DETERMINATION OF MICROBIAL CONTAMINANT (BACTERIA) ON SOME VEGETABLES IRRIGATED WITH MUNICIPAL WASTEWATER IN KAWO IRRIGATION FARM

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Abstract: The presence of microbial pathogens in untreated wastewater are very numerous, and dangerous organisms can be dictated, micro-organisms can be associated with illness or disease that are usually from human or animals faucal matter found in the wastewater or surface water sources. The study examines the determinant of microbial contaminant on vegetables irrigated with wastewater in Kawo irrigation farm. A total of 8 samples comprising of Spinach and Lettuce were collected and analyzed for bacteria. Vegetable (spinach and lettuce) samples were collected under normal conditions, from three (3) sample points of the İrrigation farm designated as Farm A, B, and C. The samples were put in paper bags, and then transported immediately to the laboratory where they were analyzed within 24hrs. The same method was also applied to a control group of vegetables (spinach and lettuce) grown using open well water under identical conditions. The standard plate count method (SPC) was employed for the analyses. The study revealed that vegetables grown within the study area are highly contaminated especially with *salmonella spp*. It was recommended that farmers should practice the use of stabilization ponds, Irrigation methods such as surface or drip Irrigation.

Keywords: Bacteria, Irrigation, Municipal- Wastewater, Vegetable

1. INTRODUCTION

Agriculture to a very large extent in many developing countries depends on wastewater as a source of Irrigation water (Khaled & Muhammad, 2016). In less developed countries, the quality of the wastewater and the conditions under which wastewater is used varies, wastewater is in the form of raw sewage (Huibers *et al.*, 2004). Most parts of Nigeria, Farmers prefer using the untreated wastewater even in a place where freshwater is abundantly available due to the simple fact that they earn higher profits, this indicates that Farmers rely more on wastewater than freshwater for irrigation. However, in most developing countries, many households cannot afford inorganic fertilizers and have little or no supply of fresh water, therefore wastewater recycling can be a solution to these problems by supplying the nutrients and water required for many household food production, this technique of Irrigation Farming is already adopted by millions of farmers across the globe and it is estimated that about 10% of the world's population consumes foods produce from wastewater Irrigation (WHO, 2006).

The presence of microbial pathogens in untreated wastewater are very numerous, and dangerous organisms can be dictated, micro-

organisms can be associated with illness or disease that are usually from human or animals faecal matter found in the wastewater or surface water sources. Irrigation water polluted with pathogens has always been the major cause of food-borne illness.

Two major health hazards associated with direct and indirect wastewater use for irrigation purposes are the rural health and safety problem for those working on the field, those living on or near the land where the water is being abstracted, and the risk that contaminated farm produce from the area may subsequently infect animals grazing around such areas or humans through consumption or handling of the product or through secondary human contamination by consuming products from animals that grazes around the area (WHO, 2000). The pathogens are transmitted to the public through the consumption of irrigated produce, especially crops eaten raw (Blumenthal *et al*., 2000). Research carried out by (Igomu,2011) reported that more than 260 people died of cholera in the northern part of Nigeria in the last quarter of 2009, also epidemiological data recorded from the public health department of the Kano State Ministry of Health revealed that the distribution and frequency of cholera epidemics in the State during the period of studies from 1995 to 2001, records 2,630 between 1995 to 1996, 847 in 1997, 2,347 in 1999, 173 in 2000 and 163 in 2001 (Usman *et al*., 2005).

Four categories of microbiological quality have been assigned based on standard plate counts, levels of indicator organisms, and the number or presence of pathogens. These are satisfactory, marginal, unsatisfactory, and potentially hazardous as indicated in Table 1.0 below:

Table 1. Guideline levels showing the microbiological quality of ready-to-eat foods

Test	Microbiological Quality (CFU per gram)			
	Satisfactory	Marginal	Unsatisfactory	Potentially Hazardous
Indicators				
Enterobacteriaceae	$<10^2$	10^2 -10^4		$\geq10^4$
Escherichia	<3	3-100	≥100	
Pathogens				
*Coagulase +ve staphylococc*i	$<10^2$	10^2 -10^3		10^3 -10^4 $\geq10^4$
Bacillus cereus and other pathogenic Bacillus spp	$<10^2$	10^2-10^3		10^3 -10^4 $\geq10^4$
Vibrio parahaemolyticus	<3	<3-10^2		10^2 -10^4 $\geq10^4$
Salmonella spp	Not to be detected in 25g			Detected

SOURCE: ICMSF International Commission on Microbiological Specifications for Foods (2001).

Note:

Satisfactory *- No action is required.as result shows good Microbial quality*

Marginal *- In this case results are said to be at the border line i.e., it can be safe for use and can also be dangerous*

Unsatisfactory *- Results shows or indicate poor microbiological quality and not acceptable for use*

Potentially Hazardous *-At this level, food-borne illness may occur and immediate remedial actions is expected.*

High level of typhoid fever, Diarrhea and Cholera outbreak as always been the case in Kawo general hospital, with average monthly cases of 85 and 93 for children less than five years, 142 and 239 for people greater than five-year records diarrhea and typhoid fever respectively, which is considered very high compared to other health centers. (Kawo General Hospital, 2018).

The study aimed at assessing the health risk associated with the use of untreated wastewater (municipal and abattoir) for irrigating vegetables, which could be achieved by assessing the biological characteristics of domestic, abattoir, and combination of the domestic and abattoir wastewater use for irrigation, and evaluate the pathogenic microorganisms both in mature crops (Spinach and lettuce) grown using the wastewater for irrigation.

2. METHOD

2.1 Study Area

The location selected for this study is the Kawo Abattoir irrigation farm site (Figure 2.1) which lies between latitude 10^0 34' 40.8'' E and longitude 07^0 26'39.1'' N of Kaduna North Local Government Area Kaduna State Nigeria. Kaduna North lies completely in the part of Western Africa, within the northern limit of the movement of the İntertropical Convergence Zone (ITCZ). It is characterized by two distinct seasonal regimes, oscillating between cool to hot dry and humid to wet seasons. The climate here is classified as Aw by the Köppen-Geiger system *(Aw = Tropical wet and dry or savanna climate; with the driest month having precipitation less than 60 mm (2.4 in) and less than 4% of the total annual precipitation).* The average annual temperature of Kaduna State is around 25.2 °C, with about average annual rainfall of 1211mm. The driest month is January with 0 mm of precipitation, while in August the precipitation reaches its peak, with an average of 284 mm.



Figure 1. Map of Kaduna Metropolis showing the study Area. (Nona.net)

2.2. **Sample collection for vegetables**

Vegetable (spinach and lettuce) samples were collected under normal conditions, from three (3) sample points of the İrrigation farm designated as Farm A, Farm B, and Farm C. The sample was put in paper bags, and then transported immediately to the laboratory where they were analyzed within 24hrs. The same method was also applied to a control group of vegetables (spinach and lettuce) grown using open well water under identical conditions.

2.3. **For the Bacteria analysis, the following Bacteria were enumerated**

i. *Coliform (E coli) using Maconkey agar (MA)*
ii. *Bacillus spp* using plate count agar (PCA)
iii. *Vibrio spp* using thiosulfate citrate bile sucrose agar (TCBS)
iv. *Salmonella spp* using salmonella-shigella agar (SS)
v. *Staphylococcus spp* using bird parker agar (BPA)

2.4. **Experimental procedure and evaluation of bacteria on the vegetable**

The mesophilic aerobic bacteria in the product were enumerated using the Standard Plate Count method (SPC) as described by WHO/FAO (2004). 10g of the vegetable sample was weighed and blended using a RM 206 model blender containing 100mL of sterile solution for 2min under sterile conditions, the blender was carefully disinfected to prevent any cross-contamination, and this was well mixed making $1:10^4$ dilution of the sample. 1ml of each homogenate mixture of the sample was transferred into a sterile tube containing 9ml sterile ringer's solution to obtain $1:10^{-2}$ dilutions. Using separate sterile pipettes decimal dilutions of $1:10^{-3}$, $1:10^{-4}$, $1:10^{-5}$, and $1:10^{-6}$ were prepared by aseptically transferring 1ml of previous dilution to 9mls of diluent in different test tubes. 1ml of each dilution was transferred to separately marked glass-made

Petri dishes (15x100mm) in duplicate. 15ml of plate count agar (M77) cooled to 45^0C and then added to each plate and gently rocked clockwise and anti-clockwise to mix well. The plates were allowed to be set and solidified and then incubated at 30^0C for 18-28 hours in a BTI-26 model incubator. The colonies on the plate were counted and the colony-forming units per milliliter (CFU/ml) were calculated using the formula:

Count (CFU/ml) = (numbers of colonies counted X dilution factor)/volume plated (in ml)

Enumeration of all other bacteria as stated above employed the same method as described for the mesophilic aerobic bacteria except for the use of different culture media i.e., instead of plate count agar we use salmonella-shigella Agar (SS) for enumeration of salmonella etc.

3. **RESULTS AND DISCUSSION**

A total of 8 samples comprising of Spinach and Lettuce were collected and analyzed for Bacteria, Table 2.0 Shows the result for bacteria found in the samples, and the results are further discussed below.

Table 2.0 *Cultivation, Enumeration, and Isolation of Bacteria*

S/N	Code	Sample	Bacillus spp CFU/g/ml	E Coli CFU/g/ml	Salmonella spp CFU/g/ml	Vibro spp CFU/g/ml	Staphylococcus spp CFU/g/ml
1.	SP 01	Spinach Farm A	$2.0x10^5$	Nil	$2.5x10^4$	$1.4x10^2$	$3.0x10^2$
2.	SP 02	SpinachFarm B	$1.6x10^4$	Nil	$1.6x10^3$	$1.2x10^2$	$3.5x10^2$
3.	SP 03	Spinach Farm C	$1.1x10^4$	$<1.0x10^2$	$1.3x10^3$	$1.6x10^2$	$5.6x10^2$
4.	SP 04	Spinach Control	$1.2x10^3$	Nil	Nil	Nil	$4.0x10^2$
5.	LT 01	Lettuce Farm A	$1.3x10^4$	Nil	$1.5x10^3$	$<1.0x10^2$	$1.8x10^2$
6.	LT 02	Lettuce From B	$1.2x10^4$	Nil	$5.0x10^2$	$5.0x10^2$	$1.2x10^3$
7.	LT 03	Lettuce Farm C	$1.0x10^4$	$<1.0x10^2$	$3.5x10^2$	$1.8x10^2$	$8.0x10^2$
8.	LT 04	Lettuce Control	$1.1x10^3$	Nil	Nil	Nil	$3.0x10^2$

(Field data 2023)

i. Bacillus spp

As shown in Figure 2 below, *Bacillus* appeared in the entire sample with the spinach from Farm A having the highest count and lettuce control having the lowest count, this could be because *Bacillus* is a common environmental organism. The ICMSF. guideline levels for determining the microbiological quality of ready-to-eat foods for *Bacillus* is 10^3 (log CFU 3) as indicated by the horizontal line, above which is considered unsatisfactory and Potentially Hazardous.

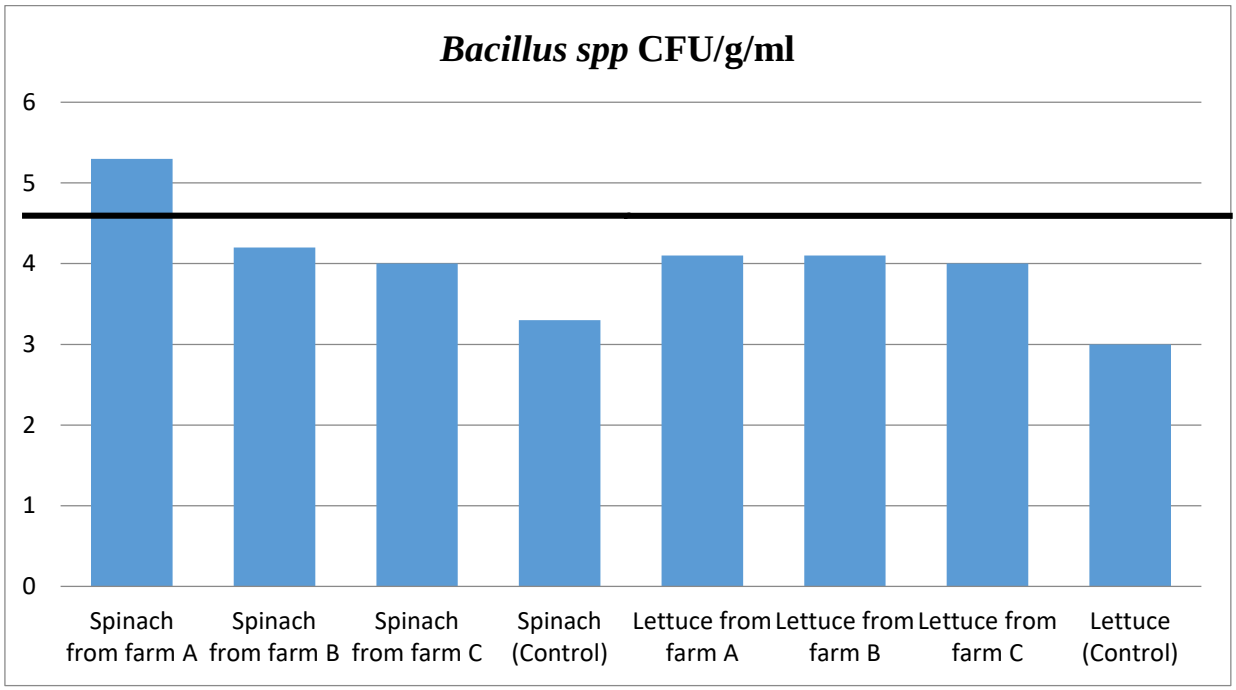

Figure 2. Sample against count in log CFU for *Bacillus spp*

ii.* Coliform *(E coli)

As shown in Figure 3 below, Coliform *(E coli)* appeared to be less in the entire sample with spinach and lettuce from Farm C having 2 counts in log CFU respectively which is within the ICMSF Guideline levels for determining the microbiological quality of ready-to-eat foods for *E Coli* is 100(log CFU 2) as indicated by the horizontal line, above which is considered unsatisfactory Potentially Hazardous.

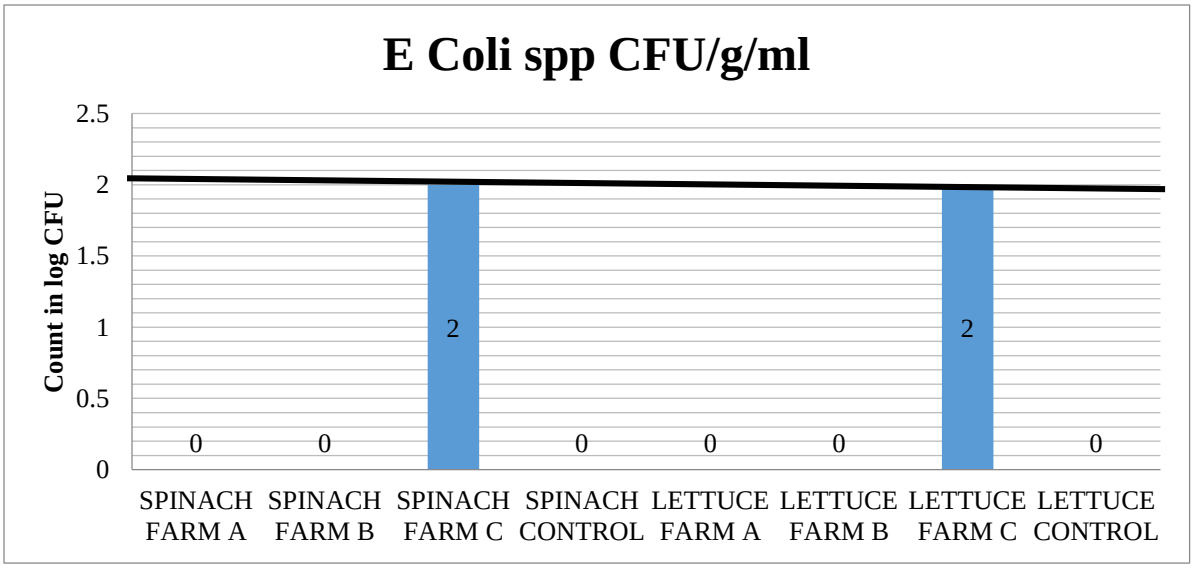

Figure 3. Sample against count in log CFU for *E Coli Spp*

iii. Salmonella SPP

Salmonella appears to be highly present in all the samples except in the controls, with spinach from farm A, having the highest value, this is far above the recommended guideline value by International Commission on Microbiological Specifications for Foods which specify that salmonella organism should not be detected in any food sample as indicated by the horizontal line in the chart (figure 4)

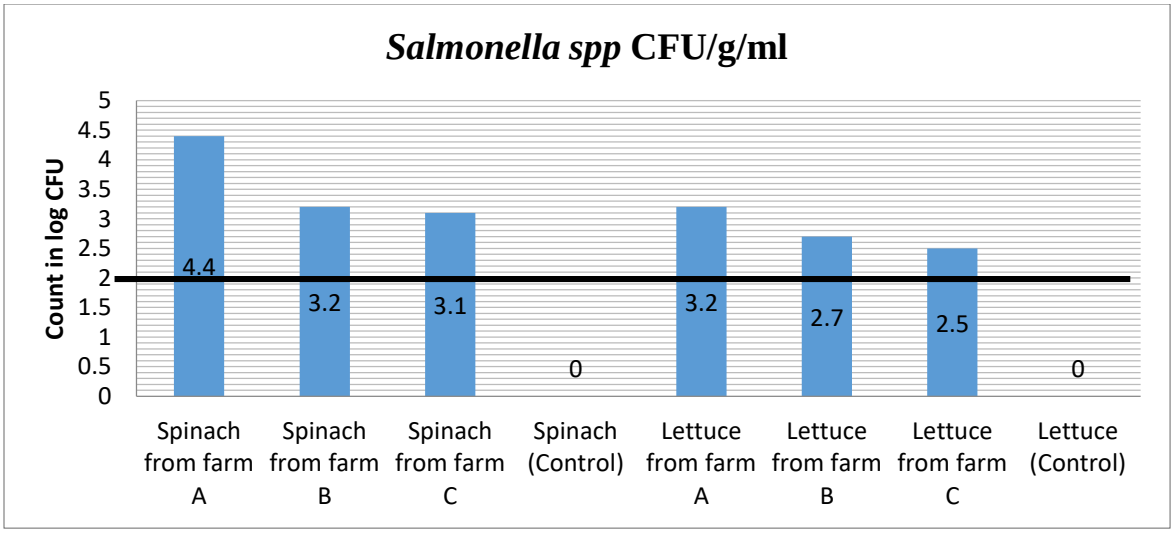

Figure 4. Sample against count in log CFU for *Salmonella spp*

iv. Vibro SPP

Vibrio spp appears in the entire sample except in the controls. The International Commission on Microbiological Specifications for Foods sets a guideline of $\geq 10^3$ (3 in $\log_{10}$)/ml for *Vibrio spp* of ready-to-eat foods as potentially hazardous as indicated with the thick horizontal line in the chart (figure 5) indicating known of the sample count reaches or exceeds the recommended limit indicating that the vegetable has low contermination level with *Vibro spp*, which could be as a result of *Vibrio spp* having low surviving time in crop (WHO, 1989).

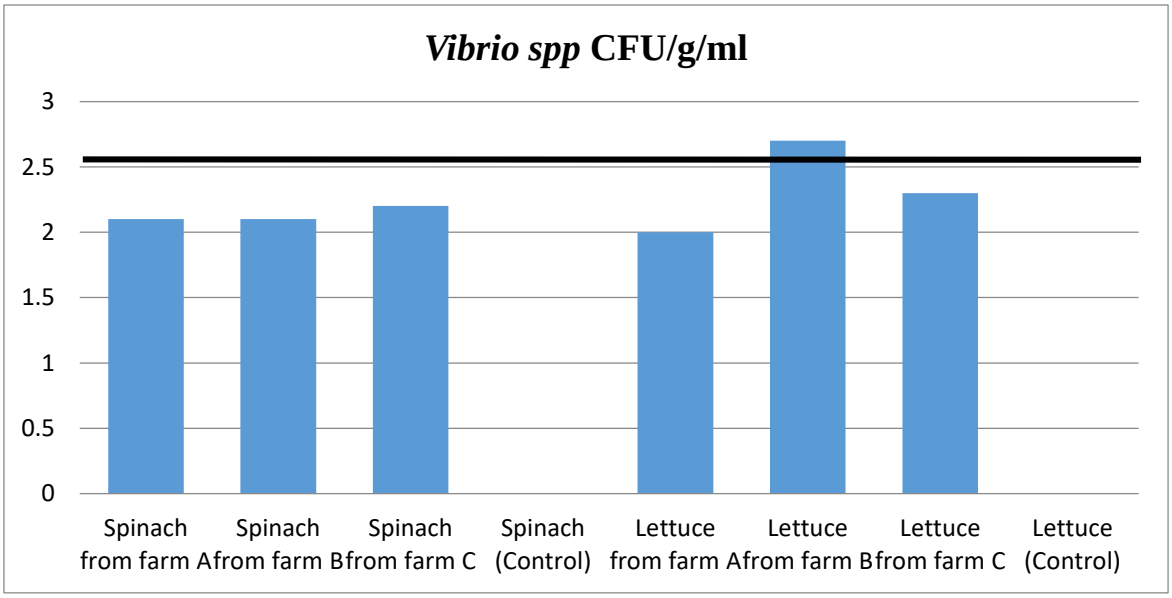

Figure 5. Sample against count in log CFU for *Vibro Sp*

V. Staphylococcus SPP

Staphylococcus appears in the entire sample except in the open well water control. The International Commission on Microbiological Specifications for Foods sets a guideline of $\geq 10^4$ (4 in $\log_{10}$)/ml for *Staphylococcus specie* of ready-to-eat foods as Potentially hazardous, from the chart (Figure 7), it is shown that *staphylococcus specie* level in the entire sample is below the recommended guideline.

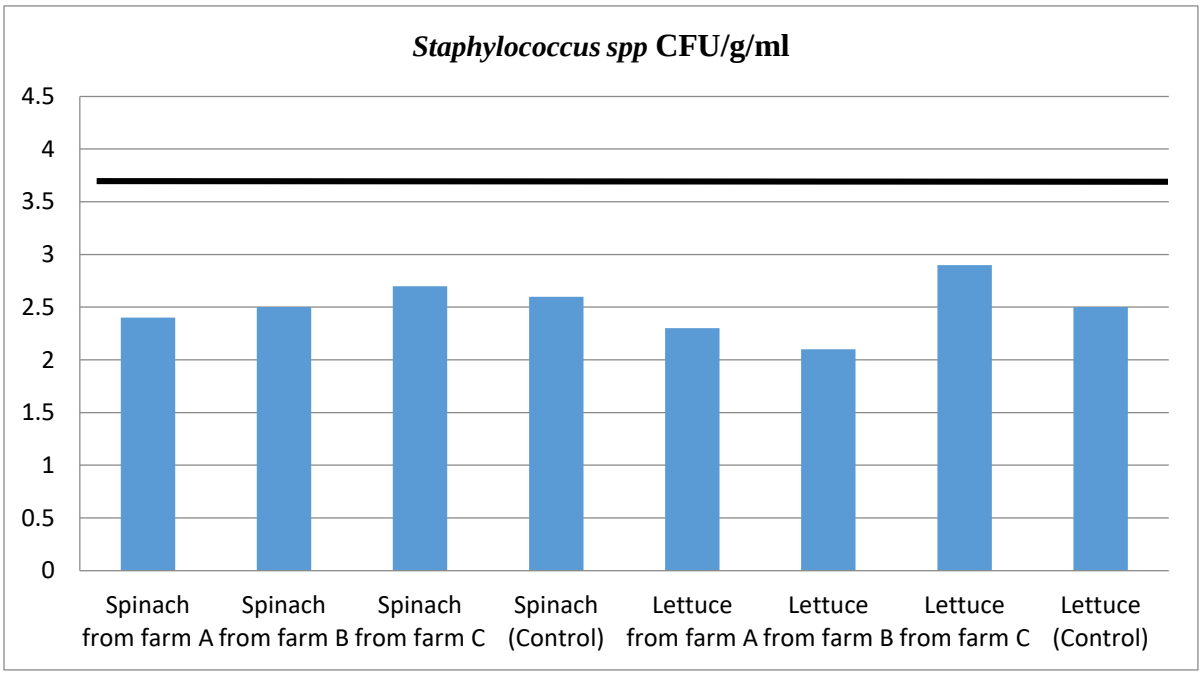

Figure 7. Sample against count in log CFU for *Staphylococcus Spp*

4. CONCLUSION AND RECOMMENDATIONS

The research carried out in the Kawo abattoir irrigation farm aimed to assess pollutants (Bacteria) in vegetables irrigated with municipal wastewater, these studies also revealed that Vegetable grown within the study area are highly contaminated most especially with *salmonella spp* which could be of a threat to consumers health and could be the reason behind the high record of typhoid fever with people around the area. It is recommended that farmers should practice the use of stabilization ponds (aerobic, facultative, and maturation) which is one of the cheapest and easiest ways of disinfecting wastewater before use for irrigation. Adoption of safer İrrigation methods such as drip or surface irrigation to minimize contact of crops with contaminants present in irrigation water especially crops that can be eaten raw.

REFERENCES

Blumenthal, U.J., Peasey, A., Ruiz-Palacios, G., & Mara, D.D. (2000). Guidelines for wastewater reuse in agriculture and aquaculture: recommended revisions based on new research evidence. WELL study, Task No: 68 Part 1.

Huibers, F.P.,Moscoso, O.,Duran, A., & Lie,r J.B.V. (2004). The use of wastewater in cochabamba, Bolivia: a degrading environment. In: Waste water use in irrigated agriculture: confronting the livelihood and environmental realitied. 135-144.

Igomu, T. (2011). Cholera epidemics: Far from being over. NBF. News. *www. Nigerian best forum.com/blog/? P=60321*. Accessed 23 August 2011.

Khaled, S. B., & Muhammad, A. (2016). Field accumulation risks of heavy metals in soil and vegetable crop irrigated with sewage water in the western region of Saudi Arabia, *Saudi Journal of biological science, 23, S32-S44* http://dx.doi.org/10.1016/j.sjbs.2015.09.023

Usman, A., Sarkinfada, F., Mufunda, J., Nyarando, P., Mansur, K., & Daiyabu, T.M. (2005). Recurrent cholera epidemics in Kano-Northern Nigeria. *Central African Journal of Medicine March-April*, 51(3-4), 34-38.

WHO. (2000). Global Water Supply and Sanitation Assessment 2000 Report. World Health Organization (WHO) United Nations Children Fund (UNICEF), Geneva and New York.

WHO. (2006). Guidelines for the safe use of wastewater, excreta, and grey water: Wastewater use in agriculture. Report of a WHO Scientific Group. WHO Technical Report Series 778. World Health Organization, Geneva, Switzerland, 2, 74-219