EAS Journal of Nutrition and Food Sciences
Abbreviated Key Title: EAS J Nutr Food Sci
ISSN: 2663-1873 (Print) & ISSN: 2663-7308 (Online)
Published By East African Scholars Publisher, Kenya

OPEN ACCESS

Volume-2 | Issue-1 | Jan-Feb-2020 | DOI: 10.36349/easjnfs.2020.v02i01.005

Research Article

Antinutritional Factor, Microbial Load and Parasite Quality of *Telfairia occidentalis* under Controlled Application of Domestic Waste Water

Oguh C.E*[1], Obiwulu E.N.O[3], Ahaiwe P.T[1], Okekeaji U[4], Musa I.G[2] and Ezugwu E.C[1]

[1]Department of Biochemistry, University of Nigeria, Nsukka, Enugu State, Nigeria
[2]Department of Biochemistry, Federal University of Technology, Akure, Ondo State Nigeria
[3]Department of Integrated Science, Delta State College of Education Agbor, Delta State Nigeria
[4]Department of Pharmaceutical Microbiology and Biotechnology, University of Nigeria, Nsukka, Enugu State, Nigeria

Article History
Received: 24.01.2020
Accepted: 20.02.2020
Published: 28.02.2020

Journal homepage:
https://www.easpublisher.com/easjnfs

Quick Response Code

Abstract: The use of domestic waste water for irrigation is a common practice in Nigeria because of the fact that wastes water enhance soil fertility for proper growth and also accommodate a wide spectrum of enteric pathogens and antinutrients that may have a negative impact on human and the environment. This study was conducted to investigate the effect of Antinutritional Factor, Microbial Load and Parasite Quality of *Telfairia occidentalis* under Controlled Application of Domestic Waste Water. *Telfairia occidentalis* seeds were planted in 18 pots and 9 pots were irrigated with waste water and the other 9 pot was irrigated with ground water which is use as control till maturity from the month of October to January. Vegetable samples were collected at random with three replications for each test sample after 4 months. The waste water, ground water, soil samples were collected and analyzed for some water and soil physicochemical properties (electrical conductivity, pH and temperature) and soil (pH, total Nitrogen, total phosphorus, organic matter, total organic carbon, and exchangeable cations K^+, Mg^+ and Na^+) using a standard methods. Microbial load such as Total heterotrophic bacteria, *E. coli*, total coliform, faecal coliform, *Staphylococcus aureus, Salmonella* and intestinal parasites were determined using standard methods of APHA. Standard titrimeric methods were used to phytate and oxalate contents while Pearson method was used for cyanogenic glycoside, tannin, and alkaloid content. Results showed a significant ($p < 0.05$) different in water and soil physicochemical properties over the control group. The microbial load in *Telfairia occidentalis* irrigated with waste water ranged between 8.9×10^5 to 12.1×10^6 cfu/g while the control site ranged between 1.3×10^5 to 4.3×10^5 cfu/g. There were significant difference ($p<0.05$) between the test group and control. The bacterial counts recorded exceeded the recommended levels by WHO and ICMSF, standards (i.e. 10 to 10^2 coliforms g-1, 10 fecal coliform g-1 and 4.9×10^6 aerobic count g-1) wet weight vegetables. Waste water led to a significant ($p < 0.05$) increased of antinutrient factors in the vegetable.The result concludes that there is a potential health risk in the consumption of vegetables grown with waste water.

Keywords: Anti-nutritional factors, Microbial Load, Parasites, *Talfairia occidentalis*, Waste water.

INTRODUCTION

The use of wastewater for crop production has been increasing worldwide due to the increase in food demand, population and change in climatic conditions, which makes food production through rain fed agriculture less reliable. The use of untreated wastewater in irrigation represents an important route for the transmission of pathogenic organisms. Approximately 70% of untreated wastewater is used for agriculture practice and may have detrimental environmental and health effects (Cytryn, 2010). Farmers generally are not bothered about the human health effect and the environmental hazards associated with irrigation of vegetables with waste water or cultivating crops around sewage dumpsite, rather they are primarily interested in the increase in yields and huge profits. The major pathogens associated with the use of highly polluted waste water are the faecal/total coliforms, *E. coli* and eggs of some helminthes such as *Ascaris lumbricoides*, whose resistant eggs can be found in the wastewater (Sahu *et al*., 2016; Miller-Robbie, 2017). Transmissions of intestinal nematode (*Ascaris lumbricoides eggs, Entamoeba histolytica cyst* and *Giardia intestinalis cysts*) and pathogenic bacteria can cause several diseases, such as diarrhea, dysentery, typhoid and cholera to farmers and consumers are the major risks. Vegetables are widely exposed to

***Corresponding Author:** Oguh C.E Email: collinsoguh(at)gmail.com

microbial, parasitic and antinutrient contamination through contact with soil, dust and water and by handling at harvest or during postharvest processing. Wastewater from domestic and industrial sources contains appreciable number of nutrients, which increase crop yield without the use of fertilizer and also reduce cost of production. However, the waste water contains a variety of toxic substances such as heavy metals and microbial loads (Khaled, 2016; Oguh *et al*., 2019a). A wide range of microbial pathogens have been found in waste water and can be transferred to crops during irrigation.

Telfairia occidentalis Hook f. commonly called fluted pumpkin occurs in the forest zone of West and Central Africa, most frequently in Republic of Benin, Nigeria and Cameroon. It belongs to the family Cucurbitaceae and it is propagated using the seeds (Akoroda, 1990). Its seed is housed in another greater covering or hard shell which protects it from harm. It has different traditional names; it is known as Ugu in igbo, in Yoruba, it is known as Ewe Iroko, Kabewa in hausa, Ubong in Efik, Umee in Urhobo and Umeke in Edo. *Telfairia occidentalis* is a good source of some micronutrients such as mineral elements (Fe, Cu, Mg, Na and K), antioxidants (such as β Carotene , flavonoid, selenium, vitamin C and vitamin E), vitamins (such as thiamine, riboflavin, nicotinamide and ascorbic acid), phyto-chemicals such as phenols which are required for normal metabolic activity of the body (Kayode *et al*., 2009; Benzie and Choi, 2014). Therefore, the vegetables are used to alleviate the problems of micronutrients deficiency prominent in West African Countries.

Anti-nutritional factors are compound found in food that interfere with absorption of beneficial nutrients, minerals, metabolic processes and reduce the bioavailability of nutrients from plants or plant products used as human foods (Oguh *et al*., 2019b). Antinutrient can causes infertility, cancer, gastrointestinal and neurological disorder when consume in high concentration (Awomukwu et al., 2015). High concentration of cyanogenic glycoside prevent cells from using oxygen and eventually these cells die when deprive of oxygen especially the heart, respiratory system and central nervous system are most susceptible to cyanogenic poisoning (Ellenhom and Barcelonx, 1988). Phytate decreases the bioavailability of proteins and essential elements such as calcium, magnesium, zinc, iron, and phosphorus by forming insoluble complexes, which are not readily absorbed by the gastrointestinal tract with the attendant health problems such as oxalemia (Agbaire and Oyewole, 2012). Oxalate exhibit it effect by binding to calcium to form insoluble calcium oxalate crystals which may precipitate in the kidney to form kidney stone and oxalemia. Alkaloids are often toxic to human at high concentrations and causes infertility, gastrointestinal and neurological disorder (Olayemi, 2007; Awomukwu *et al*., 2015). Tannins can bind to macromolecules such as proteins and carbohydrates which results to the reduction in digestibility of these macromolecules and thus inhibit of microbial growth (Dei *et al*., 2007; Nwogu *et al*., 2008).

Both old and young requires vegetables because they are dietary source of nutrients, micronutrients, vitamins and fibre for humans and are thus vital for health and reduce the risk of several diseases. These nutrients repairs worn out tissues, lower cholesterol levels, normalize digestion time, improve clear vision, protect human from oxidant stress and cardiovascular diseases, prevent cancer, fight free radicals, and boost immune system activity. Soil irrigated with wastewater are usually nutrient rich, which improve soil properties such as organic matter, and soil nutrients, which increases plant productivity, supply of macronutrients (N, P, and K) and reduction of cost for crop production. Thus despite the yield benefits, outbreaks of human infections and environmental pollution are associated with the consumption of vegetables grown with waste water. Hence, the study investigates the concentrations of Antinutritional Factors, Microbial Loads and Parasites Quality of *Telfairia occidentalis* under Controlled Application of Domestic Waste Water. The results are expected to create awareness among the public on the effect of using raw domestic waste water for crop production.

MATERIALS AND METHODS

Experimental Site

The study was carried out in Makolo farm, Chanchaga Minna Niger State, Nigeria. Chanchaga is situated at $9^{\circ}34^{'}$North latitude, $6^{\circ}33^{'}$East longitude, with an area of $72km^2$ (Fig. 1) and a population of 201,429 at the 2006 census. It has a moderate climate with a very high temperature during the dry season and average rainfall during the rainy season.

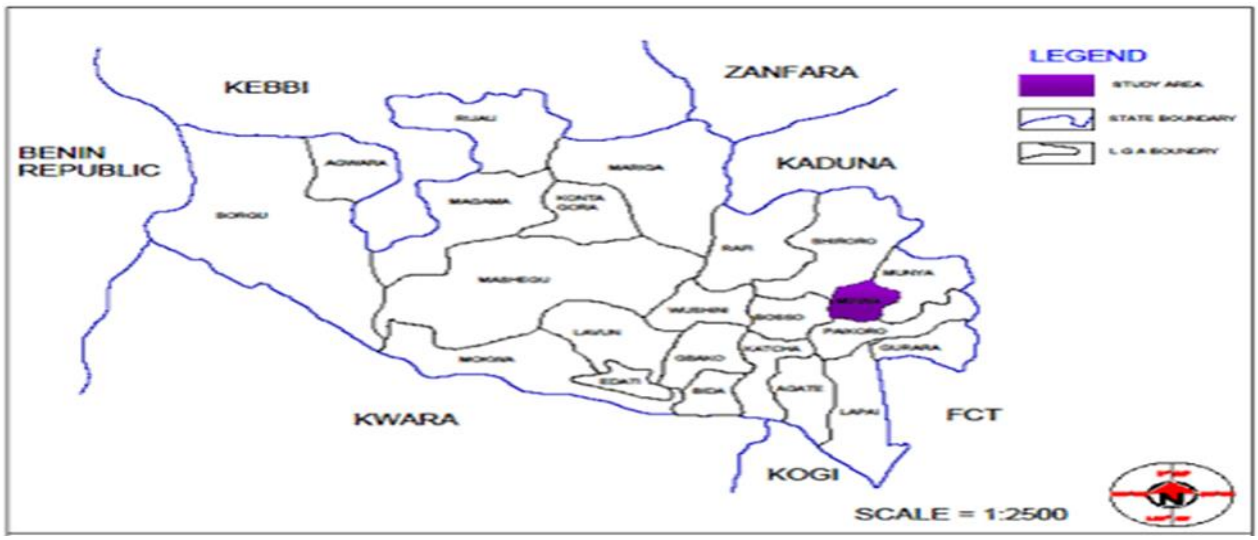

Figure 1: Map of Niger State showing study area in purple

Experimental Design

The seeds of *Telfairia occidentalis* were bought from the market from same source and 3 seed each was planted in 18 pots. Soil sample were gotten from a land free from activities, and was divided equally into the 18 pots. Nine 9 pots were irrigated with waste water three times in a week and the remaining 9 were irrigated with ground water which served as control untill maturity from the month of October to January. The control post and the test pots were kept far apart in the farm to avoid spread of microbe.

Sample Collection

Vegetable samples were collected at random with three replications for each test sample after 4 months and analyzed for microbial load, presence of a few specific parasites, and antinutrient following standard procedures. The waste water, ground water, soil samples were collected and analyzed for some water and soil physicochemical properties (electrical conductivity, pH and temperature) and soil (pH, Total Nitrogen, Total phosphorus, Organic matter, total organic Carbon, and Exchangeable cations K^+, Mg^+ and Na^+) using a standard methods. Leaves were randomly sampled within the farm to get a representative sample. All samples were collected aseptically in a sterilized universal container and plastic bags. The samples were cooled during transportation using a cooler box to keep the normal conditions of the micro flora of vegetables. Analysis was conducted within 24 hours of arrival at the Laboratory.

Physico-chemical Properties of soil use for planting

The Physico-chemical properties of the soil were analysed in order to evaluate the biodegradable process. Physicochemical parameters of the soil samples were determined according to the procedure used by Nimyel *et al*., 2015.

Soil pH

A triplicate of air-dried (20 g) soil samples were weighed into two separate groups of six 50 mL beaker and 20 mL of distilled water was added to one group and 30 ml of 1M KCl_2 to the other group. The mixtures were allowed to stand for 30 minutes with occasional stirring using a glass rod. The electrode of the calibrated pH meter was inserted into mixtures and the pH value was read using pH meter. The results were reported as soil pH in 1M KCl_2 and soil pH in water (H_20) in three readings.

Organic Matter

Soil samples were grounded to pass through 0.5 mm sieve after which 1g was weighed in triplicate and transferred to 250 mL Erlenmeyer flasks. Exactly 10 mL of 1M potassium dichromate was pipetted into each flask and swirled gently to disperse the soil. Then 20 mL of concentrated tetraoxosulphate (IV) acid (H_2SO_4) was added to each flasks and swirled gently until soil and reagents were thoroughly mixed. The mixture was allowed to stand for 30 minutes in a glass plate to allow for the oxidation of potassium dichromate to chromic acid. Exactly 100 ml of distilled water was added, 3- 4 drops of ferroin indicator or 1 ml of diphenylamine indicator was added, then titrated with 0.5 M ferrous sulphate solution or ferrous ammonium sulphate untill the colour changed from blue-violet to green or bright green. A blank titration was similarly carried out.

The percentage of organic matter is given by the following equation:

% organic matter = $(M1V1K_2Cr_2O_7 - M2V2FeSO_4) \times 0.0031 \times 100 \times F/Mass(g)$ of air dried soil

F = correction factor (1.33), M1 = mole of $K_2Cr_2O_7$, V1 = volume of $K_2Cr_2O_7$, M2 = mole of $FeSO_4$, V2 = volume of $FeSO_4$.

Total Organic Carbon

Exactly 1g of soil sample was accurately weighed into three 500 mL conical flask and 10 mL of 1M potassium dichromate ($K_2Cr_2O_7$) solution was added to each of the sample using a bulb pipette, 20 mL of concentrated sulphuric acid (H_2SO_4) was added while gently swirling the flask in a fume cupboard. It was allowed to stand and cool slowly on insulated pad like sheet of asbestos for about 25 minutes after which 200 mL of distilled water was added using a measuring cylinder. After this, 1 g of crystal sodium fluoride (NaF) was added to avoid interference by complexing $Fe^{3+,}$ obtaining a black colour mixture which was shaken vigorously. Finally, 1 mL of 1 % diphenylamine was added as an indicator and the mixtures were titrated immediately with 1 M ferrous sulphate ($FeSO_4$) solution in the burette. A blank without soil was prepared alongside the sample and titrated sameway. End point was indicated as a colour change from deep purple to green.

% Total Carbon content = (BT)×M×0.003×100×1.33/weight of soil sample taken.

Where;

B = Blank titre,
T = Test sample titre,
M = Molarity of $FeSO_4$,
1.33 = Correction factor,
0.003 = mg equivalent of carbon.

Total Phosphorus

Air-dried soil 2 g was weighed and dispensed in 20 ml of (0.025N HCl + 0.03N NH_4F) solution, shaken for 5 minutes and then filtered using whatman filter paper. After filtration, 3ml of the clear filtrate was put into a test tube, 3 ml of (0.87N HCl, 0.38N ammonium molybdate, and 0.05% H_3BO_3) solution and 5 drops of 2.5 g of 1–amino 2- tetraoxosulphsate (vi) acid, 5.0 g Na_2SO_3, and 146 g $Na_2S_2O_5$ solution were sequentially added to the prepared clear sample. A colorimeter (at wave length of 660 nm) was then used to take readings for total phosphorus.

Total Nitrogen

The total Nitrogen was determined using the kjeldahl digestion method. Exactly 20 ml of concentrated tetraoxosulphate (VI) acid was added to a 1 g measurement of air dried soil. A catalyst known as Kjeldahl TAB was also added and the solution was digested. After digestion, a clear solution was observed; this clear solution was distilled and subsequently titrated with 0.01M HCL.

Sodium, Magnesium and Potassium ion (Na^+ $Mg^{2+}$and K^+)

The exchange ions was determined calorimetrically using Flame photometer. Soil sample (5 g) was accurately weighed into No. 1 filter paper fitted into a funnel on a leaching rack with 100 mL volumetric flask for collecting the leachate. The soil sample was leached with 1 N NH_4OAC solution obtaining 100 mL volume of leachate. The Optical density readings for Na^+ Mg^{2+} and K^+ were obtained from the flame photometer.

$Na^+/mg^{2+}/K^+$ meq/100g = Optical density× correction factor×100/5

PHYSICO-CHEMICAL PROPERTIES OF WATER

pH

The pH scale was calibrated with buffer solutions of known pH values before use. About 75 ml of water sample is taken in a 100 ml beaker. The suspension was stirred at regular intervals for 30 minutes and the pH is recorded. The suspension was well stirred before the electrode was immersed and three readings were taken and then mean of it was calculated.

Temperature

Water temperature was determined using a mercury-in-glass thermometer.

Electrical Conductivity

The electrical conductivity was determined using the Richards, 1954 methods. About 5 ml water sample was mixed and shaken for 30 minutes, allowed to settle then the conductivity measured with a temperature-compensated probe. An approximate soluble salts value may be derived from the conductivity using the empirical relationship:
Soluble salts (%) = Conductivity (dS/m) × 0.35.

Microbiological Analysis

Test and control vegetable samples were ramdomly collected and mixed together for each group. Exactly 10 g of Vegetable samples were homogenized in 90 ml of sterile normal saline in a blender for 1-2 minutes. The homogenate 1 ml was mixed with 9 mls of sterile distilled water in a test tube, it was mixed very well, and then 1 ml portion of it was transferred aseptically into another test tube containing 9 mls of sterile distilled water and mixed. The dilution was done in series to the fifth dilution (10^{-5}). Inocula of 0.1 ml were taken from the third (10^{-3}) and inoculums was aseptically placed on the surface of the sterile solid Nutrient Agar (NA) medium, Eosin methylene blue agar, Salmonella shigella agar, violet red bile agar (oxoid) medium, and Manitol Salt Agar (MSA) to determine total heterotrophic bacteria/Aerobic mesophilic bacterial count, Escherichia coli count and total coliform counts, Salmonella shigella count, faecal coliforms, and *Staphylococcus aureus*. Respectively. The inverted agar plates for bacteria were incubated at 37°C for 24 to 48 hours and plates showing 30-300 colonies were used for quantitation of bacterial load as cfu/g. All samples were processed following standard methods of APHA, 2012.

Parasitological Analyses of Vegetables

About 100 g of each fresh vegetable sample was chopped into small pieces and put into a clean beaker containing physiological saline solution (0.85 % NaCl), enough to wash the vegetable sample. Fragments of the vegetable sample were removed from the washing saline using starile forceps and were kept for about 24h for sedimentation to take place. After 24h sedimentation, the top layer of the washing saline was carefully discarded leaving 5 ml of the sediment. This was finally centrifuged at 2000 rotations per minute for 5min and the supernatant discarded. The pellets/residue was mounted on slides, stained with Lugol‟s iodine solution and examined under the compound light microscope to examine the samples for intestinal parasites. *Ascaris lumbricoides eggs, Entamoeba histolytica cyst* and *Giardia intestinalis cysts* with the following characteristics, oval or spherical in shape and are 45-75 µm, oval or spherical in shape 10-20 micro meter in diameter, pear shape with two nuclei, and four pairs of flagella 10-12 µm long respectively.

Anti-nutrient Analysis

Fresh leaves of the vegetables were sorted to remove damages and defective ones. The sorted fresh samples were washed with running tap water to remove dust and dirt and then with double distilled water. Titrimetric method of Association of official analytical chemist AOAC, (1995), was used to estimate oxalate and phytate content while Pearson, 1976 method was use to estimate cyanogenic glycoside, tannin and Alkaloid content.

Phytate Content

According to the titrimetric method used 2 g of blended vegetable samples was weighed into a 250 cm^3 conical flask, and 100 cm^3 of HCl 2% added, and allowed to stand for 3 hours. The resulting solution was filtered through double layer of hardened whatman No. 1 filter paper. About 50 ml each of the filtrate was placed in another 250 cm^3 conical flask and 100 cm^3 distilled water was added in each case to give a proper acidity, while 10 ml of 0.3% ammonium thiocyanate (NH_4SCN) solution was added into each solution as indicator. This was titrated with standard solution of iron (iii) chloride ($FeCl_3$) which contains 0.00195 g/cm^3 (1.95 g). The end-point was reached when slightly brownish – yellow colour was observed which persisted for about 5 minute. The concentration of phytic acid in g/100 g sample was calculated using the formula:

Concentration of phytic acid = $\frac{\text{Titre value} \times 0.00195 \times 1.19 \times 100 \times 3.55}{\text{weight of sample}}$

Cyanogenic Glycoside Content

About 0.5 g of grounded sample was weighed into a test tube. The sample was macerated in 20 ml of phosphate buffer pH 6 for 10 minutes. The test tube was allowed to stand for an hour with shaking at every 10 minutes intervals. It was centrifuged for 5 minutes and 1 ml of the supernatant (clear liquid) was transferred into triplicate tubes. Than 4 ml of alkaline picrate was added and boiled for 5 minutes in a water bath. The tube was cooled in cold water and the absorbance was taken using a colorimeter at 470 nm against a reagent blank.

Oxalate Content

Vegetable samples were ground into slurry and 1.0 g of sample were weighed into a crucible dish, and was extracted with 10 cm^3 of distilled water, 1 cm^3 of concentrated H_2SO_4 was added and allowed to stand for an hour. The volume was made up to 50 cm^3 with distilled water. The resulting solution was filtered and 25 cm^3 of the filtrate was pipetted into a conical flask heat to 90^oC and titrated against potassium permanganate $KMnO_4$ in a burette. A colour change was noted which indicates the end point and the reading of the burette was taken when the red colour remained steady for some seconds. The concentration of oxalate (mg g^{-1}) in each of the sample was gotten by:

Oxalate (concentration) = Average volume used × 11.5

Tannin Content

The vegetable samples were ground into slurry and 0.5 g of the sample was weighed and into in a test tube. The sample was macerated in 20 ml of methanol for 10 minutes and centrifuged for 5 minutes at 3000 r. p. m. and 5 ml of the supernatant was transferred into triplicate tubes. Also 0.3 ml of 0.1 m ferric chloride in 0.1 m hydrogen chloride was added and mixed and 0.3 ml of 0.0008 m potassium ferricyanide was added, mixed, and the absorbance taken using a colourimeter after 5 minutes at 720 nm against a blank.

Alkaloid Content

The leaves samples were ground into slurry and 1 g of the sample was weighed, put in a test tube, and macerated in 10 ml of 20 % sulphuric acid and 10 ml of ethanol for 10minutes. The tube was allowed to stand for an hour with intermittent shaking, and subsequently centrifuged for 5 minutes. about 0.5 ml of the supernatant was transferred into 3 testtubes, 2.5 ml of 60 % sulphuric acid was added and mixed. exactly 2.5 ml of 0.5% formaldehyde was subsequently added and the test tubes were allowed to stand for 3 hours. The absorbance was taken using a colourimeter at 565 nm against a blank.

Statistical Analysis

The data obtained were analyzed using IBM Statistical Product and Service Solution (SPSS) version 20 and Microsoft excel 2013. The results were expressed as mean ± standard error (SE). One way analysis of variance (ANOVA) was carried out as $p<0.05$ considered statistically significant.

RESULTS

Physicochemical properties of soil samples

The texture of soil used is loamy. The pH of the soil in water (H_2O) and KCL_2 were 7.13 and 7.29 respectively. Nitrogen, phosphorus, organic carbon, organic matter, and exchangeable cation (K^+, Mg^{2+}, and

Na^+) of soil were 2.37, 13.13, 2.45, 3.83, 3.29, 3.31 and 5.27 respectively Table 1.

Table 1: Physicochemical properties of soil Concentrations

Soil properties	Loamy
Texture	Loamy
pH in H_2O	7.13 ± 0.08
pH in KCL_2	7.29 ± 0.01
Total Nitrogen %	2.37 ± 0.07
Total Phosphorus %	13.13 ± 0.05
Total Organic Carbon %	2.45 ± 0.03
Organic Matter %	3.83 ± 0.09
K^+ meq/100g	3.29 ± 0.09
Mg^{2+} meq/100g	9.31 ± 0.04
Na^+ meq/100g	5.27 ± 0.23

Results expressed as Mean ± SD. n=3

Physicochemical Properties of water samples

The values of physicochemical properties of waste water and ground water were pH (5.15 and 7.06), Temperature (34.54 and 26.21 ºC), and electrical conductivity (662.04 and 498.61 mg/l) respectively which are significantly different (P<0.05) shown in Table 2.

Table 2: Physicochemical properties of water sample

Physicochemical Properties	Water Samples		WHO, 2006**, 2010* Permissible Limit
	Waste water	Ground water	
pH	5.15 ± 0.09	7.06 ± 0.14	6.5 – 8.5*
Temperature (ºC)	34.54 ± 0.15	26.21 ± 0.52	20 – 30 ºC*
Electrical Conductivity (mg/l)	662.04 ± 6.45	498.61 ± 3.14	500 mg/l**

Results Expressed as Mean ± SD.

Microbiological Analysis on Vegetable

The total microbial load in the vegetable samples from vegetables irrigated with waste water and vegetable irrigated with ground water are shown in Table 3. The total microbial load in *Telfairia occidentalis* irrigated with waste water ranged between 8.9×10^5 to 12.1×10^6 cfu/g with total heterotrophic bacteria more dominant while the control site ranged between 1.3×10^5 to 4.3×10^5 cfu/g with heterotrophic bacteria more dominant. There were more microbial loads in the vegetables irrigated with waste water than the control which shows a significant difference (p<0.05).

Table3: Bacterial loads in *Telfairia occidentalis* leaf.

Microbial load (cfu/g)	*Telfairia occidentalis* Samples		
	Waste water (A)	Ground water (B)	WHO and ICMSF limit
Total heterotrophic B	$12.1\times10^6 \pm 7.5\times10^3$	$4.3\times10^5 \pm 4.2\times10^3$	4.9×10^6
E.coli	$9.7\times10^5 \pm 3.3\times10^3$	$1.5\times10^5 \pm 3.3\times10^3$	10
Total coliform	$11.4\times10^6 \pm 5.6\times10^3$	$3.3\times10^5 \pm 6.5\times10^3$	10×10^2
Faecal coliform	$9.7\times10^5 \pm 6.8\times10^3$	$3.0\times10^4 \pm 6.7\times10^3$	10
Staphylococcus aureus	$10.6\times10^6 \pm 6.8\times10^3$	$4.0\times10^4 \pm 4.2\times10^3$	100
Salmonella	$8.9\times10^5 \pm 4.8\times10^3$	$1.4\times10^5 \pm 6.9\times10^3$	10

B = bacteria; A = irrigated with waste water; B = irrigated with ground water; n = 3. Results expressed as Mean ± SD: Column mean values carrying different letter are significantly different (P<0.05).

Parasitological Analyses of Vegetables

Table 4 shows the parasitological analyses of vegetable irrigated with waste water and a control sample irrigated with ground water. The study showed that out of 12 sample examined, 75 % of *Ascaris lumbricoides* (9), 25 % of *Entamoeba histolytica cryst* (3) and 8.3 % of *Giardia intestinalis cysts* (1) parasit was dictated from *Telfairia occidentalis* irrigated with waste water. On the controls only 16.6 % of *Ascaris lumbricoides* (2) was seen and others were absent in the vegetable.

Table 4: Ova of intestinal helminth parasite encountered in *Telfairia occidentalis*

	Intestinal parasite						
Samples	Total examined sample	*Ascaris lumbricoides eggs*	%	*Entamoeba histolytica cyst*	%	*Giardia intestinalis cyst*	%
Test	12	9	75	3	25	1	8.3
Control	12	2	16.6	-	-	-	-

Antinutrient Factors on Vegetables Grown on Sewage Dump Site and control site

The cyanogenic and oxalate levels from waste water irrigation are 2.22 mg/100g and 23.97 mg/g^{-1} and are within the limits in vegetables 0.5 - 3.5 mg/kg and 500 mg/100g respectively. Phytate (8.65), Alkaloid (0.53) and tannin (1.81) contents in vegetable irrigated with waste water were above the threshold in vegetables 0.035 %, 0.02 % and 0.25 g/l respectively. The content of all antinutrient from vegetable irrigated with ground water were all below the permissible limit except phytate (3.42) which is above the limit of 0.035 %.

Table 5: Antinutrient factors on vegetables irrigated with waste water and a control

Antinutrient Factors (mg/100g*)	Samples		Limits	Source
	Leaves with Waste water	Leaves with ground water		
Cyanogenic*	2.22 ± 0.03	0.56 ± 0.01	0.5 – 3.5 mg/kg	Fowomole, 2010
Phytate(g/100g)	8.65 ± 0.23	3. 42 ± 0.23	0.035 %	Abdoulaye et al., 2011
Oxalate (mg/g^{-1})	23.97 ± 0.21	9.29 ± 0.15	200–500 mg/100g	Pearson, 1976
Alkaloid*	0.53 ± 0.01	0.02 ± 0.12	0.02 %	Adhikari et al., 2005
Tannin*	1.81 ± 0.04	0.19 ± 0.23	0.25 g/l	laconelli and Simmen, 2002

DISCUSSION

The pH in water and KCL_2 are toward the range of neutral. Research have shown that heavy metals are generally more mobile at pH < 7 than at pH > 7 (Oguh *et al*., 2019c). The moderate pH of the soil may probably be due to no activities around the area the soil was collected. Metal solubility tends to increase at lower pH and decrease at higher pH values. The high total nitrogen in the soil sample is as a result of the nitrogenous decay plant and animals materials. However, the results reported by (Osazee *et al*., 2013) had the range 3.476 to 4.522 % which is also significantly higher than the concentration reported in this research. The soil recorded a decrease in total phosphorus content which may be due to the nature of the soil because no dump or activity around the location. The alteration or change on physicochemical properties of the soil is therefore expected to affect the survival of certain species and hence their diversity. This change can increases the heavy metals in the soil, which is then likely transferred to plants that grow on such soils, with the associated risks of long term toxicity to humans that consume them and other biota in the ecosystem.

The waste water recorded a low pH concentration than ground water (5.15 and 7.06) respectively. Low pH level accelerate the release of pollutant such as microbial loads, antinurient and heavy metals which are as a result of waste in the waste water. The World Health Organization (WHO, 2010) recommended a pH value of 6.5 -8.5 for domestic water to prevent corrosion. Plants are often influenced by the temperature around them because high temperature affect plants. Water temperature according to Lenntech, 2014 affects the Electrical conductivity (EC) so that its value increases from 2 up to 3% per 1°C. The increase in EC in waste water more than the permissible limit of EC is 500 mg/l, may be due to waste materials such as nitrate ion and waste product such as sewage and sludge in the water. Lower pH also increases electrical conductivity.

It was observed that the microbial load on *Telfairia occidentalis* from the waste water were higher when compared to the vegetable irrigated with ground water. The sequence of occurrence is Total Heterotrophic bacteria (THB) > Total coliform (TC) > *Staphylococcus aureus* > Faecal coliform (FC) > *Escherichia coli* (*E-coli*). The result was in accordance with the findings of Samuel *et al*., 2013 who recorded highest level of contamination of total coliform, faecal coliform, *E. coli* and helminth eggs on lettuce. The Mean values of total coliforms (TC), faecal coliform (FC), *E. coli* and Helminthes eggs on lettuce were 4.1 ± 0.5, 3.7 ± 0.5, 3.3 ± 0.6 log10 CFU·g−1 fresh weight and two helminth eggs respectively. Samuel recorded a total coliform composition of wastewater ranged from 3.19 to 4.82 log CFU/100 ml with a mean of 4.4 log CFU/100 ml. Faecal coliform bacteria ranged from 3.36 to 4.33 log CFU/100 ml with a mean of 4.0 log CFU/100 ml. Vegetable samples were mostly contaminated with Total Heterotrophic bacteria (12.1 x 10^6cfu/g) due to the waste water use in irrigation. The data further showed that all the bacterial counts recorded in this study exceeded the recommended levels by WHO, 2006 and International Commission on Microbiological Specifications for Food (ICMSF), 1998 standards (i.e. 10 to 10^2 coliforms g-1, 10 faecal coliform g-1 and 4.9×10^6 aerobic count g-1) wet weight vegetables. Intestinal parasites are common in fresh vegetables especially vegetable irrigated with waste water. The consumption of contaminated vegetables plays an important role in the transmission of human parasitic infection (Tiimub *et al*., 2012; Farahat *et al*., 2017). The intestinal helminth parasite encountered were abundant at vegetable irrigated with waste water than the one irrigated with ground water. *Ascaris lumbricoides* were found to be more dominant in vegetable irrigated with waste water. Epidemiological studies have shown that the actual risk of infection for people exposed to wastewater is highest for intestinal nematodes such as roundworm (*Ascaris lumbricoides*) which can lead to various chronic diseases, particularly in elevated concentrations or in prolonged dietary intakes of this vegetable.

Large doses of cyanogenic glycoside prevent cells from using oxygen and causes heart, respiratory system and central nervous system poisoning (Ellenhom and Barcelonx, 1988). Phytate decreases the bioavailability of proteins and essential elements such as calcium, magnesium, zinc, iron, and phosphorus by forming insoluble complexes, which are not readily absorbed by the gastrointestinal tract with the attendant health problems such as oxalemia (Akande and Ajayi, 2017). Oxalate binds to calcium to form insoluble calcium oxalate crystals which may precipitate in the kidney to form kidney stone and oxalemia. Alkaloids cause infertility, gastrointestinal and neurological disorder (Olayemi, 2007; Awomukwu *et al*., 2015). Tannins can bind to proteins and carbohydrates resulting in the reduction in digestibility of these macromolecules and thus inhibition of microbial growth (Dei *et al*., 2007; Nwogu *et al*., 2008). The results showed that the cyanogenic glycoside, phytate, oxalate, alkaloid and tannin content in *Telfairia occidentalis* samples irrigated with waste water were generally higher than that of the control samples. The results also indicates that the waste water led to a significant ($p < 0.05$) increased of antinutrient factors in the vegetable.

CONCLUSION

The present research shows that vegetables irrigated with waste water were all contaminated with high microbial loads, parasite, and antinutrient. The microbial loads and antinutrient factors on the test vegetables were above ICSFM and FAO recommended limits for vegetables. People who eat these contaminated vegetables raw or half cooked, stand a high chance of contracting gastrointestinal diseases like typhoid, cholera and dysentery and disruption of numerous biochemical processes. To prevent an eminent outbreak efforts have to be made to discouraged farmers from the use of wastewater for irrigation. Awareness also should be created on the dangers of consuming vegetables irrigated with waste water.

REFERENCES

1. Abdoulaye, C., Brou, K., & Jie, C. (2011). Phytic Acid in Cereal Grains: Structure, Healthy or Harmful Ways to Reduce Phytic Acid in Cereal Grains and Their Effects on Nutritional Quality. *American Journal of Plant Nutrition and Fertilization Technology*, **1**(1): 1-22.
2. Adhikari, K .M, Sweetingham, M.W., & Buirchell, B. (2005). Yellow lupin breeding in Western Australia. Proceedings of Agribusiness Crop Updates, *Lupins and Pulses 12*–14.
3. Agbaire, P.O., & Oyewole, A. (2012). Levels of anti-nutritional factors in some common leafy edible vegetables of southern Nigeria. *Journal of Food Science Technology*, 3: 99-101.
4. Akande, F. O., & Ajayi, S. A. (2017). Assessment of Heavy Metals Level in Soil and Vegetables Grown in Peri-Urban Farms around Osun State and the Associated Human Health Risk. *International Journal of Environment, Agriculture and Biotechnology* (IJEAB), **2**(6): 2456- 1878. http://dx.doi.org/10.22161/ijeab/2.6.61.
5. Akoroda, M. O. (1990). "Ethnobotany of *Telfairia occidentalis* (Curcurbitacae) among Igbos of Nigeria." *Economic Botany*, *1*:29-39.
6. Association of Official Analytical Chemist (AOAC), (1995). Official methods of Analysis, 15th edition, Washington.
7. Awomukwu, D.A., Nyananyo, B.L., Ikpeama, A.I., & Adieze, C.U. (2015). Comparative Chemical Constituents of some Cassia Species and their Pharmacological Importance in South Eastern Nigeria. *Science Journal of Chemistry*, *3*(3), 40-49.
8. Benzie, T.F., & Choi, S.W. (2014). Antioxidants in food: content, measurement, significance, action, cautions, caveats, and research needs, and research needs. *Advances in Food and Nutrition Research*, 71, 1-53.
9. Cytryn, E. (2010). Effect of treated wastewater irrigation on antibiotic resistance in agricultural soil bacteria. Environ. Health Problem, <http://www.ehf.org.il/en/grant/effect-treated-wastewater-irrigation-antibiotic-resistance-agricultural-soil-bacteria-dr-eddie>.
10. Dei, H.K., Rose, S.P., & Mackenzie, A.M. (2007). Shea nut (*vitellaria paradoxa*) meal as a feed ingredient for poultry. *World's poult Science journal*, *63*(4), 611 – 624.
11. Elllenhom, M.J., & Barcelonx, D.G. (1988). Medical toxicology; Diagnosis and treatment of human poisoning. *Elsevier Science Publishing Co. New York, USA.*
12. Farahat, E.A., Galal, T.M., Elawa, O.E., & Hassan, L.M. (2017). Health risk assessment and growth characteristics of wheat and maize crops irrigated with contaminated wastewater. *Environmental Monitoring Assessment*, 189, 535.
13. Fowomola, M.A. (2010). Some nutrients and antinutrients components of mango (mangifera indica) seed. *African Journal of Food Science*, *4*(8), 472 – 476.
14. International Commission for the Microbiological Specifications of Foods. (1998). Microbial Ecology of Food Commodities. Microorganisms in foods. Blackie Academic and Professional.
15. Kayode, O.T., Kayode, A.A., & Odetola, A.A. (2009). Therapeutic effect of Telfairia occidentalis on protein energy malnutrition-induced liver damage. *Research Journal of Medicine and Plant*, *3*, 80-92.
16. Khaled, S.B. (2016). Microbial contamination of vegetables crop and soil profile in arid regions under controlled application of domestic wastewater. *Saudi Journal of Biological Sciences*, *23*, 83-92.

17. Laconelli, S., & Simmen B. (2002). Cite as Taste Thresholds and Suprathreshold Responses to Tannin-Rich Plant Extracts and Quinine in a Primate Species (*Microcebus murinus*), *Journal of Chemical Ecology*, *28*(11), 2315–2326.
18. Lenntech, B.V. (2014). Water treatment solutions. Delft, The Netherlands.
19. Miller-Robbie, L., Ramaswami, A., & Amerasinghe, P. (2017). Wastewater treatment and reuse in urban agriculture: Exploring the food, energy, water, and health nexus in Hyderabad, India. Environmental Res. Lett, 12, 075005. [CrossRef]
20. Nimyel, D.N., Egila, J.N., & Lohdip, Y.N. (2015). Heavy Metal Concentrations in Some Vegetables Grown in a Farm Treated with Urban Solid Waste in Kuru Jantar, Nigeria. *British Journal of Applied Science and Technol.*, ***8***(2), 139-147.
21. Nwogu, L.A., Igwe, C.U., & Emejulu, A.A. (2008). Effects of *Landolphia owariensis* leaf extract on the liver function profile and haemoglobin concentration of albino rats. *African Journal Biotechnology*, *2*(12): 240-242.
22. Oguh, C. E., Ubani, C. S., Berebon D. P., Okekeaji U., & Eze C. C. (2019b). Evaluation of Microbial loads, parasites and Antinutrient factors in *Talinum triangulare* grown on Sewage Dump Site in University of Nigeria, Nsukka Nigeria. *IOSR Journal of Environmental Science, Toxicology and Food Technology* (IOSR-JESTFT), *13*(5): 51-58. http://dx.doi.org/10.9790/2402-1305015158.
23. Oguh, C.E, Ubani C.S, Osuji C.A., & Ugwu V.C. (2019a). Heavy metal risk Assessment on the Consumption of Edible Vegetable *Talinum triangulare* Grown on Sewage Dump Site in University of Nigeria, Nsukka. *Journal of Research in Environmental Science and Toxicology*. *8*(2), 104-112. http://dx.doi.org/10.14303/jrest.2019.034.
24. Oguh, C.E., Uzoefuna, C.C., Ugwu, C.V., Ubani, C.S., Musa, A.D., & Okunowo, W.O. (2019c). Evaluation and Ecological Risk Assessment of Selected Heavy Metal Pollution of Soils and *Amaranthus cruentus* and *Telfairia occidentalis* Grown around Dump Site in Chanchaga Minna, Niger State, Nigeria. *Asian Journal of Environment & Ecology*, *10*(2), 1-16. http://dx.doi.org/10.9734/ajee/2019/v10i230114.
25. Olayemi, F.O. (2007). Evaluation of the reproductive and toxic effects of *cnestis ferruginea* (de candolle) root extract in male rats. *Journal of Pharmacology and Toxicology 33*, 46 – 51.
26. Osazee, O. J., Obayagbona, O.N., & Daniel E.O. (2013). Microbiology and physicochemical analyses of top soils obtained from four municipal waste dumpsite in Benin City, Nigeria. *Int. J. of Microbiology and mycology*, *1*(1), 23-30.
27. Pearson, D. (1976). The chemical analysis of foods. 7th edition, Churchill, livingstone, p.493.
28. Richards, L.A. (1954). Diagnosis and Improvement of Saline and Alkali Soils. U.S. Salinity Laboratory, U.S. Dept. *Agric. Hbk. 60*, pp. 160.
29. Sahu, M., & Kacholi, D.S. (2016). "Heavy metal levels in Amaranthus species from Chang'ombe-Mchicha area in Temeke District, Dar es Salaam, Tanzania,*"Asian Journal of Chemistry, 28*(5), 1123–1126.
30. Samuel, J.C., Mohammed, C.K., Joseph, K.K., & Mark, O.A. (2013). Microbial Contamination in Vegetables at the Farm Gate Due to Irrigation with Wastewater in the Tamale Metropolis of Northern Ghana. *Journal of Environmental Protection*, *4*, 676-682.
31. Tiimub, B.M., Kuffour., R.A., & Kwarteng, A.S. (2012). Bacterial Contamination Levels of Lettuce Irrigated with Waste Water in the Kumasi Metropolis. *Journal of Biology, Agriculture and Healthcare*, *2*,10.
32. WHO – World Health Organization. (2006). Guidelines for the Safe use of Wastewater, Excreta and Grey water. Volume 2.Wastewater Use in Agriculture. WHO, Geneva.
33. WHO. (1989). Health guidelines for the use of wastewater in agriculture and aquaculture. Report of a WHO Scientific Group. Geneva, (Technical Report Series, No. 778).
34. WHO. (2010). Guideline for Drinking Water Quality.3rd Edn., World Health Organization, Geneva, Switzerland.