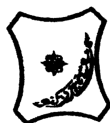
*Special Conference Edition, November, 2018*

http://dx.doi.org/10.4314/bajopas.v11i1.26S



*Bayero Journal of Pure and Applied Sciences, 11(1): 161 - 166*
**ISSN 2006 – 6996**

# COMPARATIVE STUDY OF THE LEVEL OF BACTERIAL/HELMINTHS CONTAMINATION OF VEGETABLES PRODUCED FROM POLLUTED AND UNPOLLUTED IRRIGATION SITE OF KANO

**Lambu, Z. N., Adamu, R. T., Ahmed, I. G., Isah, A. A., Shehu, A. A., Umar, S. F., Mahmud, T., Isyaku, J., Karfi, I. A. and Adamu, S.[1],**

Department of Microbiology, University of Science and Technology, Wudil, P M B 3244. Kano, Nigeria.

[1]Department of microbiology, federal university, Wukari, Taraba State.

Corresponding author:zakarinuhulambu@gmail.com

*ABSTRACT*

***A comparative study was conducted to compare the microbiological/helminths contaminations of five major crops (Lactuca veriso, Darcus carota, Solanum lycopersicum, Phylanthus amarus, Alium cepa) produced from polluted (industrial/residential effluents) Sharada and unpolluted (tube well water)Yarimawa using the method described by FAO, (1979). The result shows that the five crops, produced with polluted water viz, Lactuca veriso, Darcus carota, Solanum lycopersicum, Phylanthus amarus, Alium cepa, had mean aerobic plate count of 2.0 x $10^2$ cfu/g, 8.40 x $10^2$ cfu/g , 1.13 x $10^3$ cfu/g , 5.8 x $10^4$ cfu/g cfu/g, 5.7 x $10^1$ cfu/g, respectively, and all the five crops had mean coliform count of 180 MPN/g, while that of unpolluted water had mean aerobic plate count of of 1.22 x $10^2$ cfu/g, 5.1 x $10^2$ cfu/g , 1.4 x $10^2$ cfu/g, 1.43 x $10^2$ cfu/g, 1.32 x $10^1$ cfu/g respectively, and had mean coliform count of 30, 21,21, 21 and 21MPN/g respectively. The organisms isolated in all the crops produced with polluted water were E. coli, Salmonella sp, Shigella sp, S. aureus, Ascaris egg, Strongloides and Ancylostoma while E. coli, Shigella sp, S. aureus, were the organisms isolated in the crops produced with unpolluted water, only Lactuca verosa and Solanum lycopersicum, shows the presence of helmiths in the crops produced with the unpolluted water.***

***Key words: Kano, Bacteria, Helminth, effluent, Yarimawa, Sharada***

## INTRODUCTION

Rapid development and growth in industrialization has adversely affected the environment and surrounding ecosystem. Besides release of toxic gases, noise pollution, inadequate or non-availability of sewerage system and expensive wastewater treatment methods, huge quantities of untreated wastewater is disposed off in the drainage system creating a number of environmental problems. Industrial and commercial activities are expanding to meet the growing demands of increasing population ultimately leading to a number of problems like safe drinking water. Safe disposal of wastewater and several other related issues (UN, 2017).

Wastewater contains pathogenic microorganisms such as bacteria, viruses and parasites, which cause disease. Some toxic elements in the wastewater used for irrigation may enter in the food chain and may result in high salinity levels of soils that affect the yield of salt sensitive crops. Prolonged use of wastewater is hazardous for soil as it may deteriorate the soil structure(UN, 2017). The crop growth and yield may be affected by the use of untreated wastewater, due to high concentration of nitrogen. phosphorus and potassium (NPK). The impacts of wastewater on soil are due to the presence of high total dissolved solids and heavy metals which deposit in the soil with the passage of time, causing soil and groundwater pollution (Andersson *et al.*, 2016)

Research findings such as that of Ajayi and Osibanji, (1981); Joroen *et al.* (2002); Shahalum *et al.* (1998); Howe and Wagner, (1996); Ali, (1987); Marten *et al.* (1980) established a lot of facts on the effects of using untreated wastewater for irrigation along the river that receives industrial effluent. These include spreading of diseases which are of microbial or chemical origin. In a country like Nigeria where the health care system is deficient, the disease caused through this are seldom understood and in some cases not adequately treated.

The aim of the research is to compare the level of bacterial/helminthes contaminations of vegetables produced from polluted and unpolluted irrigation sides of Kano State.

## MATERIAL AND METHODS

### Study Area

Because of the very large size of Kano and presence of many irrigation sites, two study areas were selected these are Gidan Maza irrigation site representing polluted area and Yarimawa representing unpolluted site in Kano municipal and Tofa Local Government respectively. Samples were analyzed for Bacteriological/helminthes contamination of some representative crops namely. *(Lactuca veriso, Larcus carota, Solamum lycopersicum, Phylanthus amarus, Alium cepa)* at microbiological laboratory of Bayero University, Kano, Nigeria.

### Sample Collection

Freshly harvested vegetable samples *(Lactuca veriso, Daucus carota, Solanum lycoperiuin, Phyllanthus amarus and Allium cefa,)* were collected from the study area, packed in labeled paper bags and transported straight to the laboratory for analysis as described by Jimoh and Ali (2011).

### Detection and Enumeration of Helminths

200g vegetable samples were transferred to a petri-dish with the capacity of 2000ml then 200m1 of tap water was poured into the dish. Both sides of the leaves were washed carefully with a hand brush and then removed from the dish (Choi *et al.,* 1972). Sedimentation method was used to analyze the helminthes as follows;

The vegetables washed water was transferred to a sedimentation flask with a capacity of 500m1. The dishes and brushes were rinsed twice with 1 80m1 of tap water. After sitting undisturbed for 10 hours for sedimentation. The supernatant which formed in the flask was decanted using a funnel with a layer of iron gauze inserted for straining. The sediment was transferred to a graduated centrifuged tube. The tube was then centrifuged for five minutes at 2,500rpms. The supernatant was removed from the tube and sediment was used as a sample (Choi *et al.,* 1972).

In order to determine the mean number of parasite per 200gm of vegetable the total amount of sediment in a graduated centrifuge tube was measured and adjusted to 1ml adding tap water immediately after stirring the number of egg and larvae in 0.lml of the sediment was counted microscopically (Choi *et al.,* 1972).

### Microbiological Analysis

Microbiological analysis was carried out to determine microbial load and isolate some microorganism of public health importance using the method described by (FAO, 1979).

### Sample Preparation and Serial Dilution

The sample preparation was carried out according to the method described by FAO, (1979). In the method 25g of sample (vegetable) was weighed and homogenized by blending in 225m1 peptone water at 2000 rpm. This was labeled as 1: 10 dilution which is also the stock or the homogenate. This was further serially diluted to l: $10^5$. By taken 1ml of the homogenate and transferred to the next test tube (1:$10^2$. It was continued serially up to (1:$10^8$).

### Total Aerobic Bacterial Plate Count

This was carried out according to the method of FAO (1979). In this 1ml of inoculum from ($10^{-3}$, $10^{-4}$, $10^{-5}$, $10^{-6}$,) dilutions were transferred into duplicate Petri dishes which were labeled accordingly. This was followed by pouring aseptically about l5ml of molten nutrient agar at 35°C. The culture was homogenized by swirling the plates and later allowed to solidify. The plate were incubated at 37'C for 24hrs, after incubation, plates containing 30
300 colonies were selected and colonies counted. The average was taken and the number obtained was multiplied by the inverse of dilution factor. This gave the number of colony forming units per gram of a sample (cfu/g).

### Detection and Enumeration of Coliforms

This was carried out according to method described by Atlas (1997). In this method a set 9 test tube s each containing 9m1 of lactose broth and an inverted Durham tube were placed in a test tube rack. Thus were autoclaved to sterilize and to expel air. Inoculation was made from the serially diluted samples as follows: from the 1:10 dilution. 1ml of inoculum was transferred to the first three of the 9 test tubes containing 9m1 of lactose broth. Then 1ml also was transferred from 1: 100 dilutions to the second set of three test tubes of lactose broth and finally 1ml of inoculum was transferred from 1:1000 dilutions to each of the last three tubes. All the 9 test tubes were incubated at 37°C for 24 hours and other 24 hours in the absence of gas (Presumptive test).

Following 24hrs of incubation, the tube were observed for gas production and number of gas positive tubes was compared with the most probale number (MPN) table to estimate the most probable number of coliforms per gram samples. A loopful of inoculum from gas positive tubes was streaked onto Eosine methylene blue (EMB) agar plate and incubated at 37°C for 24hrs (confirmed test). Following incubation, colonies which formed bluish black colonies with green metallic sheen, and reddish colonies were noted and isolated on agar slant. This is called a confirmed test. Also colonies showing metallic sheen on EMB were structured into tubes of lactose broth and incubated at 3 7°C. The tubes were observed after 24hrs for gas production. This is the completed test for coliforms.

**Biochemical Tests for the Characterization of the Microorganisms**

**Isolation and identification of bacteria**

Brilliant green bile broth medium was used to confirm the presence of coliforms. Eosin methylene blue agar as used to identify lactose fermenting bacteria (e.g. *E. coil, Kiebsiella, Enterobacter, Citrobacter etc).* These organisms were differentiated through biochemical reactions such as citrate utilization, methyl red, indole, motility, **Voges. Proskauer reactions**

Other microorganisms were identified on the basis of their colonial, morphological and biochemical properties.

**Citrate utilization test**

24.28g of Simmon citrate agar was dissolved in 1 litre of distilled water and sterilized (Biomark, India). The Agar medium was inoculated with broth culture using sterile straight needle, using a stab and a streak. This was then incubated for 24 hours at 3 5°c.

A blue colour indicated positive result while no change in color was recorded as negative result (APHA, 1999).

**Methyl red test**

Ten milliliter portion of medium from a pure culture was inoculated into MRVP medium and incubated at 35°c for 5 days. To 5ml of the culture, 5 drops of methyl red indicator solution (PS Park. Northampton, UK) was added. Red colour was recorded as methyl-red positive, while a distinct yellow colour was recorded as methyl red negative (APHA. 1999).

**Voges - Proskauer test**

Five milliliter of medium was inoculated into test-tubes. These were incubated for 48 hours at 35°C. To 1ml of culture O.6m1 naphthnl solution was added and gently shaked for 1 minute. O.2m1 KOH solution was added and the tubes were again gently shaked for 1 minute. The tubes were then placed in a test tube rack and kept without disturbing for 20 minutes. Red color formation at the surface in 10-20 minutes indicated positive VP, while unchanged after 20rninutes indicated negative VP (Cappuccino, 2002).

**Motility Indole-ornithine (MIO)**

Motility Indole-ornithine (MIO) of 31.5g (Scharlau Chemie, S.A. Spain) was dissolved in 1litre of distilled water then selected colonies from a turbid broth culture were inoculated into the tubes of MIO medium. This was done by using straight needle to stab the centre of the medium to about one-half its length. The tubes were then incubated aerobically with the caps loosed (to allow air to enter ) at 35°C for 24 hours. Tubes were observed for motility and ornithine production. After reading these reactions, then few drops of Kovacs reagent were added and then observed for indole production. Turbidity or fuzzy growth away from the line of inoculation indicated motility. Bile growth only along the stabbed line indicated a negative test for motility. Dark, turbid purple color indicated ornithine positive result while yellow throughout the medium indicated negative result. Pink to red color denotes positive indole test after addition of Kovacs reagent, while yellow color denotes a negative test after the addition of Kovacs reagent (Macfaddin, 1980).

**Catalase Test**

A 2.3ml quantity of hydrogen peroxide was poured into a test tube. A sterile wire loop was used to remove se era] colonies. The rest organisms were mixed in the hydrogen peroxide solution. Bubbling of gases was recorded as positive test (Cheesebrough. 2000).

**Coagulase Test**

The test was conducted by placing a drop of normal saline (0.9%wv) on clean glass slide. A small portion of the isolate was emulsified in the drop of normal saline, a drop of plasma was added to the suspension and the complex was rocked gently. On observation. Coagulase positive isolate showed agglutination within ten (10) seconds, and the result was recorded (Cheesebrough, 2000).

**Indole Test**

This was carried out according to Cheesbrough, (2000). The test organism was inoculated in Bijou bottle containing 3ml of sterile tryptone water, incubated at 35 — 37°C for up to 48hrs. This was followed by the addition of 0.5rnl Kovacs reagent. Red color on the surface layer within 10 minutes indicated positive test for indole.

**Methyl Red Voges Proskauer Test (MR-VP)**

This was carried out according to Fowale and Oso (2001). Two different tubes each of MR-VP broth were inoculated with the suspected E. colt and E. aerogens.

These were incubated at 37 C for 2 3 days. Five drops of methyl red indicator were added to each tube. Red color gave positive (acid) test and yellow color indicated negative (alkaline) test. On the second tube of each organism VP test was carried out by addition of 1ml u.-napthol solution followed by 1ml of 40% potassium hydroxide (KOI-I) solution. This was agitated and allowed to stand for about 1hour. and then observed. A pink to red color indicated the presence of acetyl methyl carbinol (VP- positive). A reddish brown color indicated negative (i.e. VP negative).

**RESULTS AND DISCUSSION**

The result of analysis of helminthes of polluted crops showed that *Lactuca veriso* had 3, 10, 4 of Ascaris Eggs, *strongloides* and hookworm respectively. *Darcus carota* had 1, 2, 2 of Ascaris Eggs, *strongyloides* and hookworm respectively. *Solanum lycopersicun* had 2, 1,0 of Ascaris Eggs, *strongyloides* and hookworm respectively. *Phyllanthus amarus* had 10, 4, 4 of Ascaris Eggs, *strongyloides* and hookworm respectively. *Alium cepa* had 1.1,0 of Acaris Eggs. *strongyloides* and hookworm respectively.The result of analysis of helminths of unpolluted crops showed that. *Lactuca veriso* had 1,1,1 of Ascaris eggs, *Strongyloides* and *Ancylostoma* respectively. *Daucus carota* had 0,0,0 of Ascaris eggs, *Strongyloides* and *Ancylostoma* respectively. *Solanum lycopersicum* had 0,0,1 of Ascaris eggs, *Strongyloides* and *Ancylostoma* respectively. *Phyllanthus amarus and Alium cepa* did not possess any of Ascaris egg, *strongyloides* and *Ancylostoma.* While the bacteriological analysis polluted crops shows that *Lactuca veriso* had mean microbial count of 2.0 x $10^2$ cfu/g and 180MPN/g of aerobic plate count and coliform count. The organism identified include *E. coli, Salmonella,* and *Shigella, Darcus carota* had mean microbial count of 8.40 x $10^2$ cfu/g and 180 MPN/g of aerobic plate count and coliform count and the organism identified are *E. coli, salmonella* and *shigella. Solanum lycopersicum* had mean microbial count of 1.13 x $10^3$cfu/g and 180 MPN/g of aerobic plate count and coliform count, the organism identified were *E. coli selmonnella* and *shigella. Phyllanthus amarus* had mean microbial count of 5.08 x $10^4$ cfu/g and 180 MPN/g of aerobic plate count and coliform count, the organism identified are *E. coli, salmonella and shigella. Alium cepa* had mean microbial count of 5.70 x $10^1$ cfu/g and 180 MPN/g of aerobic plate count and coliform count, the organism identified are *E. coli, salmonella* and *shigella.* While the bacteriological analysis of unpolluted crops shows that *Lactuca veriso* had mean microbial count of 1.22 x $10^2$ cfu/g and 30 MPN/g of aerobic plate count and coliform count, the organism identified are *E. coli, S. aureus* and *shigella., Dacus carota* had mean microbial count of 5.1 x $10^2$ cfu/g and 21MPN/g of aerobic plate count and coliform count, no any organism identified, *Solanum lycopersicum* had mean microbial count of 1.4 x $10^2$cfu/g and 21MPN/g, *Phyllanthus amarus* had mean microbial count of 1.43 x $10^3$ cfu/g and 21 MPN/g of aerobic plate count and coliform count, no any organism identified , *Alium cepa* had mean microbial count of 1.32 x $10^1$ cfu/g and 21 MPN/g of aerobic plate count and coliform count, no any organism identified . The aerobic mesophilic count, coliform and fungal counts were high exceeded the FAO (1979) limit, this may be due to higher levels of biodegradable organic matter in the waste water from industries and residential houses, while the higher total coliform count of 180 MPN/ml is more likely to have come from faecal matter which might have contaminated the untreated effluent. All the crops irrigated with effluent were found to contain some groups of pathogenic microorganisms of public health important like *Salmonella sp, Shigelia sp, Staphylococcus sp,* and helmimyths eggs. This agrees with surveys conducted by the International Water Management Institute (IWMI) in Kumasi, Accra and Tamale that it was difficult to find any vegetables like (lettuce, onion and cabbage), that were not contaminated with faecal coliform. Helminths eggs were also commonly found on such vegetables. Hundred samples of vegetables that were collected, processed and examined. *Phyllantus amarus and Lactuca veriso* had highest parasite contamination, 10 and 10 respectively which was followed by *Dacus carota* and *Solanum lycopasicum* which had 2 and 2, the Onion recorded the least parasitic contamination.

**Table 1: Showing the result of helminths analysis of polluted crops**

| | *Lactuca verisa* | *Daucus carota* | *Solanum lycopersicum* | *Phyllanthus amarus* | *Alium cefa* |
|---|---|---|---|---|---|
| Ascaris Eggs | 3 | 1 | 2 | 10 | 1 |
| Strongloides | 10 | 2 | 1 | 4 | 1 |
| Hookworm | 4 | 2 | 0 | 4 | 0 |

**Table 2: Showing the result of helminths analysis of unpolluted crops**

| *n=100* | *Lactuca verosa* | *Daucus carota* | *Solanum lycopersicum* | *Phyllanthus amarus* | *Alium cefa* |
|---|---|---|---|---|---|
| Ascaris Eggs | 1 | 0 | 0 | 0 | 0 |
| Strongloides | 1 | 0 | 0 | 0 | 0 |
| Hookworm | 1 | 0 | 1 | 0 | 0 |

**Table 3: Showing the result of microbiological analysis of polluted crops**

| Sample n=20 | Microbial count (cfu/g) | | Identified microorganism | | | | |
|---|---|---|---|---|---|---|---|
| | APC (cfu/g) | CC (MPN/g) | E.coli | | Salm. | Shig. | Staph. |
| **Unpolluted crops** | | | | | | | |
| **Lactuca veriso** | $2.0x10^{2}$ | 180 | + | | + | + | + |
| **Daucus carota** | 8.40x10[illegible] | 180 | - | - | - | - | - |
| **Solanum lycopersicum** | $1.13x10^{2}$ | 180 | + | + | + | + | + |
| **Phyllantus amarus** | $5.8x10^{4}$ | 180 | + | + | + | + | + |
| **Allium cefa** | $5.7x10^{1}$ | 180 | - | - | - | - | - |

Key
APC: Aerobic plate count, FC:Fungal Count
CC: Coliform count
MPN: Most probable number

**Table4: Showing the microbiological analysis of unpolluted crops**

| Sample n=20 | Microbial count (cfu/g) | | Identified microorganism | | | | |
|---|---|---|---|---|---|---|---|
| | APC (cfu/g) | CC (MPN/g) | E.coli | | Salm. | Shig. | Staph. |
| **Unpolluted crops** | | | | | | | |
| **Lactuca veriso** | $1.22x10^{3}$ | 30 | + | | - | + | + |
| **Daucus carota** | $5.1x10^{2}$ | 21 | - | - | - | - | - |
| **Solanum lycopersicum** | $1.43x10^{2}$ | 21 | - | - | - | - | - |
| **Phyllantus amarus** | $1.43x10^{4}$ | 21 | - | - | - | - | - |
| **Allium cefa** | $1.32x10^{3}$ | 21 | - | - | - | - | - |

**Key**
APC: Aerobic plate count, FC:Fungal Count
CC: Coliform count
MPN: Most probable number

## CONCLUSION

The research conclude that crops irrigated with polluted water had higher microbial load, presence of helminthes and some bacteria of public health importance as compared to the crops irrigated with unpolluted water that had lower bacterial load, the research recommends primary treatments of polluted water before use for irrigation.

## REFERENCES

Ajayi, S.O. and Osibanjo, 0. (1981). Pollution studies on Nigerian rivers II: Water quality of some Nigerian rivers. Environmental Pollution Series B 2: 87 — 95.

Ali, I. (1987). Wastewater criteria for irrigation in arid regions. *Journal of Irrigation and Drainage Engineering* 113: 173 — 18.

American Public Health Association (APHA, 1998). Standard methods for the examination of water and wastewaters. 20th edition American Waterworks Association and Water Environment Federation, USA. Parts I 000 10,000. 10145pp.

Andersson, K., Rosemarin, A., Lamizana, B., Kvarnström, E., McConville, J., Seidu, R., Dickin, S. and Trimmer, C. (2016). Sanitation, Wastewater Management and Sustainability: from Waste Disposal to Resource Recovery. Nairobi and Stockholm: United Nations Environment Programme and Stockholm Environment Institute. ISBN 978-92-807-3488-1, p. 56 https://en.wikipedia.org/wiki/Wastewater

Atlas, R.M. (1997). Principles of microbiology second edition C. Brown Publishers. Pp 802 — 803.

Cappuccino, S. (2002). Microbiology, a laboratory manual. 6th ed. Benjamin Camings, 149 — 155.

Cheesebrough, lvi. (2000). District Laboratory Practice in Tropical Countries. Part 2. Cambridge University Press. Pp 76-100.

Choi D.W. S.D. Park JW Kim DH Ahn Kyungpook (1972). National University Hospital Patients. Korean I Farast. 9: 47 — 54.

Food and Agriculture Organization of the United Nations FAQ (1979). Manuals of Food Quality Control 4. Microbiological Analysis.

Fowale, M.O. and Oso, B.A. (2001). Laboratory manual of microbiology. Spectrum books ltd. P 79-80.

Howe, J., Wagner M.R. (1996). The effect of peppermill wastewater irrigation and gypsum soil amendments on sedum accumulation by cottonwood and soil. Agricultural Water Management, 31(3): 295 — 306.

Joroen H., Ensink J., Wirn van der Hoek, Yutaka Mastno, Munir S., Aslarn MR. (2002). Use of untreated wastewater in pen-urban agriculture in Pakistan: Risk and opportunities. Research Report 6I. International Water Management Institute.

MacFaddin, J.F., (1980). Biochemical Tests for identification of medical bacteria, 2 ed., Williams, Baltimore, Pp. 1 - 2.

Marten, G.C., Larson W.E., Clapp C.E. (1980). Effects of municipal wastewater effluent on performance and feed quality of maize vs. reed ceanrygrass. Journal of Environmental Quality. 9: 1347 — 141.

Shahalum, A., Abuzahara B.M., Jaradat A. (1998). Wastewater irrigation effect on soil and crop and environment — a pilot scale study at Ibrid, Jordan. Water, Air, and Soil Pollution. Pp 425 — 445.

United nation, (2017). The United Nations World Water Development Report 2017. Wastewater:TheUntappedResource.Paris.ISBN978-92-3-100201-4. https://en.wikipedia.org/wiki/Wastewater.