staphylococcus aureus, Salmonella spp, Shigella dysenteriae, Klebsiella pneumoniae, Aeromonas hydrophila* and *Enterobacter aerogenes*. This study shows that irrigation waters are possible sources of contamination of edible vegetables; therefore the irrigation of food crops with water of unknown microbial quality should be avoided.

Keywords: Pathogenic bacteria, Microbial quality, Irrigation water, Contamination and Vegetables.

INTRODUCTION

Vegetables constitute an indispensable constituent of human diet in Africa. They may be consumed raw or as cooked complements to the major staples like cassava, cocoyam, rice and plantain. When land is available, families grow vegetables for home uses. Otherwise, they are purchased from local farmers and retail outlets for further processing at home or at restaurants and other food-service facilities (Monge *et al.,* 1998). Consumption of fresh or raw vegetables (especially those used for salads), fruits and fruit juices has been associated with outbreaks of food-borne diseases (Evans *et al.*, 2003, Moro *et al.*, 2003). Pathogenic organisms that are capable of causing human diseases include bacteria, viruses and parasites which may be present in water used for irrigation or in soil in which the produce is grown (Beuchat, 1996a). *Salmonella spp, Shigella spp, Bacillus spp, Campylobacter spp, Clostridium spp, Staphylococcus aureus, Enterococcus spp, Pseudomonas aeruginosa and Actinebacter calcoaceticus* have been associated with diseases resulting from consumption of raw vegetables (Adebolu and Ifesan, 2001; Evans *et al.*, 2003; Amoah *et al.*, 2006). Sources of preharvest contamination include faeces, soil, irrigation water, improperly composted manure, air, wild and domestic animals, and human handling (Beuchat, 1996a; Steele and

Odumeru, 2004).

The purpose of this study is therefore to investigate the microbiological contamination of leafy food crops irrigated with microbial contaminated water.

MATERIALS AND METHODS

Cultivation and harvesting of vegetables

The experiment was conducted in a Completely Randomized Design with four treatments and four replicates. Seeds of lettuce vegetable (*Lactuca sativa*) and fluted pumpkin vegetable (*Telfairia occidentalis*) were planted in plastic pots filled with sterile garden topsoil. The plants were raised in the screen house and watered daily with sewage -polluted stream water, shallow well water, tap water and rain water, using spray irrigation method. Rain water served as control water. The irrigation was done for about 60d, after which the plants were harvested aseptically by cutting with the aid of sterile scissor at 5.00cm above the ground level.

Microbiological analysis

One gram leaf sample of each vegetable was chopped with 10.0ml of sterile peptone water. After then, series of dilutions were made by mixing 1.0ml of the suspension in 9.0ml of the sterile peptone water to obtain 10^{-1} dilution. The dilution was then made to 10^{-2}, 10^{-3} and 10^{-4}. The serial dilutions of each water sample were also made.

Total bacterial counts of the different water sample and the leaves of vegetable samples were determined by enumerating the colony forming units (cfu) by plating in a standard plate count agar (oxoid) and incubating at 37^0C for 48 h. The detection of coliforms was carried out by pour plating of the diluents using Mac-Conkey agar (oxoid) and incubating at 37^0C for 48h. Colonies typical of coliforms were subjected to Indole, Methyl red, Voges proskaur and Citrate (IMVC) tests.

For the detection of *Listeria monocytogenes*; 1.0ml of the diluent was spread over the plate of Blood agar and incubated at 37^0C for 48h. Typical colonies were subjected to series of biochemical tests for identification. Enrichment of *S. aureus* was performed using nutrient broth supplemented with 7.5% NaCl. The inoculated broth was incubated at 37^0C for 24. After 24h, a loopful of culture broth was streaked onto a plate of Mannitol salt agar (oxoid) and incubated at 37^0C for 48h. The pathogenic character of the organism was ascertained by coagulase test.

Enrichment of *Salmonella* and *Shigella* species was performed using alkaline peptone water. The inoculated broth was incubated at 37^0C for 24h. After 24h, a loopful of culture broth was streaked onto the plates of Salmonella – Shigella agar (oxoid) and Deoxycholate citrate agar (oxoid) and incubated at 37^0C for 48 h. Suspected colonies were subjected to series of biochemical tests.

Enrichment of *Aeromonas spp* was performed in alkaline peptone water and incubated at 37^0C for 24h. A loopful of culture broth was streaked onto the plates of Ampicillin Blood agar and Ampicillin starch agar, and then incubated at 37^0C for 48h. Typical colonies were subjected to series of biochemical tests for identification.

Statistical analysis

All samples prepared were replicated two times. Plate count data of vegetable samples were expressed as the Log_{10} mean cfu/g value ± standard deviations while those of the water samples were expressed as the log_{10} mean cfu/ml values ± standard deviations, obtained from duplicated plates. Significant differences in plate count data were established by Duncan Multiple Range test at 5% level of significance.

RESULTS

Bacteriological quality of irrigated vegetables and irrigation waters:

Tables 1 and 2 summarize the results of total microbial counts of irrigation water samples and irrigated vegetable leaves respectively, while table 3 shows the pathogenic bacteria isolated from the irrigation water samples and irrigated vegetable leaves. The total bacterial counts of the irrigation water samples ranged from 5.60 log_{10} cfu/ml to 6.12 log_{10} cfu/ml. Sewage- polluted stream water sample had the highest bacterial load (6.12log_{10} cfu/ml) while rain water had the lowest load (5.60log_{10} cfu/ml) (Table 1). The total bacterial counts of the irrigated vegetable samples ranged from 5.46log_{10} cfu/g to 6.11log_{10} cfu/g (Table 2). *Telfairia* vegetable irrigated with polluted stream water had the highest total bacterial counts (6.11 log_{10} cfu/g) while *Lactuca* vegetable irrigated with rain water had the lowest counts (5.46 log_{10} cfu/g). Generally, the vegetables irrigated with sewage- polluted stream water had higher bacterial loads while the vegetables irrigated with rain water had the lowest bacterial counts.

The total coliform counts of the irrigation water samples ranged from 0.00 to 5.48 log_{10} cfu/ml. Sewage- polluted stream water had the highest coliform counts of 5.48log_{10} cfu/ml and no coliform bacteria was isolated from rain water. Also, the total counts of coliform bacteria in irrigated vegetables ranged from 0.00 to 5.43log_{10} cfu/g with *Lactuca* and *Telfairia* vegetables irrigated with sewage- polluted stream water having the highest coliform counts of 5.43 log_{10} cfu/g and 5.40log_{10} cfu/g respectively. No coliform was isolated from the vegetables irrigated with rainwater (Table 2).

All the irrigation water samples and irrigated vegetables contained pathogenic bacteria with the exception of rain water and vegetables irrigated with rain water which did not contain food-borne bacteria (Table 3). Bacteria isolated from water samples and vegetables include *Escherichia coli*, *Staphylococcus aureus, Klebsiella pneumoniae*, *Enterobacter aerogenes, Salmonella paratyphii, Shigella dysenteriae, Pseudomonas aeruginosa, Streptococcus spp., Aeromonas hydrophila* and *Bacillus spp.* (Tables 3). *Micrococcus luteus* was isolated from irrigation water samples but not from the vegetables. *Listeria spp.* was neither isolated from the irrigation waters nor the irrigated vegetable samples. The presence of these pathogenic bacteria in any food indicates that such food has been contaminated and it is unsafe for human consumption.

Table 1: Total Microbial counts of irrigation water samples.

Water samples	Total bacterial counts (Log10 cfu/ml ± SD)	Total coliform counts (Log10 cfu/ml ± SD)
Stream water	6.12 ± 0.15ab	5.48 ± 0.04a
Well water	6.11 ± 0.05cd	5.40 ± 0.02b
Tap water	5.70 ± 0.13ac	5.15 ± 0.08ab
Rain water	5.60 ± 0.10bd	0.00ab

Note: Means with the same letters along the columns are significantly different (b< 0.05).

Table 2: Total Microbial counts of irrigated vegetable leaf samples

	LS	LW	LT	LR	TS	TW	TT	TR
Total bacterial counts (Log10 cfu/g ± SD)	6.08 ± 0.06a	6.04 ± 0.09b	5.75 ± 0.23abcd	5.46 ± 0.07abcd	6.11 ± 0.03c	6.03 ± 0.08d	5.73 ±0.14abcd	5.48 ± 0.10abcd
Total coliform counts (Log10 cfu/g ± SD)	5.43 ± 0.17a	5.34±0.11b	5.00 ± 0.12ab	0.00ab	5.40 ± 0.06	5.23 ± 0.05a	4.95 ± 0.07ab	0.00ab

Note: Means with the same letters along the rows are significantly different (p<0.05).

LS and TS: *Lactuca* vegetable and *Telfairia* vegetable irrigated with polluted stream water.

LW and TW: *Lactuca* vegetable and *Telfairia* vegetable irrigated with well water.

LT and TT: *Lactuca* vegetable and *Telfairia* vegetable irrigated with tap water.

LR and TR: *Lactuca* vegetable and *Telfairia* vegetable irrigated with rain water.

Table 3: Incidence of bacterial isolates on vegetables and irrigation waters

Isolates	Tap water	Vegetables irrigated with tap water	Rain water	Vegetables irrigated with rain water	Well water	Vegetables irrigated with well water	Stream water	Vegetables irrigated with stream water
Escherichia coli	+	+	-	-	+	+	+	+
Staphylococcus aureus	+	+	+	+	+	+	+	+
Klebsiella pneumonia	+	+	-	-	+	+	+	+
Enterobacter aerogenes	+	+	-	-	+	+	+	+
Salmonella paratyphii	-	-	-	-	+	+	+	+
Shigella dysenteriae	-	-	-	-	+	+	+	+
Pseudomonas aeruginosa	+	+	+	+	+	+	+	+
Streptococcus spp	-	-	-	-	-	-	+	+
Aeromonas hydrophila	-	-	-	-	-	-	+	+
Bacillus spp	-	-	+	+	+	+	+	+
Micrococcus luteus	+	-	+	-	+	-	+	_
Listeria spp	-	-	-	-	-	-	-	-

Note: + : Presence
- : Absence

DISCUSSION

The outbreak of diseases caused by food-borne pathogens that are associated with fresh farm produce has necessitated a review of food safety measures during the growth and processing of these products. The present study has shown that irrigation water that is contaminated with human and animal wastes may be a source of pre-harvest contamination of food crops growing on the field. The result showed that *E. coli* and other pathogenic bacteria were transferred to vegetable plants during irrigation. This is because the vegetable plants irrigated with polluted stream water and well water were more contaminated with *E. coli* and other pathogenic bacteria than those irrigated with tap water and rain water. The bacterial pathogens isolated from the vegetables irrigated with water from different sources were *Escherichia coli, Salmonella paratyphii, Shigella dysenteriae, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus spp., Enterobacter aerogenes and Aeromonas hydrophila.* All these bacteria are of public health significance and their presence in any food indicates that such food has been contaminated with faecal materials and it is unsafe for human consumption. All these bacteria were isolated from vegetables irrigated with polluted stream water and well water; few of them were isolated from vegetables irrigated with tap water while the indicator organisms and other pathogens were not isolated from the vegetables irrigated with rain water. Since *E. coli* is regarded as primary indicator for microbiological quality of water and food, the presence of *E. coli*, *Salmonella spp., Shigella spp.* and *Klebsiella spp.* on vegetables irrigated with polluted stream water and well water is an indication of faecal contamination. The possible source of faecal contamination to these vegetables was primarily from the irrigation waters contaminated with faecal materials from nearby abattoir, pit latrines and human wastes. The results of this study therefore support the previous findings that irrigation of food crops with contaminated water resulted in contamination of the crops (Sadovski *et al.*, 1978; Beuchat, 1996a; Solomon *et al.,* 2002; Wachtel *et al.,* 2002; Steele *et al.*, 2005; Heaton and Jones, 2008).

The absence of *E. coli*, *Salmonella spp.* and other pathogens on vegetables irrigated with rain water makes the vegetables safe for human consumption and ranks rain water as the best source of water to irrigate food crops.

CONCLUSION

In conclusion, the result of this study revealed that irrigation of food crops with poor – quality water is one way that vegetables can become contaminated with food – borne pathogens. Rain water is of good microbial quality and can be used for irrigation. Groundwater or well water is also generally of good microbial quality, unless it is contaminated with surface run off while human waste water or polluted stream water is usually of very poor microbial quality and requires extensive treatment before it can be used safely to irrigate crops. Therefore, in order to increase the microbial safety of raw fruits and vegetables, it is suggested that regardless of the irrigation method used, crops can become contaminated from the irrigation water, thus the irrigation of food crops with water of unknown microbial quality should be avoided. However, if polluted water is to be used for irrigation, such water should be treated in order to remove the pathogenic bacteria present in it. It is also recommended that standard guidelines governing the microbiological quality of irrigation water should be set up, so as to reduce the risk of disease transmission by food-borne pathogens present in irrigation water.

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