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# Studies on the microbial load of selected vegetables irrigated with untreated waste water in Allahabad district

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**Abstract**
Vegetables can become contaminated with pathogenic organism during growth, harvest, postharvest handling or distribution. The microbial (Total Coliforms, Faecal coliforms, *Staphylococcus aureus*, *Listeria* spp., *Pseudomonas* spp, *Salmonella* spp) quality of vegetables (Spinach, Cucumber, Ladyfinger and Ridge guard) irrigated with waste water in around Yamuna river of Allahabad district investigated. Total coliform and faecal coliform were observed positive in all vegetables, The ANOVA tables appended with appendix showed Non-significant result of *Staphylococus aureus* for site one (Mahewa Path) but in site two(Arail Ghat), significant result was obtained for *Staphylococcus aureus*. Non-significant result of *Listeria spp* for site one but in site two significant results was obtained for *Listeria spp.* There was significant result of *salmonella spp*in site one similarly in site two. The quality of the waters and the irrigated vegetables exceeded standard microbiological limits.

**Keywords:** microbial load, waste water, vegetables.

## Introduction
Vegetables can become contaminated with pathogenic microorganism during growth, harvest, postharvest handling or distribution. The use of untreated wastewater in irrigation represents an important route for transmission of these pathogenic microorganisms. The major pathogens associated with the use of highly polluted water. According to Ensink (3), farmers and irrigation workers can acquire helminthes infections and parasitic diseases due to direct contact with untreated wastewater and contaminated soils especially if exposed for a long duration. It has also been reported that irrigation of salad crops with untreated wastewater caused excess diseases (e.g. Shigellosis in England) in those who consumed them. Numerous opportunities exist for attachment and penetration of pathogenic bacteria on vegetables in the field, as well as during harvesting, processing and handling especially when a contaminated product is exposed to water or is damaged.

Wastewater reuse in irrigation is largely considered an inevitable option to compensate water shortages in developing countries. Hence, crop irrigation with wastewater is a widespread practice in these countries (Sou *et al*., 2011)[5]. In the urban areas the use of wastewater in agriculture is a centuries-old practice that is receiving renewed attention with the increasing scarcity of freshwater resources in many arid and semi-arid regions (Ackerson *et al*., 2012)[1]. The growing wastewater volumes are driven by rapid urbanization (Scott *et al*., 2004)[4]. Economic and agronomic advantages are sometimes promoted in wastewater reuse but there are several studies warning about health risks and environmental impacts (Sou *et al*., 2011)[5]. One of the most economically feasible agricultural uses of reclaimed water is the irrigation of Vegetables which typically have high returns per volume of water invested in it (Toze *et al*., 2006)[6].

## Materials and methods
### Study area
The present work entitledwas conducted in research laboratory of Warner School of Food and Dairy Technology, The sample of vegetables was collected from waste water irrigated Yamuna banks where crops are irrigated with wastewater which is coming directly from the municipality. Our study area is situated in the left side of the Yamuna River around 10 km south of Allahabad city. The common vegetables grown in this area is cucumber, spinach, ladyfinger, ridge gourd and etc. which are supplied to the all vegetables market in Allahabad and the rest entire the common market.

Vegetable samples were collected by hand using sterilized poly ethylene gloves carefully packed into polyethylene bags and the whole plant body was brought to the laboratoryon the month of October 2014 from both sites (Mahewa path and Arail Ghat) where waste water is using as irrigation for the vegetables. Vegetable samples were quantifyingfor the determination of Total *Coliforms, Faecal coliforms,Staphylococcus aureus*, *Listeria* spp, *Pseudomonas* spp, *Salmonella* spp. Vegetable samples rinsed vigorously, the water resulting from the rinsing was used for the determination of Total Coliforms, Faecal coliforms, *Staphylococcus aureus*, *Listeria* spp., *Pseudomonas* spp, *Salmonella* spp.

**Preparation of sample**

**Total and Faecal coliforms**

Enumeration was done by Most Probable Number (MPN) method. Serial dilutions of $10^{-1}$ to $10^{-6}$ were prepared from each vegetable sample. One ml of each dilution was inoculated in triplicate into 5 ml tubes. Tubes showing acid and gas production after incubation for 24 h at 37°C.All tubes showing a yellowish color development after gentle agitation were recorded as positive for total coliforms.

The Multiple Tube Fermentation also referred to as Most Probable Number (MPN) method was used to determine the number of total and faecal coliform bacteria populations in the samples [2]. Aliquot of 1 ml of sample was pipetted into 9 ml of sterile distilled water. The serial dilution prepared for the analysis ranged from $10^{-1}$ to $10^{-6}$ from each dilution, 1ml was taken and carefully added in order into triplicate tubes containing 5 ml sterile Mac-Conkey broth with inverted Durham tubes. The tubes were later incubated at 37°C for 24 to 48 hrs for deter-mination of total coliform and at 44.5°C for the determination of faecal coliforms. The number and distribution of positive tubes (acid and/or gas production) from incubated samples were used to obtain the population of coliform bacteria from MPN Table [2].

***Staphylococcus aureus***

Plating was done on Baird's Parker agar media. Plates were incubated at $37^0$C for 24 hours, and observation was noted.

***Listeria* spp.**

Isolation was performed on Tryptone soya yeast extract agar in all samples and the observation noted carefully by using the colony counter. Plates were incubated at 37 $^0$C for 48 hours.

***Pseudomonas* spp.**

Isolation was done by using*Pseudomonas* isolation agar in all vegetables and observations were noted**.** Plates were incubated at 31 $^0$C for 48 hours.

***Salmonella* spp.**

Salmonella was enumerated by using *Shigella Salmonella* (SS) Agar in both sites all vegetables and data recorded by counting the number of the colonies.

**Results and Discussion**

Microbiological testing of vegetables irrigated with waste water, included *Staphylococcus aureus, Listeria spp, Pseudomonas spp, Salmonella spp* and presence and absence of *Total and faecal coliform* were done by using different growth media. Each sample were tested for the above mentioned microorganisms.

**Table 1:** Bacterial count of vegetables irrigated with untreated waste water obtained from Mahewa Path.

| Treatments | *Staphylococcus aureus* ($10^{-6}$) | *Listeria spp* $10^{-6}$ | *Salmonellaspp*$10^{-6}$ | *Psedomonas spp*($10^{-6}$) | Total *coliform* | *Faecal coliform* |
|---|---|---|---|---|---|---|
| Spinach | 100.000 | 258.667 | 190.667 | - | + | + |
| Cucumber | 257.333 | 197.333 | 74.667 | - | + | + |
| Ladyfinger | 149.333 | 93.333 | 121.333 | - | + | + |
| Ridge guard | 88.000 | 289.333 | 189.333 | - | + | + |
| Mean | 148.667 | 209.667 | 144.000 | - | + | + |
| F- test | NS | NS | S | - | | |
| S. Ed.(±) | 120.049 | 57.650 | 9.444 | - | | |
| C. D. (P=0.05) | | | 19.841 | - | | |

Total coliform and faecal coliform were observed positive in all vegetables in all three replication. An important reason as to why it is difficult to draw conclusion from the data is that the test for total coliform and faecal coliforms was only a presence/absence test and therefore gives no indication of the actual number in all vegetables i.e. whether heavily or slightly contaminated.

There was maximum number of *Staphylococus aureus* coloniesin Cucumber (257.333), followed by Ladyfinger (149.333), Spinach (100) and minimum number of *Staphylococus aureus* colonies was in Ridge guard, the average mean *Staphylococus aureus* colonies were (148.667) for all vegetables, The ANOVA tables appended with appendix showed non-significant result of *Staphylococus aureus* in $10^{-6}$ dilution.

Maximum number of *Listeria spp.*coloniesin Ridge guard was (289.333), followed by Spinach (258.667), cucumber (197.333) but there was minimum number of *Listeria spp* colonies in Ladyfinger ( 93.333), the average mean *Listeria spp.*colonies were (57.650) for all vegetables, The ANOVA tables appended with appendix showed non-significant result of *Listeria spp.*in $10^{-6}$ dilution.

The Maximum number of *Salmonellaspp*colonieswas in spinach(190.667), followed by ridge guard (189.333), ladyfinger (121.333) but there was minimum number of *Salmonellaspp*colonies in cucumber (74.667), the average mean *Salmonellaspp.*colonies were (144) for all vegetables, The ANOVA tables appended with appendix showed significant result of *Listeria spp.*in $10^{-6}$ dilution.

*Staphylococcus aureus* has been detected on fresh produce and ready-to-eat vegetable salads and is transmitted through food handlers. However, enter toxigenic*S. aureus* does not compete well with other microorganisms normally present on fresh produce, so incipient spoilage caused by nonpathogenic micro biota would likely precede the development of high populations of this pathogen (U. S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, 2001)[7].

The genus *Salmonella* has over 2700 serotypes. Animals and birds are the natural reservoirs. Surveys of fresh produce have revealed the presence of several *Salmonella* serotypes capable of causing human infection. (U. S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, 2001)[7].

**Table 2:** Bacterial count of vegetables irrigated with untreated waste water obtained from site two Arail Ghat.

| Treatments | *Staphylococcus aureus* ($10^{-6}$) | *Listeria spp* $10^{-6}$ | *Salmonella spp* $10^{-6}$ | Pseudomonas spp($10^{-6}$) | Total coliform | Faecal coliform |
|---|---|---|---|---|---|---|
| 4k1m | 189.333 | 24.000 | 397.333 | - | + | + |
| Cucumber | 328.000 | 121.333 | 614.667 | - | + | + |
| Ladyfinger | 317.333 | 140.000 | 434.667 | - | + | + |
| Ridge guard | 358.667 | 58.667 | 354.667 | - | + | + |
| Mean | 298.333 | 86.000 | 450.333 | - | + | + |
| F- test | S | S | S | - | | |
| S. Ed.(±) | 27.947 | 3.220 | 8.692 | - | | |
| C. D. (P=0.05) | 58.717 | 6.766 | 18.262 | - | | |

Total coliform and faecal coliform were observed positive in all vegetables in all three replications. An important reason as to why it is difficult to draw conclusion from the data is that the test for total coliform and faecal coliforms was only a presence/absence test and therefore gives no indication of the actual number in all vegetables i.e. weather heavily or slightly contaminated.

There was maximum number of *Staphyloccus aureus* coloniesin Ridge guard (358.667), followed by Cucumber (328.000), Ladyfinger (317) and minimum number of *Staphyloccus aureus* colonies was in Spinach (189.333), the average mean *Staphyloccus aureus* colonies was (298.333) for all vegetables, The ANOVA tables appended with appendix showed significant result of *Staphyloccus aureus* in $10^{-6}$ dilution.

Maximum number of *Listeria spp* coloniesin ladyfinger was (140.333), followed by cucumber (121.333), ridge guard (57.667) but there was minimum number of *Listeria spp* colonies in spinach ( 54), the average mean *Listeria spp*colonies were (86.000) for all vegetables, The ANOVA tables appended with appendix showed non-significant result of *Listeria spp* in $10^{-6}$ dilution.

The Maximum number of *Salmonellaspp*colonieswas in Cucumber (614.667), followed by Ladyfinger(434.667), Spinach (397.333) but there was minimum number of *Salmonellaspp*colonies in Ridge guard (354.667), the average mean *Salmonellaspp* colonies were (450.333) for all vegetables, The ANOVA tables appended with appendix showed significant result of *Listeria spp* in $10^{-6}$ dilution.

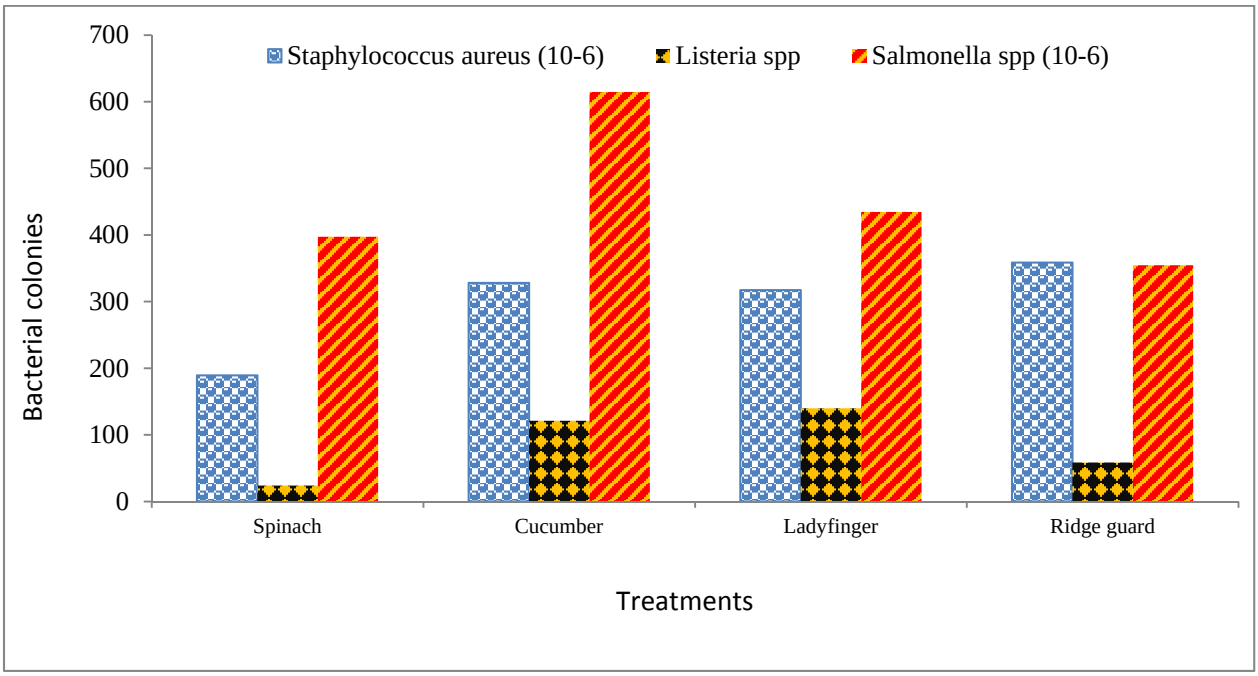


**Fig-1 Bacterial colonies in vegetables irrigated with untreated waste water in site one (Mahewa Path)**

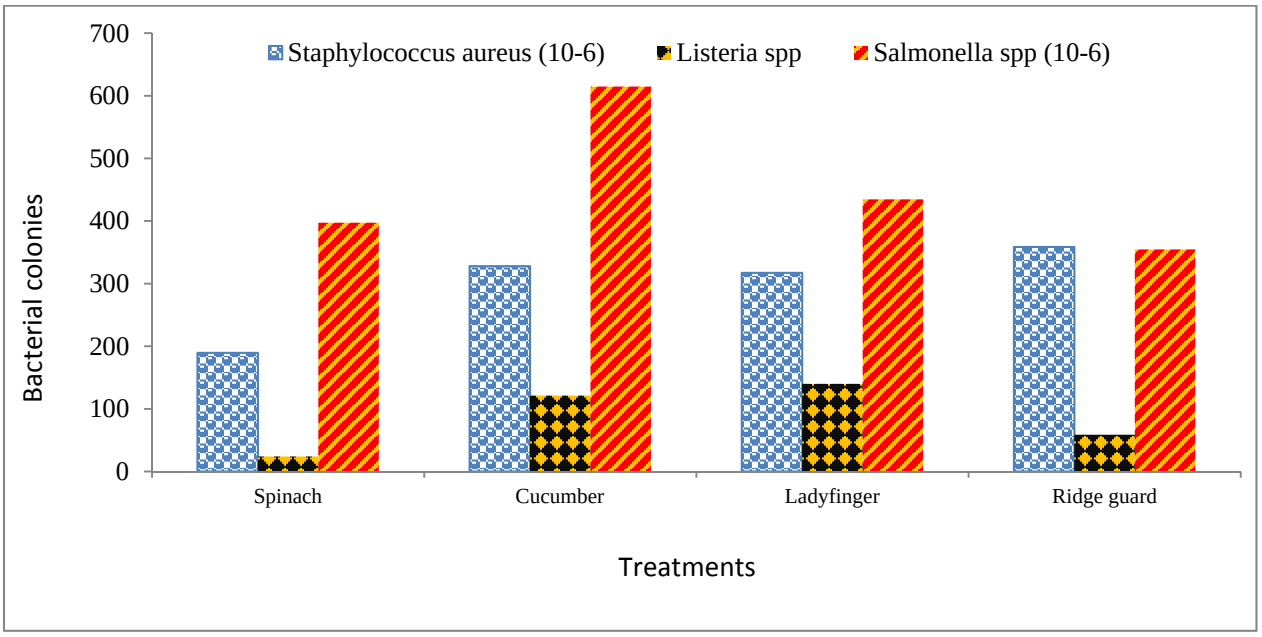


**Fig-1 Bacterial colonies in vegetables irrigated with untreated waste water in site one (Arail Ghat)**

## Conclusion

The study revealed that there was bacterial contamination of vegetables (spinach, cucumber, lady finger and ridge guard) grown in (Mahewa path and Arail Ghat) irrigated with untreated waste water. The quality of the waters and the irrigated vegetables exceeded standard microbiological limits. We recommend that the government enforce strict rules and legislation on adequate treatment of wastewater and effluents before discharge to the environment. Proper washing and disinfection of vegetables before consumption is strongly advised for the public health perspective.

## References

1. Ackerson NOB, Awuah E. Microbial risk assessment of urban agricultural farming: A case study on Kwame Nkrumah University of Science and Technology campus, Kumasi, Ghana. International Journal of Engineering and Technology.2012; 2(3): 356-363.
2. APHA, AWWA, WEF. Standard Methods for Examina-tion of Water and Wastewater, 22nd Edition, Washing-ton DC, 2001.
3. EnsinkJHJ. Water Quality and the Risk of Hook- worm Infection in Pakistani and Indian Sewage Farm- ers, Ph.D. Thesis, University of London, London, 2006.
4. Scott T, LippE, RoseJ. The effects of climate change on waterborne disease In *Microbial waterborne pathogens*, ed. E. Cloete, J. Rose, L. Nel, and T. Ford. London: IWA Publishing, 2004.
5. Sou M, Yacouba H, Mermoud A.Fertilising value and health risks assessment related to wastewater reuse in irrigation, case study in a Soudano-Sahelian city. Ouagadougou. Journees Scientifiques du. 2011; 21E(6): 4-8.
6. Toze S. Reuse of effluent water benefits and risks. Agricultural water management.2006; 80:147-159.
7. FoodUS. Drug Administration Center for Food Safety and Applied Nutrition Analysis and Evaluation of Preventive Control Measures for the Control and Reduction/Elimination of Microbial Hazards on Fresh and Fresh-Cut Produce.2001. www.cfsan.fda.gov/~comm/ift3-4a.html.