Microbiological, Physicochemical and Heavy Metals Assessments of Soils and Selected Vegetables Grown on Rumde-Doubeli Irrigated Farmland in Yola Nigeria

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Abstract

Leaf parts of vegetable plants grown on irrigated farmlands and are easily accessed by contaminants; pose greater risks of being contaminated with heavy metals. Soil, irrigation water and edible vegetable plants were collected and evaluated for concentrations of heavy metals using Atomic Absorption Spectrophotometry (AAS) and standard laboratory methods. Physicochemical parameters of irrigation water, compared with WHO limits for chloride (326.14 $\pm$ 7.03 mg/L, 250 mg/L), total hardness (196.0 $\pm$ 8.72 mg/L, 150 mg/L), chemical oxygen demand (286.4 $\pm$1.35 mg/L; 150 mg/L) and alkalinity (171.0 $\pm$ 4.36 mg/L, 120 mg/L) were significantly ($p < 0.05$) higher than the control sites and respective reference values. Zinc (0.92 $\pm$ 0.06 mg/L, 2.00 mg/L) and biological oxygen demand (115.7 $\pm$ 6.46 mg/L, 200 mg/L, except for site C, which had 211.5 $\pm$ 1.11 mg/L, 200 mg/L) had lower values compare to the control sites and respective reference values. For the control site, the physicochemical parameters were within the permissible limits but with increase of trace metal contents in soil and consequently into the vegetables ($p < 0.05$). Microbiological analysis revealed a high count of faecal coliforms, above the WHO recommended limit. Three significant pathogens identified included *Salmonella, Klebsiella, Pseudomonas, Staphylococcus,* and *Streptococcus* species. Farmers should be enlightened on the need for hygienic water for irrigation, and proper disinfection of raw vegetables before its consideration for consumption.

Keyword: Wastewater, Heavy Metal, Pollution, Physicochemical, Irrigation, Toxicity.

Introduction

Contamination of vegetables and other plants with heavy metals and other toxic materials occurs mostly at their respective sites of harvests (Mark et al. 2019). Vegetable leaves such as *Lactuca sativa* (lettuce), *Spinacia oleracea* (spinach) and *Corchorus capsularis* (jute) are of high importance to people's diet in Nigeria as they are the major ingredients in several native soups, taken daily alongside carbohydrates. They are therefore often grown and harvested widely in urban farms, home gardens and fallow plots during the dry season in varying levels (Agbenin et al. 2009). Water is an essential component of life, but it is a susceptible and finite resource that has qualitative vulnerability and quantitative limitations (Khalid et al. 2018, Karanja et al. 2010). Many countries are struggling to

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balance water uses among municipal, industrial, agricultural and recreational purposes. In areas where fresh water is in short supply, wastewater is frequently used for plant irrigation. Although not legally permitted in most countries, the use of untreated wastewater for plant irrigation is been practiced worldwide due to the shortage of good quality water (Jaramillo and Restrepo 2017, Khalid et al. 2017). The use of wastewater for crop production has been on the increase worldwide due to the increasing food demands and the changing climatic conditions that are making crop production through rain-fed agriculture less reliable as reported by the Food and Agricultural Organization (FAO 2006).

Wastewater irrigation provides essential nutrients and organic matter, saving water and reducing water contamination (Murtaza et al. 2010). Besides these benefits, a number of drawbacks are associated with the use of wastewater for plant irrigation (Murtaza et al. 2010, Khalid et al. 2017). Wastewater contains potentially toxic elements, which can induce severe risks to human health and the environment (Mark et al. 2019, Shahid 2017). Depending on the concentrations of the metals and properties of the soil, uptake of heavy metals into plants is inevitable, which can pose toxic threats to biota, cause soil degradation and decrease plant yields (Luo et al. 2011). Mobile metals can be taken up by vegetables from the soils whilst scavenging for nutrients at varying degrees (Luo et al. 2011). Studies have shown that urban and garden soils can contain high amounts of trace metals (Charlesworth et al. 2011). These soils are therefore considered one of the notable sites for human exposure to trace metals (De Miguel et al. 2007). With regards to human exposure, soil to plant transfer represents a major pathway for contamination and eventual disease manifestation (Jolly et al. 2013). Studies have shown that chronic intake of even low levels of toxic metals over time can lead to nervous, cardiovascular, renal, and neurological impairments as well as bone diseases (Järup 2003). Therefore, the determination of the concentrations of heavy metals such as chromium (Cr), iron (Fe), lead (Pb), copper (Cu), zinc (Zn) and cadmium (Cd) and physicochemical parameters in some vegetable plants and soil at a typical irrigation site that has been on wastewater irrigation during the summer of which this study is aimed at, is of paramount importance.

Materials and Methods
Study area and sampling

The leaf parts of vegetable plants, 25 g each sample of *Lactuca sativa* (lettuce), *Spinacia oleracea* (spinach) and *Corchorus capsularis* (jute). Three (3) samples each of *Lactuca sativa* (lettuce), *Spinacia oleracea* (spinach) and *Corchorus capsularis* (jute) were collected from points A, B and C; giving a total of 75 g per sample in total from each point and soil samples were collected from three sampling points chosen, viz. A-upstream (1 g), B-midstream (1 g) and C-downstream (1 g). All samples were collected between November and December, 2014 from Rumde-Doubeli bye-pass in Jimeta/Yola, Adamawa State, Nigeria where dry season farming is normally practiced along the river bank using municipal wastewater to grow edible plants. Sampling of wastewater was carried out at a depth of 10 cm below the water surface between 8:00 and 10:00 am in the morning in line with farmer's irrigation schedules. Ten millilitres (10 mL) of ground water was collected from a farming site that uses ground water for irrigation to serve as control. All samples were collected in 500 mL sterile bottles for laboratory analysis and were immediately transported to the Microbiology Laboratory, stored in the refrigerator at a temperature of 4 °C prior to processing and further analysis.

Determination of physicochemical parameters

The temperature, pH and electrical conductivity of wastewater and groundwater samples were determined using portable hand held thermometer, Jenway digital pH meter electrical conductivity meter, respectively. Other physicochemical properties determined were alkalinity, total hardness, total solids, suspended solids, dissolved solids, dissolved oxygen (DO), chemical oxygen demand (COD), biochemical oxygen demand (BOD) and chlorides according to standards recommended by the Association of Official Agricultural Chemists (AOAC 1990).

The soil particle distribution analysis was carried out by adopting the Bouyoucos hydrometer method where the textural classification was interpreted using the textural triangle into percentage sand, silt and clay (APHA 2012). Soil pH and electric conductivity (EC) were measured in soil suspension (1:5 w/v), using a digital pH-meter and electric conductivity meter, respectively. Organic carbon was determined using the American Standard for Testing of Materials method, while organic matter content was determined according to the method described by Gupta et al. (2008). Available nitrogen was determined using the Kjeldahl method as described by APHA (2012). The method for determination of exchangeable cations was adopted from APHA (2012). Following extraction using ammonium acetate, sodium and potassium, were determined by flame photometer, while calcium and magnesium were determined by EDTA titration.

Heavy metals analysis

The soil sample was dried and crushed gently then sieved with a 2 mm mesh to separate stones, roots and gravels from the mineral soils. One gram of the sample was weighed in a 100 mL beaker and digested using the procedure recommended by Gupta et al. (2008). Water used for irrigating vegetables was digested using the procedure of APHA

(2012). The vegetables were washed with distilled water to eliminate suspended particles. The leafy stalks were removed from all samples, sliced and dried to eliminate excess moisture, then carefully blended into fine powder. A sample weighing 0.5 g of vegetable materials was taken in a 50 mL Pyrex beaker and digested using the procedure of Linkon et al. (2015).

Determination of heavy metals by atomic absorption spectrophotometry

Water, soil and vegetable filtrates were taken to the Atomic Absorption Spectrophotometer (iCE FIOS double beam, Thermo Fisher Scientific Pvt. Ltd. Mumbai, India) using air acetylene flame integrated mode for reading of the metals. The elements analysed by the AAS were; chromium (Cr), iron (Fe), lead (Pb), copper (Cu), zinc (Zn) and cadmium (Cd). The calibration curves were prepared separately for all the metals by running different concentrations of standard solutions. The instrument was set to zero by running the respective reagent blanks. The AAS was calibrated using standards for each heavy metal. Each element was read using specific lamps depending on the elements. For samples reading higher than the highest standard, a dilution was made and used to bring to volume. The standard solutions were prepared immediately before analyses to avoid adsorption of metals by the glass containers and decomposition. Also, the same instrumental conditions were used to run all the samples at a time.

The samples (digest of wastewater, soil and vegetables) were aspirated into the nebulizer through a capillary tube where they were converted into aerosol before entering the atomizer. The respective concentrations were determined from the linear calibration curve for each metal. Analysis of each sample was carried out three times to obtain representative results. The standard solutions were run after each 6 samples, to check signal drift. When the relative error increased above 5%, the instrument was recalibrated by running the standards (Mahar et al. 2015). A

blank sample was read and the value of the blank was used in correcting the readings of the samples. After the concentrations of the samples have been read on AAS, calculations were then made for the elements in the original sample.

Microbiological analyses
Soil samples from irrigated fields
All media used were prepared according to manufacturer's specifications. One gram each of contaminated and control soil sample were serially diluted in to 10 fold, 7 series. Zero point one (0.1) mL aliquot from 10^{-4}, 10^{-5} and 10^{-7} dilutions were inoculated in plate count agar (PCA) and eosin methylene blue (EMB) agar by spread plate method in triplicate plate for total count and fecal coliforms count, respectively. PCA plates were incubated at 37 °C for 24 hours while EMB plates were incubated overnight at 44.5 °C for faecal coliforms due to the presence of some indispensable thermotolerant genes expressed at high temperature as described by Ofor et al. (2009). Bacterial counts in soil samples were determined by direct plate count after incubation, and culture responses expressed as colony forming units per gram of soil (CFU/g).

Vegetables
Twenty five grams of each vegetable sample was soaked in 100 mL distilled water for 15 minutes and washed by shaking thoroughly. One millilitre each of the rinsed water was serially diluted in 10 fold, 5 series. Zero point one mL each from 10^{-2}, 10^{-4} and 10^{-5} dilutions was inoculated as described for the soil samples. Colonies were then counted using colony counter and expressed as colony forming unit per gram of vegetable.

Irrigation water
An aliquot (1 ml) of irrigation water was transferred into 9 mL of distilled water and diluted serially in ten folds up to 7 series. Zero point one millilitre (0.1 mL) aliquots of 10^{-4}, 10^{-5} and 10^{-7} dilutions were inoculated

in triplicate plates of PCA and EMB agar using the spread plate method. PCA plates were incubated at 37 °C for 24 hours for the enumeration of total heterotrophic bacteria while EMB plates were incubated at an elevated temperature of 44.5 °C for 24 hours to isolate faecal coliforms. After an appropriate incubation period, colonies which developed on the plates were counted, recorded and calculated as colony forming unit per millilitre (CFU/mL) of water sample.

Data analysis
The generated data were processed using Microsoft excel 2007 software package and analysed by one way analysis of variance (ANOVA). The results were presented in mean $\pm$ standard deviation.

Results
Physicochemical parameters and of wastewater
The physicochemical parameters of water samples from the study and control areas are presented in Table 1. The pH of wastewater samples in the study area ranged from 7.30 $\pm$ 0.88 (sample C) – 7.70 $\pm$ 0.43 (sample A). The average pH value of water samples in the control area was 6.98$\pm$0.87, hence below those of the study area. The conductivity values recorded across the wastewater irrigated points ranged from 803.0 $\pm$ 6.2461 µs/cm (sample C) to 1016 $\pm$ 3.61 µs/cm (sample A). All conductivity values recorded across the three sampled wastewaters sources were significantly higher (p < 0.05) than that of ground water (130.0 $\pm$ 7.00) in the control site. The total hardness recorded ranged between 178.7 $\pm$ 7.85 and 196.0 $\pm$ 8.72 mg/L, which were higher than the value of 64.11 $\pm$ 3.61 obtained in ground water (p < 0.05). The chloride levels obtained in all the wastewater samples were in general above the recommended limit for irrigation water (Table 1). The chloride values recorded from wastewater ranged from 290.7 $\pm$ 2.52 to 326.14 $\pm$ 7.03 mg/L. The chloride value at each sampling point was higher than 78.03 $\pm$

1.02 mg/L recorded in ground water. The BOD values recorded across the sampled wastewater sources ranged from 110.0 ± 6.14 to 211.5 ± 1.11 mg/L, and they were all significantly higher than the BOD recorded in pipe water (45.7 ± 3.11 mg/L) ($P < 0.05$). The mean BOD values of the wastewater samples were within the recommended limit of 200 mg/L except for sample C. The values of COD recorded across the wastewater sampling points ranged between 285.6 ± 5.40 and 288.4 ± 2.59 mg/L and were higher than the value recorded in ground water (140.8 ± 2.75 mg/L).The results for heavy metal concentrations in water samples from both the study and control areas for different sampling points are also presented in Table 1.

The concentrations of trace metals in the wastewater samples were found to be in the following order Zn > Cu > Pb > Cd. Cadmium values recorded from the three wastewater sources ranged between 0.06 ± 0.08 mg/L (sample A) and 0.58 ± 0.07 mg/L (sample C), and were higher than that of ground water (not detected), as well as 0.01 mg/L the WHO limit. Zinc was detected in all the sampling points and had the highest concentration of 0.92 ± 0.06 mg/L in sample C. Also, the zinc values recorded across the three wastewater sampling points were not significantly different to that recorded in ground water. More so, the zinc values from each point did not exceed 2.0 mg/L the WHO tolerable limit.

Table 1: Physicochemical properties of wastewater samples

Parameter	A (n = 3)	B (n = 3)	C (n = 3)	Ground water (n = 3)	WHO limits
Temperature (°C)	26.3 ± 0.70[c]	26.2 ± 0.95[c]	27.9 ± 0.92[b]	29.5 ± 1.8[a]	–
pH	7.70 ± 0.43[a]	7.50 ± 0.36[a]	7.30 ± 0.88[a]	6.98 ± 0.87[a]	6.5–8.5
Conductivity (μs/cm)	1016 ± 3.61[a]	900 ± 41.76[b]	803.0 ± 6.24[c]	130.0 ± 7.00[d]	–
Total hardness (mg/L)	185.2 ± 10.06[a]	178.7 ± 7.85[a]	196.0 ± 8.72[a]	64.11 ± 3.61[b]	150
Alkalinity (mg/L)	171.0 ± 4.36[a]	168.0 ± 9.54[a]	152.0 ± 4.58[b]	35.00 ± 3.61[c]	120
Dissolved oxygen (mg/L)	8.25 ± 0.43[b]	10.30 ± 0.82[a]	11.65 ± 1.00[a]	6.10 ± 0.26[c]	5–14
Biological oxygen demand (mg/L)	110.0 ± 6.14[b]	115.7 ± 6.46[b]	211.5 ± 1.11[a]	45.7 ± 3.11[c]	200
Chemical oxygen demand (mg/L)	285.6 ± 5.40[a]	288.4 ± 2.59[a]	286.4 ± 1.35[a]	140.8 ± 2.75[b]	150
Chloride (mg/L)	326.14 ± 7.03[a]	312.0 ± 1.04[b]	290.7 ± 2.52[c]	78.03 ± 1.02[d]	250
Cadmium (mg/L)	0.06 ± 0.08[b]	0.12 ± 0.03[b]	0.58 ± 0.07[a]	nd[b]	0.01
Zinc (mg/L)	0.86 ± 0.09[a]	0.83 ± 0.03[a]	0.92 ± 0.06[a]	0.60 ± 0.23[a]	2.00
Lead (mg/L)	0.12 ± 0.03[a]	0.05 ± 0.09[b]	0.06 ± 0.10[b]	nd[c]	0.01
Copper (mg/L)	0.45 ± 0.26[b]	0.32 ± 0.11[c]	0.89 ± 0.09[a]	0.07 ± 0.04[d]	0.02

Note: Values are mean ± standard deviation; Alphabet; a, b, c, d that differed from others indicated significant difference among water sources ($p < 0.05$). Wastewater Sources: {A: Upstream, B: Midstream; C: Downstream}; nd = not detected and each parameter was analysed in triplicate (n = 3).

Physicochemical properties of soils

Table 2 reveals the physicochemical properties of soils from wastewater irrigated lands and control sites. The textural class was loam in sample A and B and clay loam in sample C, while the control soil had clay soil. The pH values recorded across the

wastewater irrigated soil ranged from 5.67 ± 0.27 to 5.90 ± 0.6 and was not significantly different from the WHO limit of pH in ground water irrigated soil. The electrical conductivity (EC) values recorded across the farms of the study area ranged from 0.06 ± 0.03 to 0.08 ± 0.03 dS/m and were not

different from the EC values observed in the control area ($p < 0.05$). The highest EC value of 0.08 ± 0.03 dS/m was recorded in sample A, while the lowest value of 0.06 ± 0.03 dS/m occurred in sample C. The values of organic carbon from the study site ranged from 0.81 ± 0.04 to $0.92 \pm 0.21\%$ and were significantly higher than the control soil ($p < 0.05$). More so, the values of organic matter recorded across the wastewater irrigated soil ranged from 1.39 ± 0.08 to $1.59 \pm 0.36\%$,

which were significantly higher than $0.36 \pm 0.03\%$ recorded for soil irrigated with ground water ($p < 0.05$). Similarly, the available nitrogen recorded across the wastewater irrigated farms ranged from 1.68 ± 0.21 to $2.36 \pm 0.51\%$ which were higher than $0.36 \pm 0.19\%$ recorded in the ground water irrigated farms ($p < 0.05$). The values of exchangeable bases from wastewater irrigated soil were found to be in the following order $K^+ > Ca^{2+} > Mg^{2+} > Na^+$ (Table 2).

Table 2: Physicochemical properties of soil from wastewater irrigated farms and control sites

Parameter	A (n = 3)	B (n = 3)	C (n = 3)	WHO Limits
Sand (%)	38.8 ± 1.67^b	35.2 ± 2.19^c	35.2 ± 0.10^c	44.2 ± 0.62^a
Clay (%)	24.0 ± 2.65^c	26.0 ± 2.65^c	29.0 ± 2.65^b	41 ± 2.65^a
Silt (%)	37.2 ± 0.44^a	38.8 ± 1.15^a	35.8 ± 1.18^b	14.8 ± 1.43^c
Textural Classes	Loam	Loam	Clay loam	Clay
pH	5.90 ± 0.63^a	5.67 ± 0.27^a	5.70 ± 0.44^a	6.05 ± 0.23^a
Electrical conductivity (dS/m)	0.08 ± 0.03^a	0.07 ± 0.03^a	0.06 ± 0.03^a	0.04 ± 0.03^a
Organic carbon (%)	0.90 ± 0.22^a	0.81 ± 0.04^a	0.92 ± 0.21^a	0.21 ± 0.02^b
Organic matter (%)	1.55 ± 0.37^a	1.39 ± 0.08^a	1.59 ± 0.36^a	0.36 ± 0.03^b
Available nitrogen (%)	1.82 ± 0.29^b	1.68 ± 0.21^b	2.36 ± 0.51^a	0.36 ± 0.19^c
Calcium (cmol/kg)	12.16 ± 1.53^a	13.36 ± 0.04^a	11.28 ± 0.29^a	8.80 ± 0.36^b
Magnesium (cmol/kg)	6.32 ± 0.43^a	5.12 ± 0.31^a	5.28 ± 0.04^a	2.72 ± 0.49^b
Sodium (cmol/kg)	3.89 ± 0.32^a	3.83 ± 0.42^a	3.75 ± 0.03^a	2.30 ± 0.09^b
Potassium (cmol/kg)	21.50 ± 0.63^b	20.90 ± 0.31^a	16.90 ± 0.21^c	8.28 ± 0.09^d
Total exchangeable acidity (cmol/kg)	2.28 ± 0.41^a	2.40 ± 0.05^a	2.24 ± 0.28^a	1.40 ± 0.44^b

Note: Values are mean ± standard deviation; Alphabets; a, b, c, d that differed from others indicated significant difference ($p < 0.05$). Soil sources: {A: Upstream, B: Midstream; C: Downstream}, and each parameter was analysed in triplicate (n = 3).

Heavy metals status of wastewater irrigated farmlands

Table 3 reveals the elemental distribution in wastewater from both the study and control areas. The concentrations of cadmium and lead in all the wastewater samples from the study sites were significantly higher ($p < 0.05$) than in samples from the control site. Both chromium and lead were not detected in

the control plots. The mean concentrations of Cd in all the wastewater from the study area were outside the recommended limits as established by WHO, while Fe, Zn and Cu were well within the WHO tolerable limits. However, the levels of metals in wastewater of the control area were all within the normal range for metals in plant leaves.

Table 3: Heavy metals status of wastewater irrigated farmlands and control site (concentration in mg/kg)

Heavy metal	A (n = 3)	B (n = 3)	C (n = 3)	Control soil (n = 3)	FAO limits
cadmium	8.90 ± 0.12^a	9.50 ± 1.14^a	10.33 ± 1.11^a	4.34 ± 0.12^b	3 .0
Zinc	62.80 ± 2.05^c	84.39 ± 0.18^a	74.37 ± 1.07^b	28.92 ± 0.03^d	300
Lead	6.86 ± 0.88^a	8.63 ± 0.43^a	7.18 ± 0.56^a	3.50 ± 0.48^b	300
Copper	12.47 ± 0.61^c	15.39 ± 0.05^b	21.20 ± 1.09^a	11.32 ± 0.08^c	135

Note: Values are mean ± standard deviation; Alphabets a, b, c, d that differed from others indicated significant difference among sources (p < 0.05). Soil sources: (A: Upstream, B: Midstream; C: Downstream), and each parameter was analysed in triplicate (n = 3).

Heavy metals contents of vegetable crops

Table 4 reveals the elemental distribution in vegetables (lettuce, spinach and jute) from both the study and control areas. The concentrations of chromium (5.41 ± 0.44 mg/kg) and lead (2.61 ± 0.31 mg/kg) in all the vegetable samples from the study sites were significantly higher (p < 0.05) than the control site (not detected). Both chromium and lead were not detected in the control plots. The mean concentrations of Cr in all the vegetables from the study area were outside the recommended limits as established by WHO, while Fe, Zn and Cu were well within the WHO tolerable limits. However, the levels of metals in vegetables of the control area were all within the normal range for metals in plant leaves.

Table 4: Elemental contents of vegetables from wastewater irrigated farms (mg/kg)

Vegetables	Chromium	Iron	Cadmium	Zinc	Lead	Copper
Lettuce						
A	5.41 ± 0.44^a	228.7 ± 15.6^a	0.32 ± 0.06^a	31.90 ± 1.09^b	2.61 ± 0.31^a	30.2 ± 1.34^a
B	5.10 ± 0.44^a	187.5 ± 7.52^b	0.16 ± 0.09^b	38.81 ± 0.90^a	0.04 ± 0.07^b	20.1 ± 0.36^b
C	4.81 ± 1.04^a	139.5 ± 0.54^c	0.14 ± 0.08^b	29.14 ± 0.88^b	0.09 ± 0.15^b	28.8 ± 0.55^a
Control	0.00 ± 0.00^b	138.1 ± 4.24^c	0.09 ± 0.04^b	12.60 ± 1.37^c	0.00 ± 0.00	8.50 ± 1.06^c
Spinach						
A	5.81 ± 1.13^a	121.1 ± 1.11^a	0.42 ± 0.03^a	29.48 ± 0.09^a	0.24 ± 0.14^a	28.7 ± 0.95^a
B	4.73 ± 0.96^a	118.3 ± 1.34^a	0.39 ± 0.02^b	32.72 ± 1.03^a	0.07 ± 0.13^b	27.6 ± 0.29^a
C	5.75 ± 0.69^a	87.52 ± 0.02^b	0.28 ± 0.01^c	31.33 ± 1.04^a	0.14 ± 0.13^a	21.2 ± 1.16^b
Control	0.00 ± 0.00^b	89.63 ± 0.03^b	0.03 ± 0.05^d	23.20 ± 1.00^b	0.00 ± 0.00	14.3 ± 1.30^c
Jute						
A	5.13 ± 0.69^a	9.40 ± 0.29^a	0.03 ± 0.05^a	28.73 ± 0.79^a	0.21 ± 0.04^a	28.9 ± 0.99^a
B	2.84 ± 0.13^b	8.60 ± 1.24^a	0.01 ± 0.02^a	30.85 ± 0.04^a	0.06 ± 0.11^b	26.8 ± 1.27^a
C	4.68 ± 0.33^a	8.10 ± 0.99^a	0.01 ± 0.02^a	30.10 ± 0.98^a	0.00 ± 0.00	28.4 ± 1.01^a
Control	0.00 ± 0.00^c	6.60 ± 0.40^b	0.02 ± 0.03^a	12.80 ± 0.66^c	0.00 ± 0.00	15.2 ± 0.07^b
WHO limits	0.30	425.00	0.10	100.00	0.30	73.00

Note: Values are mean ± standard deviation; Alphabets a, b, c, d that differed from others indicated significant difference (p < 0.05).

Bacterial loads of soil, wastewater and vegetables tested

The bacteria loads and faecal coliform counts of the vegetables and wastewater tested are presented in Table 5. The highest heterotrophic counts range was recorded in wastewater ($9.8 \times 10^6 – 4.8 \times 10^9$) followed by lettuce ($8.5 \times 10^4 – 4.8 \times 10^7$), while soil ($2.46 \times 10^6 – 6.2 \times 10^9$) had the lowest. Similarly, wastewater ($4.4 \times 10^6 – 3.1 \times 10^9$) had the highest content of faecal coliform counts range, followed by lettuce ($3.5 \times 10^4 – 3.3 \times 10^7$), while soil ($1.22 \times 10^6 – 3.6 \times 10^9$) had the lowest value. Meanwhile, no

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significant (p < 0.05) growth was observed in the control samples.

Table 5: Total bacteria and faecal coliform counts from wastewater and vegetables from waste irrigated farm (CFU/mL)

Samples	Total heterotrophic count	Faecal coliforms count
Soil	$2.46 \times 10^6 - 6.2 \times 10^9$	$1.22 \times 10^6 - 3.6 \times 10^9$
Control	$1.80 \times 10^6 - 3.3 \times 10^9$	$0.0 \times 10^9 - 3.0 \times 10^6$
Wastewater	$9.8 \times 10^6 - 4.8 \times 10^9$	$4.4 \times 10^6 - 3.1 \times 10^9$
Control	$3.2 \times 10^6 - 3.4 \times 10^9$	NSG
Lettuce	$8.5 \times 10^4 - 4.8 \times 10^7$	$3.5 \times 10^4 - 3.3 \times 10^7$
Control	$5.0 \times 10^4 - 3.6 \times 10^7$	$0.0 \times 10^7 - 3.2 \times 10^4$
Spinach	$8.0 \times 10^4 - 4.2 \times 10^7$	$3.1 \times 10^4 - 3.0 \times 10^7$
Control	$4.2 \times 10^4 - 4.4 \times 10^7$	NSG
Jute	$7.0 \times 10^4 - 4.3 \times 10^7$	$3.2 \times 10^4 - 3.2 \times 10^7$
Control	$5.3 \times 10^4 - 3.2 \times 10^7$	NSG

Key: NSG = No Significant Growth

Occurrence and identity of bacteria isolates on vegetable plants

Occurrence and identities of bacterial species isolated in the vegetable samples are represented in Table 6. A total of 19 different bacterial species were isolated from the vegetables analysed. Notable isolates included *Escherichia coli, Micrococcus luteus, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris, Shigella sonnei,* and *Klebsiella pneumoniae* which are notable causes of infections. Table 6 also shows that lettuce had the highest number (28) of isolates followed by spinach (23), while jute (13) had the least number of isolates.

Table 6: Occurrence and identity of bacteria isolates on vegetable plants

Isolates	Lettuce	Control lettuce	Spinach	Control spinach	Jute	Control jute
Escherichia coli	4 (14.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Salmonella sp.	2 (7.1%)	0 (0%)	0 (0%)	0 (0%)	2 (15.4%)	0 (0%)
Micrococcus luteus	2 (7.1%)	2 (14.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Staphylococcus aureus	5 (17.9%)	3 (21.4%)	5 (21.7%)	3 (25%)	0 (0%)	0 (0%)
Pseudomonas aeruginosa	5 (17.9%)	0 (0%)	4 (17.4%)	0 (0%)	2 (15.4%)	0 (0%)
Enterobacter aerogenes	3 (10.7%)	3 (21.4%)	3 (13%)	2 (16.7%)	2 (15.4%)	1 (16.7%)
Proteus vulgaris	0 (0%)	2 (14.3%)	2 (8.7%)	3 (25%)	2 (15.4%)	0 (0%)
Klebsiella pneumoniae	0 (0%)	2 (14.3%)	2 (8.7%)	0 (0%)	0 (0%)	0 (0%)
Bacillus subtilis	0 (0%)	0 (0%)	2 (8.7%)	2 (16.7%)	2 (15.4%)	2 (33.3%)
Bacillus cereus	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Streptococcus faecalis	3 (10.7%)	0 (0%)	2 (8.7%)	0 (0%)	0 (0%)	0 (0%)
Shigella sonnei	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Yersinia enterocolitica	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Bacillus megaterium	2 (7.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Staphylococcus epidermidis	2 (7.1%)	0 (0%)	3 (13%)	0 (0%)	3 (23.1%)	2 (33.3%)
Serratia marcescens	0 (0%)	2 (14.3%)	0 (0%)	0 (0%)	0 (0%)	1 (16.7%)
Aeromonas hydrophilia	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Citrobacter sp.	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Proteus mirabilis	0 (0%)	0 (0%)	0 (0%)	2 (16.7%)	0 (0%)	0 (0%)
Total	28 (100%)	14 (100%)	23 (100%)	12 (100%)	13 (100%)	6 (100%)

Discussion

The physicochemical parameters of water samples from study and control areas are presented in Table 1. pH is important in determining the corrosive nature of water (Gupta et al. 2009). The wastewater was slightly alkaline, while the control water was near neutral in this study. The pH of wastewater samples in the study area ranged from 7.30 ± 0.88 (sample C) to 7.70 ± 0.43 (sample A). However, pH value of water samples in the control area was 6.98 ± 0.87 and hence the value was below those of the study area. The pH range observed for wastewater samples was similar to that obtained by Hong et al. (2014). However, Ogunfowokan et al. (2005) and Akan et al. (2008) reported lower pH range of 5.23–6.32 and higher pH values of 8.94–10.34 for wastewater effluents in Ile–Ife and Jakara both in Nigeria. Low pH values obtained might be due to the high levels of free CO_2 which may consequently favour the growth of indicator and pathogenic bacteria thereby affecting the bacterial counts (Pavendan et al. 2011). The chloride values recorded from wastewater ranged from 290.7 ± 2.52 to 326.14 ± 7.03 mg/L. The chloride value at each sampling point was higher than 78.03 ± 1.02 mg/L recorded for ground water. The chloride content of wastewater was quite high and hence above the recommended limit for irrigation water; this indicated that wastewater samples were polluted, since excess of chloride in water is usually taken as an index of pollution (Prasanth et al. 2012). In a similar work, Das and Acharya (2003) also reported very high content of chloride in the range of 294–311 mg/L.

The required oxygen demand expressed as BOD and COD are important parameters for the evaluation of wastewater. The BOD recorded across the sampled wastewater sources ranged from 110.0 ± 6.14 to 211.5 ± 1.11 mg/L, and were significantly higher than 45.7 ± 3.11 mg/L the BOD recorded in ground water (p < 0.05). The mean BOD values of the wastewater samples were within the recommended limit of 200 mg/L except for sample C. COD value is often greater than BOD value (Salem et al. 2011). This was also observed in this study. A low BOD is an indicator of good quality water, while high BOD indicates polluted water (Pal et al. 2016). High BOD and COD concentrations observed in wastewater might be due to elevated levels of organic pollutants (Salem et al. 2011).

Table 2 shows the physicochemical properties of soils from wastewater irrigated lands and control sites. The textural class was loam in sample A and B and clay loam in sample C, while the control soil had clay soil. These textural classes were accounted for by the low clay contents. The values of organic carbon from the study site ranged from 0.81 ± 0.04 to $0.92 \pm 0.21\%$ and were significantly higher than $0.21 \pm 0.02\%$ (p < 0.05) obtained in the control soil. Therefore, soil organic carbon was higher in wastewater irrigated farms than the control site, indicating that the farms were different in nutrient status which would require different farm management practices especially with respect to fertilizer applications. The concentrations of the exchangeable cations were higher at the study sites than the control site, which indicated that wastewater could be implicated with the amendments of soil properties of the study area. In this study, electrical conductivity, organic carbon, organic matter content, available nitrogen and exchangeable cations under wastewater irrigation conditions were higher in the study area than the control site. This agreed with Singh et al. (2012) where they observed that wastewater irrigation increased available nitrogen, potassium and organic carbon content of soil compared to well water irrigated soil.

The wastewater was contaminated with Cr, Zn, Pb and Cu and the concentrations of these heavy metals varied widely (Table 3). Cadmium values recorded from the three wastewater sources ranged between 8.90 ± 0.12 mg/kg (sample A), 9.50 ± 1.14 mg/kg (sample B) and 10.33 ± 1.11 mg/kg (sample

C), and were higher than that of ground water. The values of lead in wastewater were higher than that of ground water as well as the recommended WHO limit (p < 0.05). With reference to WHO standards for irrigation water, the heavy metals content in wastewater exceeded the safe limits specified except for Zn, while those of ground water in the control site were all within the tolerable limits. In agreement with this study, Rattan et al. (2005) reported higher concentrations of heavy metals in sewage effluents as compared to ground water. The average values of metals found in this study were lower than the corresponding values reported for wastewater channel (Zn, 7.45; Cu, 3.58; Pb, 5.23 and Cd, 2.87 mg/L, Akan et al. 2008) and (Pb, 1005-1818; Cu, 285–1390 and Cd, 290–310 mg/L; Dike et al. 2004) both in Nigeria. Most of these heavy metals have been implicated in poisonous drugs exhibiting high toxicity to human systems. Lead particularly affects the central nervous system; therefore toxicity test for plant based drugs cannot be overruled. All the studied heavy metals were found to be present in the soils in all the sites (Table 3). The cadmium levels recorded across the wastewater irrigated soils ranged from 8.9 ± 0.12 to 10.33 ± 1.11 mg/kg exceeding the FAO threshold limit of 3.0 mg/kg and was significantly higher than 4.34 ± 0.12 mg/kg cadmium value recorded in ground water irrigated soil (p < 0.05). The observed high cadmium concentrations in this study revealed a potential hazard of cadmium exposure for crops grown on this soil. Khan et al. (2010) also affirmed and reported high cadmium concentrations at low pH. Thus, pH is a key parameter controlling heavy metal transfer behaviour in soils. The concentrations of zinc, lead, and copper in the farms were below the threshold limits for agricultural soil contamination levels recommended by FAO (2006). Continuous cultivation and regular absorption by plants, possibly keep the concentrations of this heavy metals within safe limits. In agreement

with this study, Singh et al. (2010) observed that continuous application of sewage water to soil led to higher concentrations of heavy metals in the soil at wastewater irrigated sites as compared to clean water irrigated sites.

The concentration of heavy metals in wastewater irrigated vegetables were significantly higher (p < 0.05) than ground water irrigated vegetables in the control site (Table 4). This may be attributed to the higher concentrations of these metals in wastewater irrigated soils as compared to the control soils. The levels of metals in vegetables of the study area were within the normal range, except for chromium and cadmium in lettuce and spinach which had values beyond the WHO recommended threshold limits. However, the levels of metals in vegetables of the control area were all within the normal range for metals in plant leaves. The mean concentrations of cadmium, zinc, lead and copper in wastewater irrigated vegetables of the study area were higher than the values (Cd 0.059 and Cu 2.263 mg/kg) reported by Dike et al. (2005), but lower than the values (Zn 76.63 and Pb, 22.59 mg/kg) reported by Chiroma et al. (2003) in similar works. Cadmium is a notable contaminant and made concentrations that were significantly above the critical levels in lettuce and spinach, and thus might be a threat for the consumers. According to Nabulo et al. (2012), cadmium is mobile and readily available for uptake by vegetables explaining its accumulation in soil and plant parts. High concentrations of cadmium in the body exert detrimental effects on human health and cause severe diseases such as tabular growth, kidney damage, cancer, diarrhoea and vomiting (Abbas et al. 2010). Differences in metals accumulation are attributed to the differences in absorption strength and species of the vegetables despite the length of exposure (Tan et al. 2011). At high concentrations, all heavy metals have strong toxic effects on plants which results in weak growth, yield depression, disorders in

plant metabolism and reduced nutrient uptake (Ibrahim et al. 2014).

Bacteria on soil, water and vegetables from the study and control sites were enumerated mainly to assess levels of pre-harvest contamination primarily total aerobic count and faecal coliforms. Wastewater irrigated fields had comparatively higher bacteria counts than the control field irrigated with clean water. This may be because the effluent contains some nutrients which can be utilized by microorganisms in the contaminated soil that are lacking in the control soil, hence, the high microbial counts (Rabah et al. 2010). Irrigation water showed considerable variations in total bacteria and faecal coliform concentrations. Microbial loads of effluent determine the extent of contamination of the wastewater and its related environmental and public health threats. The higher counts showed by wastewater samples are an indication that the irrigation water serves as the potential source of contamination. These results therefore, reflect the exposure of the vegetables to contamination during irrigation and in particular, the existence of favourable conditions for the multiplication of microorganisms. Such contamination if not well treated could be a source of infections to consumers considering the fact that vegetables are used as raw food sources. The poor quality of wastewater in the study area may be due to anthropogenic activities such as the disposal of human and animal waste into drains, open defecation around the vegetable farms and runoffs of fresh manure used to amend soils (Cobbina et al. 2013). There is no epidemiological evidence that higher heterotrophic plate count (HPC) populations have any public health significance, some have argued that lower HPC bacterial populations in drinking water are more desirable than higher populations. The samples with low bacterial and faecal coliform counts could be considered to be of better quality. The presence of faecal coliform is an index of the bacteriological quality of water (WHO 2006). Faecal coliform bacteria have a strong correlation with faecal contamination of water from warm blooded animals (Chigor et al. 2012). The wastewater sample did not meet the recommended limit for faecal coliform bacteria in unrestricted irrigation of crops likely to be eaten raw of 10^3 to 10^5 (WHO 2006).

All vegetables sampled from the study site recorded high levels of total bacteria and faecal coliforms which indicated that microorganisms are abundant on the surface of vegetables. These high counts could be attributed to the water used in irrigation, soil and other environmental contaminations. High levels of total bacteria and coliforms in vegetables have been reported by Nma and Oruese (2013), Bukar et al. (2010) and Seow et al. (2012). Most of the vegetable samples from the study site showed high faecal coliform contamination levels ranging from 1.22×10^6 to 3.6×10^9 CFU/g (irrigated soil), 3.1×10^4 to 3.0×10^7 CFU/g (spinach), 3.2×10^4 to 3.2×10^7 (jute), 3.5×10^4 to 3.3×10^7 (lettuce) to 4.4×10^6 to 3.1×10^9 CFU/g (wastewater). The highest level of faecal coliform contamination was recorded in lettuce, which may be due to the larger surface area exposed to possible sources of contamination. However, vegetables irrigated with potable water showed lower contamination levels and hence would pose minimal health risks to farmers and consumers. The obtained results are in conformity with the report of Abdullahi and Abdulkareem (2010) with value ranges of 7.9 x 10^7–1.6 x 10^8 CFU/g; 7.2 x 10^5–1.20 x 10^8 CFU/g and Omotayo et al. (2017) with range of 2.51 x 10^3–1.31 x 10^8 CFU/g, where they reported high coliforms counts in vegetables from different parts of Nigeria. There were differences in the total bacterial and faecal coliforms counts between the samples in the study and the control site. This indicated that there were indeed effects of the wastewater and contaminated soil on the quality of the vegetables studied. Contrary to the findings

of this study however, Soriano et al. (2000) reported lower faecal coliform results in fresh vegetables (< 3 to1.1×10^4).

The presence and abundance of *Bacillus* species observed in both contaminated and control soil may not be surprising as these bacteria are indigenous to soil and are known to persist in such environment (Rabah et al. 2010). However, the presence of *Escherichia coli, Streptococcus faecalis, Enterobacter aerogenes* and *Salmonella* species in the contaminated vegetables could be attributable to the high loads of excreta in the soil and wastewater containing these organisms being used for irrigation in the study site. It is also an indication of recent faecal pollution. The presence of these organisms is a pointer to possible pollution and may have effects on the soil ecological balance and quality of vegetables used by consumers. These findings are in conformity with those of Adesemoye et al. (2006) as well as Ogbonna and Igbenijie (2006). The presence of *Pseudomonas aeruginosa* and *Klebsiella pneumonie* in soil and wastewater in this study was not surprising; the previous study by Tuméo et al. (2008) gave the same report. Bala (2006) reported the isolation of similar organisms from water sources in Jimeta-Yola that were faecally contaminated. The presence of *Staphylococcus* and *Enterobacter aerogenes* in the control water samples is unacceptable from the public health point of view, because these organisms could be pathogenic.

All vegetables sampled from the wastewater irrigated site recorded high levels of coliforms bacteria and varieties of other pathogenic microorganisms. The source of vegetables contamination seems to arise primarily from wastewater used in irrigation. The transported pathogens from wastewater may survive in soil and crops which will in turn be transported to consumers and may potentially be responsible for numerous diseases (Halablab et al. 2011). The results of this study therefore support the previous findings that irrigation of vegetables with

contaminated water could result in their contamination (Afolabi and Oloyede 2010). Diseases associated with enteric bacteria ranged from bacteria that cause mild to life threatening gastroenteritis, hepatitis, skin infections, wound infections, conjunctivitis, respiratory infections, and generalized infections (Adebayo-Tayo et al. 2012). Indiscriminate open defecation is a common practice in the study area and this could be a source of faecal coliform contamination.

The bacteria isolated in the vegetables have previously been isolated in similar studies in Nigeria and elsewhere (Wogu and Iwezeua 2013, Coniglio et al. 2016, Uzeh et al. 2009). Some of the bacteria isolated may be part of the natural flora of the vegetables or contaminants from soil and irrigation water (Ofor et al. 2009). The occurrence of *Bacillus subtilis* in vegetables have been reported as being part of the natural flora and are among the most common vegetable spoilage bacteria (Dada and Makinde 2015), though some are capable of causing food borne illness. *Pseudomonas aeruginosa* isolated from majority of the samples is majorly found in water, soil and plant surfaces. It is responsible for angular leaf spot disease of many vegetables. They are also associated with spoilage of vegetables. *P. aeruginosa* is a frequent cause of nosocomial infections such as pneumonia, urinary tract infections (UTIs), and bacteremia (Flores-Mireles et al. 2015).

The high frequency of *Staphylococcus* sp. is possibly because they are present as normal flora of humans and contaminate the vegetables as a result of poor hygiene and unsatisfactory sanitation. *S. aureus* isolated is an opportunistic pathogen and enterotoxigenic strains are known to cause serious food borne diseases (Akinyele et al. 2013). They also cause boils, abscesses, post-operative infections, toxic shock syndrome and food poisoning in man. The isolation of *E. coli, Klebsiella, Serratia* and *Salmonella spp.* coupled with high coliform counts recorded in this study suggested gross faecal

contamination of the vegetables. These poses food safety problems since they are enterotoxigenic and cause gastroenteritis (Ameko et al. 2012). Despite the high microbial counts obtained for some of the vegetable samples in this study, it is important to note that these vegetables did not show any visible signs of spoilage. Thus outward appearance may not be a good criterion for judging the microbial quality of vegetables.

Conclusion

The physicochemical analysis of the study area that uses wastewater for irrigation revealed that in most cases the values were significantly higher than the threshold limits as well as the control site irrigated with ground water, and thus, it can be concluded that the study area received more pollution loads than the control area. The study further showed that vegetables grown in sewage water could be contaminated with variable amounts of microorganisms and heavy metals above the World Health Organization (WHO) maximum limits.

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