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Assessment of Some Enteropathogenic Bacteria In Water And Irrigated Vegetables Along Selected Locations Of River Kaduna, Nigeria.

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Abstract

Irrigation Farming in Nigeria is not the only one of the most important economic sectors but also provide many job opportunities. Nowadays the sustainability of the sector has become threatened with faecal pollution of rivers used to irrigate produce. A ten months study was done to assess the enteropathogenic bacterial load and distribution in water, vegetables and fruits along some parts of river Kaduna. The results of bacteriological analysis obtained from water and irrigated vegetables sampled at the various sampling points revealed high correlation with those isolated from the river water in load and species types. *Escherichia coli* was the predominant coliform isolated with 90% frequency of occurrence in Lettuce(*Lactuca sativa*). *Salmonella* and *Shigella species* were the only enteropathogenic bacteria with high percentage of occurrence at point B. In the control sample, only two enteropathogenic bacteria *Salmonella and Shigella species* registered 5% frequency of occurrence. The preponderance of identical enteropathogenic bacteria in both vegetables and water in all the three sample points is suggestive of cross contamination by irrigation water from the river. Bring to doubts the safety value of vegetables cultivated along study area and portended health risk of food-borne infection to potential consumers of raw eaten vegetables irrigated by the water and users of the water downstream.

Key Words: Enteropathogenic bacteria, Irrigated vegetables, River Kaduna.

1. INTRODUCTION

Water is the basis of all life and primary need for all vital life processes. With increasing industrialization and population growth, water sources available for various purposes such as drinking, recreation, agriculture and aquaculture have been adulterated with industrial as well as animal and domestic wastes. As a result it has become the most important means of transmission of several infectious diseases. Polluted sewage contains solids and dissolved organic compounds that impact an offensive odour and serves as excellent medium for growth and multiplication of microorganisms (Aneja, 2005).

According to 1998 study by world health organization (WHO) at least 30,000 people die every day in developing countries of the world because of unsanitary water supplies. The WHO (1998) reported that as many as 250 million new cases are documented each year from which about 15 million deaths are recorded. The breakdown indicated that 5.5 million of the mortalities world- wide are due to dysenteric diarrhea; 1 million from enteric fever and 1.5 million from dengue fever related to consumption of biologically polluted water.

Aquatic environments that pass through cities are usually prone to over- loading with variety of pollutants either through direct or indirect discharges (DWAF, 2005). This situation may be worsened by the indiscriminate disposal of untreated wastes such as heavily laden with sewage. Sewage polluted water bodies carry microorganisms, some of which are pathogenic to human and animals (Lipp *et al.,* 2001).

River Kaduna, like many rivers in Nigeria, serves as a center for recreation, fishing, and irrigational purposes and as a sink for disposal of municipal and industrial wastes. A paucity of information exists on the extent of pathogenic bacterial contamination of the river and its public health implications. This study was therefore designed to appraise the level of bacterial contamination and potential health risks of the river to humans and animals.

2. MATERIALS AND METHODS

2.1 *Sample collection areas*

Point A: This site is situated at the point of raw water intake into malali water treatment works. Here municipal pollutants entering this point consist of faecal contamination, sewage runoff from Malali, Badarawa, Anguwan Dosa, and legislators quarters.

Point B: This site is situated at Gamji park, were river Kaduna passes through the park. Here pollutants into the river are generated from Kaduna state university, old ABU, police college and nearby settlements.

Point C**:** is located at Nasarawa bridge the along bypass. This site is a confluence point of industrial and municipal effluents flowing from industries and settlements of kakuri, Nasarawa and Makera.

2.*2* *Water sample collection*

Guidelines on sampling of rivers and stream as given by SANS 5667-6(2006) were used at the 3 sampling points of the river water and A 10ml sterile syringe was used at each site to collect the water sample for bacteriological analysis.

2.3 *Vegetable sample collection*

The randomly picked chosen fruits and vegetables samples from different irrigated farms by this river consisted of Lettuce(*lactuca sativa*), cabbage(*Brassica oleracea*), Tomato(*Lycopersicon esculenetum*), garden egg(*Solanum melongena*), carrot(*Daucus carota*). Each sample was placed in sterile polythene bag , placed in a cooler and transported to the laboratory for analysis.

2.4 *Enumeration of aerobic bacteria and coliform*

The media used for the bacteriological analysis of river water and vegetable washing include nutrient agar(NA) and Eiosin Methylene blue agar (EMB). All media used were weighed and prepared according to the manufacturer's specification . water samples and vegetable washing were serially diluted followed by plating in duplicate, using pour plate technique and incubated at 34^{o}C for 24 hour(APHA, 1957).

2.5 *Biochemical Characterization of Bacteria*

Pure culture of bacterial isolates were subjected to various biochemical characterization test such as Triple sugar iron agar test(TSI), Citrate Utilization test, Urease test and motility Indole ornithine Decarboxylation(MIO) test to determine the identity of the bacteria isolates (Aneja, 2005; Garrity et al; 1984., perry et al., 2002).

2.6 *Gram staining*

Pure culture of the bacterial isolate were identified based on their reaction to gram stains according to modified gram staining technique in standard method 9221-B(APHA, 2001).

2.7 *Data analysis*

Analysis of variance was used to test differences while mean were compared using Duncan's multiple range test(DMRT). $P<0.05$ was selected as significance difference and calculated using SPSS (version 16.0) statistical package.

3. RESULTS

Table 1 showed that, the aerobic bacterial counts of the area under investigation ranged from $30x10^2$-$37x10^2$ cfu/ml in all the sampling points. The maximum counts was $30x10^2$ cfu/ml at point C and minimum count of $30x10^2$ cfu/ml in point B. Also the Coliform counts ranged from $25x10^2$-$29x10^2$ cfu/ml. The maximum count of $29x10^2$ cfu/ml was recorded at point B and minimum of $25x10^2$ cfu/ml recorded at points A and C. The counts reflected no significant differences($p> 0.05$) between the sampling points, except the control with aerobic count of $2.6x10^2$ and Coliform with $1.0x10^2$

With respect to sampling months as shown in table 2. There was significant difference between counts of December, January, and February from others for aerobic bacterial counts while December, January, February

and march are not significantly different, but vary from other months. Bacterial colony Counts were significantly higher($p< 0.05$) in the wet months than dry months for both aerobic bacteria and coliform counts.

Seasonal variation in bacterial counts of the area under investigation illustrated in Table 3. The maximum aerobic bacteria counts was $50x10^2$ cfu/ml obtained in wet season and minimum counts $27x10^2$ cfu/ml was occurred in dry season. The maximum Coliform counts $24x10^2$ cfu/ml was registered during wet season and minimum counts $13x10^2$ cfu/ml was recorded during the dry season. With respect to seasonal variation, the counts were significantly higher ($p< 0.05$) in the wet season than dry season.

Table 4 shows that Lettuce(*L. sativa*) and Carrot (*D. carrota*) were the only irrigated vegetables with significantly different counts of aerobic bacteria but Coliform counts reflects no significant between the vegetables.

The results of percentage frequency of occurrence of bacterial isolates from water and vegetables samples are presented in figs 1 and 2 above. From the results of the bacterial isolates from water samples at different sampling points and control. *E.coli* was found to have the highest frequency of occurrence at point B with 90% followed by point A with 85%, then C with 75%. *Salmonella* species recorded highest frequency of 60% each at points A and C followed by B with 50% while control samples had the least number of positive *Salmonella* isolates with 5%. *Shigella* recorded the highest frequency of 80% at point A, followed by 20% each at points B and C while the lowest frequency of 5% was observed in the control. *Proteus* species had the highest frequency of 55% each at point A and C, followed by B with 30% while *Klebsiella* species recorded a highest frequency of 55% at point B, followed by C with 50%, then point A with 40% occurrence, respectively.

Of all the vegetables analysed , *E.coli* was the predominant bacterial isolate from lettuce(*Lactuca sativa*) with 90%, followed by tomato(*Lycopersicon esculentum*) and carrot(*Daucus carrota*) with 70% each, then cabbage(*Brassica oleracea*) with 60% and the lowest frequency was recorded in garden egg(*Solanum molongena*) with 50%. About 80% of cabbages(*Brassica oleracea*) sampled were positive for *Salmonella* species while 50% of tomato(*Lycopersicon esculentum*), 40% each of lettuce(*Lactuca sativa*) and carrot(*Daucus carrota*), then 30% of garden egg(*Solanum molongena*) revealed the presence of Salmonellae. *Shigella* species recorded highest frequency of 50% each in cabbage(*Brassica oleracea*), 40% in tomato(*Lycopersicon esculentum*), then 30% each in carrot(*Daucus carrota*) and garden egg(*Solanum molongena*). *Proteus* species had frequency of 60% in cabbage(*Brassica oleracea*), 50% in carrot(*Daucus carrota*), 30% each in tomato(*Lycopersicon esculentum*) and lettuce(*Lactuca sativa*) and the least occurrence was in garden egg(*Solanum molongena*) with 20%. In all vegetables examined for *Klebsiella* species, carrot(*Daucus carrota*) had the highest frequency of 40%, garden egg(*Solanum molongena*) with 40%, then tomato(*Lycopersicon esculentum*) and cabbage(*Brassica oleracea*) with 30% each while the lowest frequency of 20% was obtained in lettuce(*Lactuca sativa*).

4. DISCUSSION

There was significantly higher bacterial and coliforms in the wet season than in the dry season(Table 6) and all the counts recorded in the study exceeded guidelines of $1.0x10^2$ cfu/ml and zero *E.coli* for irrigation of food crops consumed raw (Tebbut, 1990 and WHO/FAO, 1996). Cornish *et al*.(1991), Keraita *et al*.(2003a), Amoah *et al*.(2005) all reported high bacterial number above limits stipulated by WHO/FAO/EU in irrigation water and irrigated vegetables usually eaten raw in farms within Kumasi, Ghana.

These high counts could be due to high levels of organic matter present in the river as a result of indiscriminate dumping of waste, and are in agreement with the findings of Tatah and Ikenebomeh (1999) who in a similar study in Ikpoba river, Nigeria attributed such high counts to the high organic matter content of the river. Fleisher *et al.* (1996) and Sequel *et al.* (2001) equally reported similar findings. During a rainfall, runoff washes organic matter as well as bacteria. Coliform counts have been found to correlate strongly with amounts of suspended solid in water which might not be unconnected with the disturbances of the sediment along with bacteria to which they adhere. This has lead to the acceptance that coliform commonly settle and are housed in the sediment; however the rise in coliform could possibly rather be due to an increase in available nutrient from disturbed sediments (Jamieson *et al*., 2005). We therefore posit that this may also explain the significantly higher counts obtained in the wet season during which periods high volume of runoffs get into the river; it would therefore appear that pollution effects the aquatic food chain. The counts above these limits are indicative of sanitary status of the river. It is also to note that high counts were recorded throughout the study period. Such

counts are highly undesirable and indicative of faecal and other domestic pollution of the river, hence the possible presence of human pathogens of medical importance at all the sampling points and also the irrigated vegetables.

5. CONCLUSION

When looking at the data discussed, it is evident that the water of river Kaduna is contaminated and does not comply with guidelines for aquaculture, and irrigation of vegetables to be eaten raw or minimally processed foods. Since potential human pathogens were isolated from this river during this study; it can be concluded that the water might pose threat to the health of individuals coming into contact with it. It is recommended that the appropriate authorities are made aware of this problem to take proactive measures on environmental legislation in Kaduna metropolis.

6. RECOMMENDATION

To be absolutely sure that organisms are indeed of the same origin, future research should make use of molecular methods such as DNA sequencing for identification and comparism of the different organism.

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Table 1: Analysis of variance of mean aerobic bacterial and Coliform counts at various sampling points along river Kaduna during the study period.

Sampling points	Aerobic Bacterial counts(cfu/ml)	Coliform counts(cfu/ml)
Pont A	$30x10^2$ a	$25x10^2$ a
Point B	$36x10^2$ a	$29x10^2$ a
Point C	$37x10^2$ a	$25x10^2$ a
Control	$2.6x10^2$ ab	$1.0x10^2$ ac

Figures with the same alphabet in the same row are not statistically significantly different($p< 0.05$) according to DUNCAN MULTIPLE RANGE TEST

Table 2: Analysis of variance of mean monthly aerobic bacterial and Coliform counts along river Kaduna during the sampling period

Months	Aerobic Bacterial counts (cfu/ml)	Coliform counts (cfu/ml)
June	$49x10^2$a	$23x10^2$ a
July	$45x10^2$a	$26x10^2$ a
August	$58x10^2$a	$25x10^2$ a
September	$49x10^2$a	$22x10^2$ a
October	$47x10^2$a	$24x10^2$ a
December	$23x10^2$ ab	$14x10^2$ ac
January	$26x10^2$ab	$11x10^2$ ac
February	$16x10^2$ac	$13x10^2$ ac
March	$31x10^2$a	$11x10^2$ ac
April	$40x10^2$a	$28x10^2$ a

Figures with the same alphabet in the same row are not statistically significantly different($p< 0.05$) according to DUNCAN MULTIPLE RANGE TEST

Table 3: Seasonal variation of mean aerobic bacterial and Coliform counts along river Kaduna during the study period.

Seasons	Bacterial counts (cfu/ml)	Coliform counts (cfu/ml)
Wet season	$50x10^2$ a	$24x10^2$ c
Dry season	$27x10^2$ ab	$13x10^2$ ac

Figures with the same alphabet in the same row are not statistically significantly different($p < 0.05$) according to DUNCAN MULTIPLE RANGE TEST

Table 4: Analysis of variance of mean aerobic bacterial and Coliform counts of irrigated vegetables during dry season along river Kaduna.

Vegetables	Bacterial counts (cfu/ml)	Bacterial counts (cfu/ml)
Lettuce *(Lactuca sativa)*	$74x10^2$ ab	$50x10^2$ d
Cabbage *(Brassica oleracea)*	$39x10^2$ b	$31x10^2$ d
Tomato *(Lycopersicum esculantum)*	$32x10^2$ b	$35x10^2$ d
Carrot *(Daucus carrota)*	$62x10^2$ ab	$44x10^2$ d
Garden egg *(Solanum molongena)*	$46x10^2$ b	$45x10^2$ d

Figures with the same alphabet in the same row are not statistically significantly different($p < 0.05$) according to DUNCAN MULTIPLE RANGE TEST

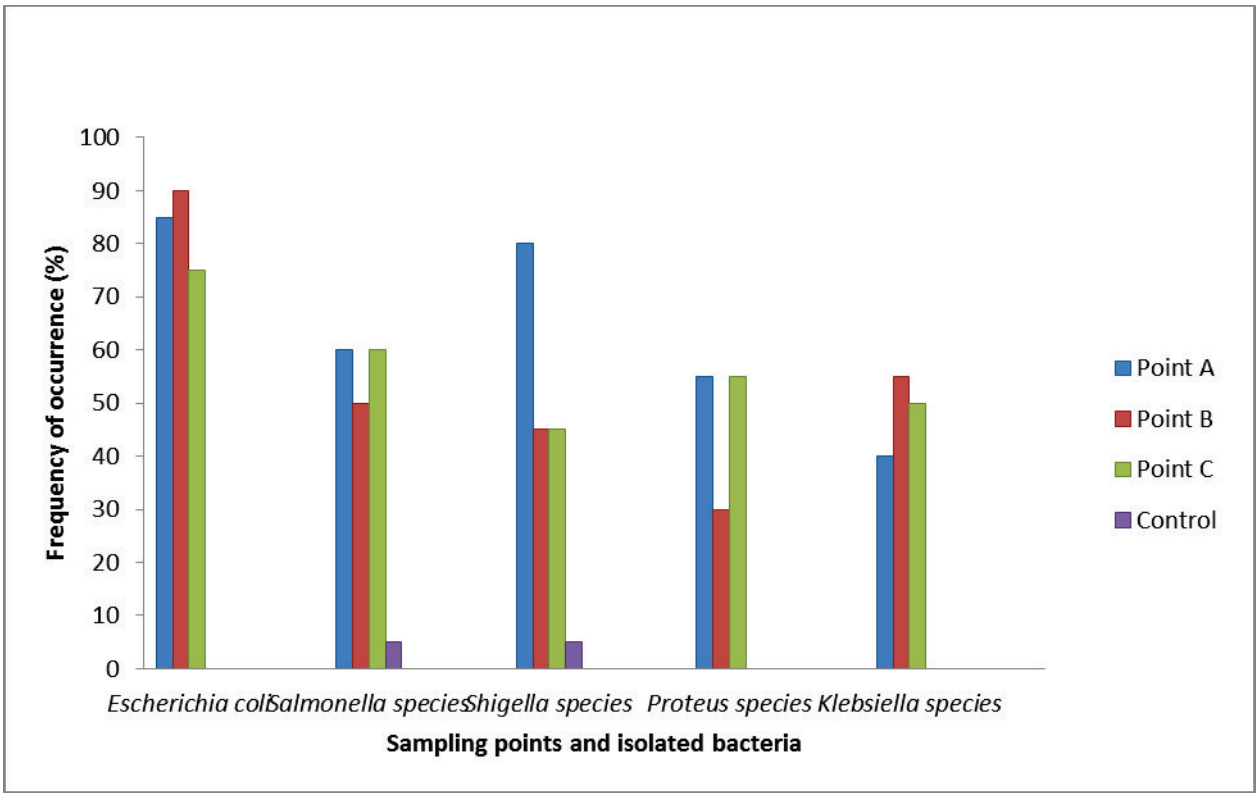

Fig 1 : Frequency of occurrence of bacterial isolates from water at different sampling points

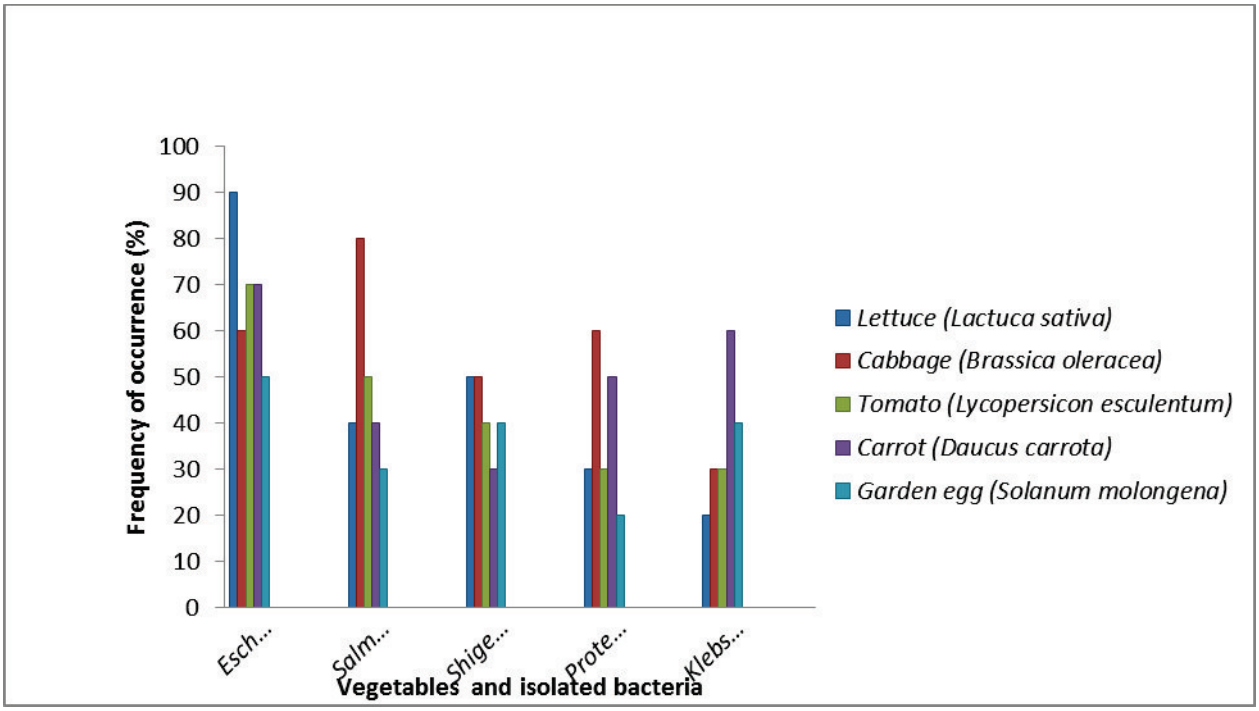

Fig 2 : Frequency of occurrence of bacterial isolates from irrigated vegetables

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