https://dx.doi.org/10.4314/ijs.v21i1.9

HEAVY METAL CONCENTRATION AND BACTERIAL CONTAMINATION ASSOCIATED WITH SELECTED LEAFY VEGETABLES IN ABEOKUTA METROPOLIS, SOUTHWEST NIGERIA

***[1]Oyebanji, F. F., [2]Ogunyemi, I. A., [3]Ojekunle, Z. O., [4]Ogundipe, O., [5]Sosanya, O. and [6]Aroyeun, T. H.**

Department of Environmental Management and Toxicology, Federal University of Agriculture, Abeokuta
Email addresses of authors:*[1]oyebanjiff@funaab.edu.ng, [2]essackyy2k3@yahoo.com, [3]oojekunle@yahoo.com, [4]Oluwasogodami@gmail.com, [5]sosanyafemi@gmail.com and [6]aroyeunth@funaab.edu.ng
*Corresponding Author's email: oyebanjiff@funaab.edu.ng

(Received: 2nd July, 2018; Accepted: 22nd December, 2018)

ABSTRACT

The assessment of heavy metal concentration (Cu, Fe, Zn, Pb, Cd, Co and Cr) and bacteriological contamination in five (5) most consumed vegetables Talinum triangulare, Celosia argentea, Vernonia amygdalina, Corchorus olitorus and Telfaria occidentalis in Abeokuta Metropolis was carried out because the consumption of vegetables on display in markets and roadsides had been associated with gastro-intestinal disorders. The vegetables were randomly purchased from six markets within the metropolis and analyzed using standard procedures involving Atomic Absorption Spectrometry (AAS) and Standard Plate Count (SPC). The levels of heavy metals in leafy vegetables varied significantly ($P < 0.05$) across locations and were within World Health Organisation (WHO)/Food and Agricultural Organisation (FAO), permissible limits except for Pb and Co found in T. occidentalis, C. argentea, T. triangulare and V. amygdalina. Eleven species of bacteria including Bacillus subtilis, Escherichia coli, Micrococcus species, Staphyloccocus aureus, Klebsiella aerogenes, Staphylococcus saprophyticus, Proteus mirabilis and Bacillus megaterium were isolated from the leafy vegetables. B. subtilis was the most abundant (93.3% occurrence) while B. megaterium and S. aureus were least abundant (8% occurrence). Total colony counts of bacteria ranged from $1.51 \times 10^7 - 58.50 \times 10^7$ (CFU/g) with V. amygdlina and Celosia argentea both from Elega having the lowest and highest values respectively.
The levels of heavy metals and bacterial contamination in selected vegetables were found to be unsafe. Public sensitisations on hygienic handling of vegetables from production to consumption in addition to frequent safety assessment are recommended to minimize human exposure to heavy metals and bacterial pathogens.

INTRODUCTION

Leafy green vegetables are highly nutritious food with rich levels of minerals, vitamins and phytochemicals. They are part of daily diets in many households forming an important source of vitamins and minerals required for human health. They are made up of chiefly cellulose, hemi-cellulose and pectin substances that give them their texture and firmness (Mohammed and Sharif, 2011). They are cultivated in the natural environment, especially in wetlands.

A toxic heavy metal is any relatively dense metal or metalloid known for potential toxicity, especially in environmental contexts (Baldwin and Marshall, 1999). The term has particular application to cadmium (Cd), mercury (Hg), lead (Pb) and arsenic (Ar) (Bánfalvi, 2011), all of which appear in the World Health Organisation's list of ten chemicals of major public concern (Barceloux, 1999). Others include manganese (Mn), chromium (Cr), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), selenium (Se), silver (Ag), antimony (Sb) and thallium (Tl). Toxic heavy metals are found naturally in the earth and become concentrated as a result of anthropogenic activities. Soils can be polluted though depositions while rivers can be polluted by industrial effluents discharge and indiscriminate disposal of untreated or partially treated sewage (Itanna, 2002).

Vegetables could be contaminated when grown in heavy metal rich soils (Sharma et al., 2007; Kawatra and Bakhetia, 2008), they absorb these metals from contaminated soils (Haiyan and Stuanes, 2003), polluted deposits though the roots which are subsequently incorporated into their tissues (Nwajei, 2009) and from deposits on different parts of the vegetables exposed to the air from polluted environments (Aderinola et al., 2014). Plants growing on heavy metal contaminated medium may bio-accumulate high

concentrations of trace elements leading to serious health risk to consumers (Long et al., 2003). Heavy metals rank highest amongst the chief contaminants of leafy vegetables (Mapanda et al., 2005). Factors such as climate, atmospheric deposition, nature of soil, type of fertilizers and irrigation systems could influence the concentration of heavy metals on and within plant tissue (Anyanwu et al., 2004).

Although, some of these heavy metals are essential for human health in small quantities however, when in excess, they become toxic. Studies have shown that fruits and vegetables consumption is the only primary pathway though which humans get exposed to these heavy metals (Sobukola et al., 2007; Otitoju et al., 2012). In addition, heavy metals can enter into human bodies though the food chain, leading to an increased incidence of chronic diseases such as deformity and cancer (Müller and Anke,1994; Tembo et al., 2006; Ramadan and Al-Ashkar, 2007). They get absorbed into plant, animal and human tissues though inhalation, diet and handling, also, they can bind to and interfere with the functioning of vital cellular components (Divya and Helen, 2017).

Similarly, vegetables are contaminated by pathogens during propagation, harvesting and transportation as reported by Xiangwu and Yaguang (2010). Microbial contamination can also occur due to high temperature and storage in contaminated bins along with pre-harvest practices such as soil fertilization with manure and irrigated water. Moreover, the common practice in Nigerian markets is the storage and display of vegetables on benches, sacks and baskets for prospective buyers where they are susceptible to microbial invasion and colonization which might lead to contamination (Muhammad et al., 2004). Contamination of vegetables by microorganisms reduces the quality of the produce and poses health risk. Consumption of leafy vegetables is commonly viewed as a potential risk factor for infection with mycotoxigenic and enteropathogenic microbes while diseases including enteritis, diarrhea, salmonellosis, aspergillosis, haemorrhagic colitis, hemolytic uremic syndrome, typhoid and dysentery can be associated with the intake of contaminated foods (Salau et al. 2014). Diseases caused by microbial pathogens have been one of the major challenges to agriculture, health as well as national economy (FAO, 2012). The risk of food borne diseases transmission is increased when consumed raw or minimally processed as is common with leafy vegetables worldwide. Therefore, it is of practical significance to assess the extent of heavy metal and microbial contamination in plants such as fruits and vegetables and this is a field of research that has gained increasing attention (Sobukola et al., 2008; 2010; Otitoju et al., 2012).

Green Leafy Vegetables (GLVs) such as Jute mallow (Corchorus olitorius Linn.), Fluted pumpkin (Telfairia occidentalis Hook. f.) are widely consumed in the south-western part of Nigeria and are usually purchased from open markets along roadsides with heavy traffic which make them prone to contaminations with potential health consequences in human body. In this research, five green leafy vegetables namely Jute mallow (Corchorus olitorius), Fluted pumpkin (Telfairia occidentalis), Lagos spinach (Celosia argentea Linn.) Water leaf (Talinum triangulare Jacq. Willd.) and Bitter leaf (Vernonia amygdalina Delile) were purchased from six selected markets within Abeokuta metropolis and were assessed to determine the level of some heavy metals (Cu, Fe, Zn, Pb, Cd, Co and Cr) and bacterial contamination.

MATERIALS AND METHOD

Study Area

The study area was Abeokuta Metropolis, the state capital of Ogun state, southwest, Nigeria. The state lies between latitude 7°00' N and longitude 3°35'E situated on the east bank of Ogun river near a group of rocky outcrops in a wooded savanna (Hoiberg and Dale, 2010). Abeokuta and the surrounding area lies within latitude 7°9'39"N and longitude 3°20'54"E and an elevation of 67 m with a population of 593,100 according to the 2006 population census

Sampling Locations

Six major markets namely: Lafenwa, Kuto, Omida, Iberekodo, Elega and Adatan situated along

roadsides with heavy vehicular traffic and characterized by open environment were selected

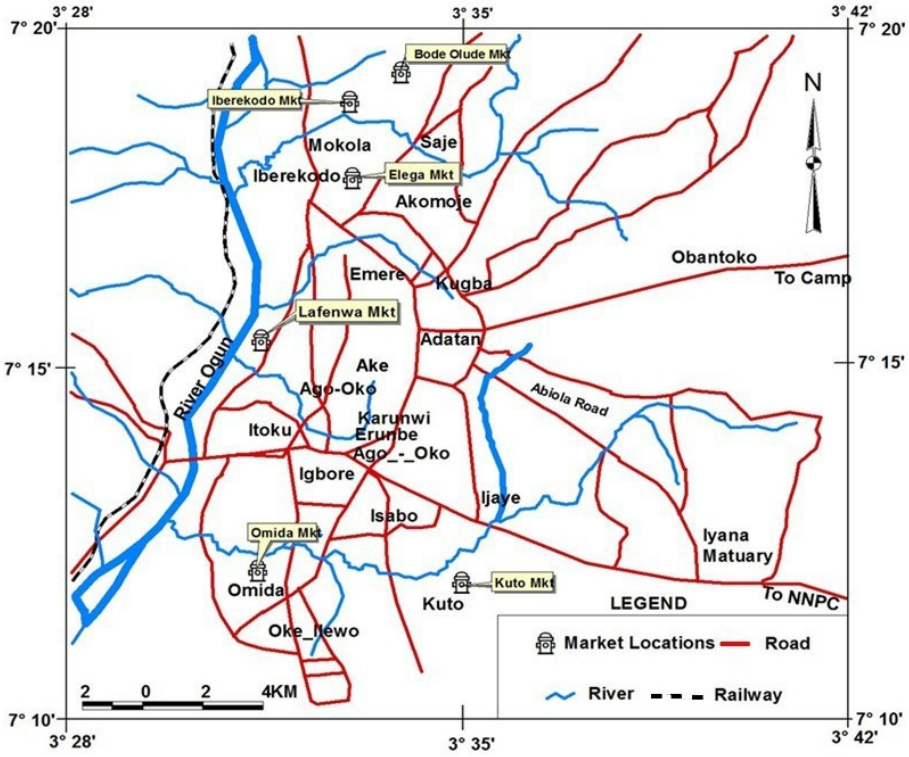

Figure 1: Locations of the Selected Markets within Abeokuta Metropolis

Sample Collection

Fresh leaf samples of vegetables listed in table 1 were collected randomly in clean sampling bags (Ziploc bags) from different marketing sites between the month of June and July, 2015. The samples were stored in ice vault/chest and taken to the Botany department of the Federal University of Agriculture, Abeokuta (FUNAAB) for proper identification. Upon identification, they were taken to Environmental Management and Toxicology department's laboratory where they were prepared for analysis. Healthy portions of the samples were used for analysis while bruised or rotten samples were removed.

Table 1: List of the Types and Names of Vegetable Samples

Scientific Name	Family	Common Name	English Name
Corchorusolitorius	Malvaceae	Ewedu	Jute mallow
Telfairiaoccidentalis	Cucurbitaceae	Ugwu	Fluted pumpkin
Celosia argentea	Amranthaceae	Shoko	Lagos spinach
Talinumtriangulare	Portulacaceae	Gbure	Waterleaf
Vernoniaamygdalina	Compositae	Ewuro	Bitterleaf

Sterilization of materials

All glass wares used were sterilized and aseptic environmental condition was maintained using standard procedures.

Heavy metal analysis

The green leafy vegetables were washed and leaf parts were chopped into small pieces. Chopped portions were first spread to air dry at room temperature and packed inside a brown envelope then dried in an oven at a temperature of 102 °C for proper drying of the samples. Dried samples of the vegetables were ground into a fine powder using a pre-cleaned pestle and mortar, followed by acid digestion according to Ladipo and Doherty (2011). The samples were analyzed for Co, Cu, Fe, Cr, Zn, Pb, and Cd using Flame Atomic Absorption Spectrophotometer (AAS, Perkin

Elmer model 2130).

Microbiological Analysis

One gram (1 g) of each leafy vegetable was suspended in a 250 ml conical flask containing 90 ml of sterile distilled water to prepare the initial dilution. Subsequent serial dilutions of the samples were made using normal saline to keep the microbes alive. One millilitre (1 ml) of the serially diluted samples (10^{-4} – 10^{-6}) were inoculated onto nutrient agar plates and incubated at 37 °C while the glass wares were oven dried for 2-h at 122 °C. The total bacterial counts for each sample were determined and population was expressed as Colony Forming Units per millilitre (CFU/g) of the samples.

Biochemical Tests

Gram Staining

A drop of distilled water was placed on a clean grease-free microscopic slide with an already sterilized inoculating loop. A loopful of the colony on the plate was placed on the microscopic slide with the tip of the inoculating loop and smeared. The smear was allowed to air-dry at room temperature after which it was heat-fixed by passing the slide over the flame twice. The smear was flooded with 1% crystal violet for 1 minute and washed properly with distilled water. It was stained with Gram's iodine solution for 1minute and washed with distilled water. Ethyl alcohol (75%) was then added and the slide was allowed to stand for 30 seconds. The alcohol acted as a decolorizer. The alcohol was washed off with water. The slide was counterstained with safranin for 30 seconds, washed off with water and air-dried. The slide was observed under the microscope at x 1000 magnification. Grams-positive bacteria appeared purple while gram negative bacteria appeared red (Fawole and Oso, 2004).

Citrate Test

The medium used is called Simmon's citrate agar. The medium was made into the slant by dispensing 15.0 ml of the medium into McCartney bottles, autoclaved at 121 °C for 20 minutes and allowed to solidify. The slants were inoculated with the test organism and incubated at 37 °C for 96 h. Colour change from green to blue indicated positive result while original green colour indicated negative result.

Oxidase Test

A piece of filter paper was soaked in few drops of oxidase reagent (Tetra-methy-p-phenylenediamine). An inoculum of the test organism was smeared on the impregnated filter paper and observed for colour change. A purple colouration indicated positive result i.e. oxidase positive while no colour change indicated oxidase negative results (Cheesbrough, 2006).

Coagulase Test

A suspension of the 18-24 h of the organism culture was made on a clean slide and a drop of fresh plasma was added and mixed. Immediate clumping of the suspension indicates a positive result (Olutiola et al., 2000).

Catalase Test

A loopful of the bacteria isolate was placed on a glass slide and few drops of 3% hydrogen peroxide were added to the isolate. Production of gas bubbles from the surface or foaming indicates a positive reaction.

Indole Test

Ten milliliter (10.0 ml) of sterile tryptone broth was aseptically inoculated with the test isolate, leaving one tube un-inoculated to serve as control. The tubes were incubated at 37 °C for 48 h. After incubation, 1.0 ml of chloroform was added to each broth culture and shaken gently. After this, 1.0 ml of Kovac's reagent was added. The tubes were then allowed to stand for about 20 minutes. Red colour at the top layer indicated positive result implying production of indole, no change in colour indicated a negative result (Cheesbrough, 2006).

Methyl Red

The test organism was inoculated into test tubes containing 5.0 ml of sterile MR-VP broth aseptically. The tubes were incubated at 37 °C for 48 h. After incubation, 5 drops of methyl red indicator were added to each test tube and the medium was observed for colour change. Red colour indicates positive reaction while yellow colour indicates negative reaction (Olutiola et al.,

2000).

Vogesprokauer Test

The test organism was inoculated into a test tube containing 5.0 ml of sterile MR-VP broth. After 48 h of incubation, 5 drops of Barrit's A (alpha-napthol) and Barrit's B (potassium hydroxide) was added. A pink-burgundy colour within 20-30 minutes indicates a positive reaction.

Urease

Urea agar (basal medium) was prepared and dispensed into tubes, then sterilized. Glucose and phenol red were added to the basal medium and steamed for 1 h. The filtered sterilized urea solution was added and all content mixed well and dispensed into sterile test tubes. The test organisms was then inoculated and incubated at 37 °C for 24-48 h. Colour change was observed. Phenol red is orange-yellow at pH < 6.8, and turns bright pinkish-red at pH > 8.1. Hence, a positive urea test is denoted by the change of medium color from yellow to pinkish-red (Vashist et al., 2013).

Isolation of Microbial Contaminants from Vegetables

One gram (1 g) of each of the leafy vegetables was suspended in 90 ml of sterile distilled water in 25 ml conical flask to prepare the initial dilution from which subsequent serial dilutions of the samples were made. One ml of the serially diluted samples (10^{-4} - 10^{-6}) were inoculated on nutrient agar plates and incubated at 37 °C. The total bacterial for each sample was counted and expressed as Colony forming units per gram (CFU/g) of the samples. Microbial colonies developing on the agar plates were subsequently isolated and continuously sub-cultured to obtain pure cultures of the isolates. Pure cultures of the bacterial isolates were identified based on morphological, cultural and biochemical characteristics including pigmentation, colony morphology, spore characteristics, nature of mycelium and gram stain reaction (Mackie and Mccartney 1999; Barnetth and Hunter 1998).

Statistical Analysis

Simple descriptive analysis of means, ranges and tables were employed for the determination of heavy metal concentrations. Inferential statistics (ANOVA) was used to determine the relationship existing between different vegetable types and location. Analysis of variance was performed on each measured variable while mean and standard error (SE) was calculated using the SPSS software package 15.0.

RESULTS AND DISCUSSION

Heavy Metals

Heavy metal concentrations as illustrated in table 3 varied significantly between market locations for different green leafy vegetables. In C. olitorius, C. argentea and V. amygdalina the maximum mean heavy metals is in the order Fe > Zn > Co > Cu > Pb > Cd > Cr. However, the concentration of heavy metals in C. olitorius were significantly ($P < 0.05$) higher in samples from Kuto and Omida but within WHO/FAO (2014) permissible limits. In C. argentea, there were also significant increase ($P < 0.05$) in samples from Adatan and Elega but within WHO/FAO (2014) permissible limits except for Pb in samples from Lafenwa and Adatan market and in V. amygdalina, significant increase was observed ($P < 0.05$) in samples from Omida although below WHO/FAO permissible limits across the markets except at Lafenwa where Pb exceeded the limit. Furthermore, the values of heavy metals in T. occidentalis samples was Fe > Zn > Co > Cu > Pb > Cr > Cd. Although, heavy metals were significantly ($P < 0.05$) higher in samples from Lafenwa and Kuto but within WHO/FAO(2014) permissible limits except for Pb at Lafenwa market. However, in T. triangulare values the maximum heavy metal is in the order Fe > Zn > Co > Pb > Cu > Cd > Cr. Heavy metals were significantly ($P < 0.05$) higher in samples from Kuto and Elega but within WHO/FAO (2014) permissible limits except for Pb and Co samples from Kuto and Elega respectively.

All the heavy metal concentrations in the selected vegetables across the markets were all within WHO/FAO (2014) permissible limits except for Pb, which exceeded limit in Telfaria (Lafenwa), Celosia (Lafenwa and Adatan), Talinum (Kuto) and Vernonia (Lafenwa) and also Co (Elega).The high level of lead and cobalt contamination might be due to the display of the vegetables on

Table 3: Variation of Heavy Metal Concentration (mg/Kg) in Green Leafy Vegetables from Selected Markets

Vegetable Type	Markets	Cu	Fe	Zn	Pb	Cd	Co	Cr
Corchorus olitorius	Lafenwa	0.35±0.01^{a}	3.10±0.01^{d}	1.13±0.01^{d}	0.23±0.01^{a}	0.03±0.01^{d}	0.35±0.01^{c}	0.07±0.01^{b}
	Kuto	0.36±0.01^{a}	2.90±0.01^{e}	1.70±0.01^{a}	0.22±0.01^{b}	0.06±0.01^{bc}	0.33±0.01^{d}	0.09±0.01^{a}
	Adatan	0.26±0.01^{c}	2.90±0.01^{e}	1.38±0.01^{b}	0.18±0.01^{c}	0.05±0.01^{c}	0.38±0.01^{b}	0.04±0.01^{c}
	Omida	0.22±0.01^{d}	4.52±0.01^{a}	1.32±0.01^{c}	0.16±0.01^{d}	0.14±0.01^{a}	0.49±0.01^{a}	0.07±0.01^{b}
	Elega	0.29±0.01^{b}	3.27±0.01^{c}	0.64±0.01^{f}	0.04±0.01^{f}	0.07±0.01^{b}	0.37±0.01^{b}	0.10±0.01^{a}
	Iberekodo	0.19±0.01^{e}	3.30±0.01^{b}	0.75±0.01^{e}	0.06±0.01^{e}	0.06±0.01^{bc}	0.34±0.01^{cd}	0.07±0.01^{b}
Telfairiaoccidentalis	Lafenwa	0.36±0.01^{a}	3.48±0.01^{e}	1.77±0.01^{a}	0.32±0.01^{a}	0.09±0.01^{a}	0.30±0.01^{e}	0.09±0.01^{b}
	Kuto	0.36±0.01^{a}	4.34±0.01^{d}	1.62±0.01^{b}	0.29±0.01^{b}	0.07±0.01^{b}	0.40±0.01^{a}	0.01±0.01^{d}
	Adatan	0.32±0.01^{b}	2.86±0.01^{f}	1.45±0.01^{c}	0.14±0.01^{c}	0.05±0.01^{c}	0.35±0.01^{c}	0.04±0.01^{c}
	Omida	0.28±0.01^{c}	6.91±0.01^{c}	1.26±0.01^{f}	0.10±0.01^{e}	0.04±0.01^{c}	0.33±0.01^{d}	0.11±0.01^{a}
	Elega	0.31±0.01^{b}	7.26±0.01^{a}	1.32±0.01^{d}	0.08±0.01^{f}	0.08±0.01^{ab}	0.37±0.01^{b}	0.09±0.01^{b}
	Iberekodo	0.37±0.01^{a}	6.93±0.01^{b}	1.29±0.01^{e}	0.10±0.01^{d}	0.08±0.01^{ab}	0.28±0.01^{f}	0.04±0.01^{c}
Celosia argentea	Lafenwa	0.26±0.01^{d}	3.00±0.01^{d}	1.27±0.01^{f}	0.31±0.01^{b}	0.07±0.01^{c}	0.36±0.01^{d}	0.12±0.01^{a}
	Kuto	0.37±0.01^{b}	2.98±0.01^{d}	1.58±0.01^{b}	0.28±0.01^{c}	0.09±0.01^{b}	0.44±0.01^{b}	0.01±0.01^{d}
	Adatan	0.47±0.01^{a}	2.58±0.01^{e}	1.58±0.01^{a}	0.34±0.01^{a}	0.16±0.01^{a}	0.50±0.01^{a}	0.05±0.01^{c}
	Omida	0.25±0.01^{d}	4.08±0.01^{c}	1.29±0.01^{e}	0.16±0.01^{d}	0.09±0.01^{b}	0.44±0.01^{b}	0.11±0.01^{a}
	Elega	0.26±0.01^{d}	6.75±0.01^{a}	1.42±0.01^{c}	0.03±0.01^{e}	0.07±0.01^{c}	0.37±0.01^{d}	0.11±0.01^{a}
	Iberekodo	0.33±0.01^{c}	5.44±0.01^{b}	1.33±0.01^{d}	0.29±0.01^{c}	0.16±0.01^{a}	0.40±0.01^{c}	0.07±0.01^{b}
Talinum triangulare	Lafenwa	0.22±0.01^{b}	3.02±0.01^{c}	1.33±0.01^{a}	0.33±0.01^{b}	0.06±0.01^{b}	0.35±0.01^{d}	0.08±0.01^{a}
	Kuto	0.28±0.01^{a}	2.99±0.01^{d}	1.23±0.01^{c}	0.40±0.01^{a}	0.10±0.01^{a}	0.44±0.01^{b}	0.00±0.01^{c}
	Adatan	0.19±0.01^{cd}	2.43±0.01^{f}	1.26±0.01^{b}	0.24±0.01^{c}	0.10±0.01^{a}	0.44±0.01^{b}	0.03±0.01^{b}
	Omida	0.13±0.01^{e}	4.79±0.01^{b}	1.10±0.01^{d}	0.18±0.01^{d}	0.06±0.01^{b}	0.39±0.01^{c}	0.08±0.01^{a}
	Elega	0.20±0.01^{c}	6.20±0.01^{a}	0.23±0.01^{f}	0.16±0.01^{e}	0.10±0.01^{a}	0.52±0.01^{a}	0.04±0.01^{b}
	Iberekodo	0.18±0.01^{d}	2.59±0.01^{e}	0.86±0.01^{e}	0.12±0.01^{f}	0.06±0.01^{b}	0.39±0.01^{c}	0.03±0.01^{b}
Vernonia amygdalina	Lafenwa	0.29±0.01^{b}	2.82±0.01^{e}	1.50±0.01^{b}	0.34±0.01^{a}	0.08±0.01^{b}	0.27±0.01^{e}	0.05±0.01^{c}
	Kuto	0.21±0.01^{d}	3.27±0.01^{d}	1.02±0.01^{c}	0.23±0.01^{c}	0.09±0.01^{b}	0.33±0.01^{d}	0.11±0.01^{a}
	Adatan	0.21±0.01^{d}	2.79±0.01^{f}	1.00±0.01^{d}	0.16±0.01^{d}	0.05±0.01^{b}	0.38±0.01^{c}	0.03±0.01^{de}
	Omida	0.41±0.01^{a}	4.67±0.01^{a}	1.59±0.01^{a}	0.25±0.01^{b}	0.11±0.01^{a}	0.45±0.01^{a}	0.07±0.01^{b}
	Elega	0.28±0.01^{b}	4.11±0.01^{c}	0.33±0.01^{e}	0.12±0.01^{f}	0.11±0.01^{a}	0.43±0.01^{b}	0.02±0.01^{e}
	Iberekodo	0.25±0.01^{c}	4.60±0.01^{b}	0.24±0.02^{f}	0.14±0.01^{e}	0.12±0.01^{a}	0.37±0.01^{c}	0.04±0.01^{cd}
WHO/FAO Limits		73:00	425:00	100.00	0.30	0.20	50.00	-

Values in the same column with different superscripts are significantly different ($P < 0.05$)

Bacterial Contamination

The bacteria isolates found in the leafy vegetable samples as shown in table 6 were Bacillus subtilis, Serratia marcescents, Bacillus megaterium, Staphylococcus aureus, Staphylococcus saprophyticus, Klebsiella aerogene, Eschericia coli, Micrococci spp, Pseudomonas fluorescens, Citrobacter aerogenesand Proteus mirabilis. The highest isolated bacteria colony was found in T. triangulare (12) while the least bacteria colony was isolated from V. amygdalina (6). The bacteria count as illustrated in table 4 showed that C. olitorus from Elega has the highest count (58.5 x 10^7 CFU/g) followed by T. triangulare from Lafenwa 48.0 x 10^7 CFU/g while T. occidentalis from Lafenwa and C.argentea from Adatan had the least with 1.51 x 10^7 CFU/g and 1.60 x 10^7 CFU/g respectively. As shown on table 5, Bacillus subtilis has the highest occurrence in the samples followed by Micrococcus species with 93.3% and 90% respectively. The least bacteria count in P. mirabilis and B. megaterium were 10% and 26.75% respectively. Also, from tables 6 and 7, the distribution of the isolates on the samples revealed the presence of bacteria in all the leafy vegetable samples.

The presence of Bacillus subtilis, Serratia marcescens and Proteus mirabilis on the vegetable samples could be linked to the fact that these microbes are widely distributed in air, dusts and soils. It is a common practice in Nigeria to display vegetables in the markets and along the streets of the vendors for prospective customers to purchase. This practice might have predisposed the vegetables to contamination by a wide range of microbes present in the display environment. The isolation of Staphylococcus aureus is indicative of human contamination during handling or improperly washed food utensils and equipment, since the microbe is known to be a normal flora of man (Onuorah et al., 1987).These pathogens could have been introduced from the handler's body either by touching, picking and nasal droplets contamination. Staphylococcus aureus is known to be common inhabitants of the human body and 40-50% of human population carries the organism in their nose and throat. The discovery of Klebisella species which is normally found in human intestinal tract on the vegetables suggests possible human faecal contamination. This study agrees with the reports of Anon (1995) that growth and activity of microorganisms principally bacteria is one of the major causes of deterioration of leafy vegetables. Deterioration was evident by loss of green color to mushiness of the leaves and very high microbial count. This is in agreement with Kendall et al., (2004) who reported loss of green pigments as a post-harvest deterioration of leafy vegetables and microorganisms as agent of deterioration. Hence, the microbial isolates are implicated in the color change of the leaves.

In addition, contaminants could have been introduced from the water used in irrigation as revealed by the finding of Ibeyessie (2007), who reported a wide range of microbial organisms in vegetables produced with waste water. A relationship between the microbial quality of the water used in irrigation and the microbial quality of vegetables was reported. Apart from this, pre-harvest and post-harvest agricultural activities such as transportation, packaging and marketing processes could also be potent sources of contamination.

Contaminated vegetables have been associated with outbreaks of food-borne diseases varying in size from a few persons to many thousands but only a small proportion of the total number of cases reported (De Roever, 1998). Consumption of vegetables from the selected market locations could therefore lead to an outbreak of food

Table 4: Total Bacteria Count (CFU/g x 10^7) in the Selected Vegetables

Markets	T. triangulare	C. argentia	V. amygdalina	C. Olitorus	T. occidentalis
Adatan	3.70	1.60	1.90	11.90	42.8
Iberekodo	34.00	18.30	7.00	12.90	9.00
Lafenwa	48.00	33.60	7.50	45.00	1.51
Omida	10.60	13.50	16.00	24.40	17.20
Kuto	13.10	18.40	25.20	25.40	37.50
Elega	44.70	9.80	6.00	58.50	11.10

Table 5: Total Viable Count of Microbial Contaminants on Leafy Vegetables

Bacteria	**Frequency of Occurrence**	**Percentage (%)**
Micrococcus spp.	27	90
Bacillus subtilis	28	93.3
Staphyloccocus saprophyticus	17	56.7
Klebsiella aerogenes	14	46.7
Staphylococcus auereus	8	26.7
Bacillus megaterium	8	26.7
Escherichia coli	21	70
Pseudomonas fluorescens	15	50
Proteus mirabilis	3	10
Serrata marcescens	10	33.3
Citrobacter aerogenes	11	36.7

Table 6: Bacteria Contaminants of Fresh Leafy Vegetables

Bacteria	T. triangulare	C. argentea	V. amygdalina	T. occidentalis	C.olitorus
M. spp	+	+	+	+	+
B. subtilis	+	+	+	+	+
S. saprophyticus	+	+	+	+	+
K. aerogene	+	+	+	+	+
S. aureus	+	+	+	+	+
B. megaterium	+	+	+	+	+
E. coli	+	+	+	+	+
P. fluorecens	+	+	+	+	+
P. mirabilis	+	+	+	+	+
S. marcescens	+	+	+	+	+
C. aerogenes	+	+	+	+	+

Key: + = present

Table 7: Species of Bacteria Isolated from the Samples

Table 7: Species of Bacteria Isolated from the Samples

Markets	Vegetables samples	Bacteria Isolated
Adatan	T. triangulare	M. sp, B. subtilis, K.aerogene, E.coli, B.megaterium, P.fluorescens
	C. argentea	B. subtilis, E.coli, P. fluorescens, C. Aerogenes
	V. amygdalina	B.subtilis,S.saprophyticus, E.coli
	C. olitorus	M. sp, B. subtilis, S. saprophyticus, K. aerogene, P. Fluorescens
	T. occidentalis	M. sp., B. subtilis, S. saprophyticus, K. aerogene, S. aureus, B. megaterium, E. coli, P. mirabilis
Iberekodo	T. triangulare	M. spp, B. subtilis, S. saprophyticus, K. aerogene, S. aureus, B. megaterium, E. coli, P. mirabilis
	C. argentea	M. spp, B. subtilis, E. coli, P. fluorescens, C. Aerogenes
	V. amygdalina	S. saprophyticus, E. Coli
	C. olitorus	M. spp, B. subtilis, K. aerogene, E. coli, P. fluorescens, P. Mirabilis
	T. occidentalis	M. spp, B. subtilis, P. Fluorescens
Lafenwa	T. triangulare	M. spp, S. saprophyticus, S. aureus, K. aerogene, B. subtilis, B. megaterium, E.coli, P. mirabilis
	C. argentea	M. spp, B. subtilis, S. saprophyticus, K. aerogene, S. aureus, B. megaterium, E. coli, P. mirabilis.
	V. amygdalina	M. spp, B. subtilis, S. saprophyticus, K. Aerogene
	C. olitorus	M. spp, B. subtilis, S. saprophyticus, K. aerogene, S. aureus, B. megaterium, E. coli, P. mirabilis
	T. occidentalis	M. spp, B. subtilis, C. aerogenes, S. Marcescens
Omida	T. triangulare	M. spp, B. subtilis, S. aureus, B. megaterium, C. Aerogene
	C. argentea	M. spp, S. saprophyticus, K. aerogene, E.coli, P. Fluorescens
	V. amygdalina	M. spp, B. subtilis, S. aureus, E. coli, P. fluorescens, C. Aerogenes
	C. olitorus	M. spp, B. subtilis, S. saprophyticus, E. coli, P. fluorescens.
	T. occidentalis	M. spp, S. saprophyticus, E. coli, P. fluorescens, P. mirabilis, C. Aerogene
Kuto	T. triangulare	M. spp, B. subtilis, P. mirabilis, C. Aerogenes
	C. argentea	M. spp, B. subtilis, S. saprophyticus, P. fluorescens, P. Mirabilis
	V. amygdalina	M. spp, S. saprophyticus, K. aerogene, S. Marcescens
	C. olitorus	M. spp, B. subtilis, S. saprophyticus, E. coli, P. fluorescens, P. Mirabilis
	T. occidentalis	M. spp, S. saprophyticus, B. subtilis, E. Coli
Elega	T. triangulare	M. spp, B. subtilis, K. aerogene, S. saprophyticus, P. fluorescens, S. Marcescens
	C. argentea	M. spp, B. subtilis, S. saprophyticus, E. coli, P. fluorescens, C. Aerogenes
	V. amygdalina	M. spp, B. subtilis, P. fluorescens, C. Aerogene
	C. olitorus	M. spp, B. subtilis, S. saprophyticus, K. aerogene, S. aureus, B. megaterium, E. coli, P. mirabilis
	T. occidentalis	M. spp, B. subtilis, K. aerogene, E.coli, P. fluorescens.

CONCLUSION

This study revealed that the levels of Co, Cu, Fe, Cr, Zn and Cd were within WHO/FAO (2014) permissible limits in all selected vegetables investigated across the markets within the metropolis except for the level of Pb and Co found in T. occidentalis, C. argentea, T. triangulare and V. amygdalina respectively. Also, the vegetables were found to be contaminated with heavy loads of eleven species of bacteria, many of which are pathogenic.

Therefore, hygienic handling of vegetables should be encouraged to reduce the level of lead contamination and more research works should be carried out in the soil samples where the vegetables are grown. Thus, control measures such as appropriate handling, packaging, transportation as well as adequate knowledge of the epidemiology of these microbes are recommended with a view to minimizing the adverse impacts they could have on consumers, as some of these microbes have been linked to production of toxins which when ingested are harmful to humans and animals alike.

ACKNOWLEDGMENT

The authors acknowledge the assistance of the multi-disciplinary central research laboratory (MDCR), UI Ibadan, Nigeria for helping in the analysis of this study.

REFERENCES

Aderinola, A. J., Adu, A. A. and Kusemiju. V. 2014. Assessment of trace metal levels in commonly edible vegetables from selected markets in Lagos State, Nigeria. Current World Environment, 9: 789-796

Anon 1995. Fruit and vegetable processing.Food and Agriculture Organization of the United Nations, Rome. 22: 13-15

Anyanwu, E. C., Ijeoma, E. J. E. and Saleh, M. A. 2004. Bioavailability of lead concentration in vegetable plants grown in soil from a reclaimed industrial site: Health Implications. International Journal of Food Safety, 6: 31-34

Baldwin, D. R. and Marshall, W. J. 1999.Heavy

metal poisoning and its laboratory investigation. Annals of Clinical Biochemistry, 36: 267–300.

Barnett, H. L. and Hunter, B. B. (1998) Illustrated Genera of Imperfect Fungi. 4th Edition, American Phytopathological Society Press, St. Paul, Minnesota USA, pp 218

Bánfalvi G. 2011. Heavy metals, trace elements and their cellular effects in Bánfalvi (ed.), Cellular Effects of Heavy Metals, Published by New York Springer Science Business. Chapter 1, pp. 3–28.

Barceloux, D. G. 1999. Chomium. Journal of Clinical Toxicology, 37: 173–94.

Cheesbrough, M. 2006. District laboratory practice in tropical countries. 2nd Edition, Cambridge University Pess, Cambridge, UK.

De Roever, C. 1998. Microbial safety evaluations and recommendations on fresh produce. Food Control, 9: 321-347.

Divya, P. C. and Helen, H. M. 2017. Studies on heavy metal contamination in Poovar estuarine water, Kerala, South West Coast of India. International Journal of ChemTech Research, 10: 343 – 347

Fawole, M.O. and Oso, B.A. 2004.Characterization of bacteria: Laboratory manual of microbiology. 4th Edition, Spectrum Book Ltd., Ibadan, Nigeria, pp 24-33.

FAO, WFP and IFAD 2012. The state of food insecurity in the world; Economic growth is necessary but not sufficient to accelerate reduction of hunger and malnutrition. Rome, FAO. 65pp.

Haiyan, W. and Stuanes, A. 2003. Heavy metal pollution in air-water-soil-plant systems of Zhuzhou city, Hunan province, China. Water, Air and Soil Pollution, 147: 79-107.

Hoiberg and Dale, H. 2010. Abeokuta In: Encyclopedia Britannica., A. Bayes (Editor) Chicago, IL: Encyclopedia Britannica Inc. pp27

Ibeyessie, J. 2007. Bacterial pathogens recovered from vegetables irrigated by waste water. Journal of Environmental Health, 37: 711-718.

Itanna, F. 2002. Metals in leafy vegetables grown in Addis Ababa, Ethiopia. Journal of Health and Development, 6: 295- 302.

Kawatra, B. L. and Bakhetia, P. 2008. Consumption of heavy metal and minerals by adult women though food in sewage and tube-well irrigated area around Ludhiana city (Punjab, India). Journal of Human Ecology, 23: 351- 354.

Kendall, P. A., Yoon, V. and Sofos, J.N. 2004. Inactivation of Salmonella during the storage of Nantes carrot slices treated with steam, water or acid blanching before dehydration. Proceeding of International Association of Food protection 91st Annual meeting. August 8-11, Phoenix, AZ. 116pp.

Ladipo, M. K. and Doherty, V.F. 2011. Heavy metal levels in vegetables from selected markets in Lagos, Nigeria. African Journal of Food Science and Technology, 2: 18 – 21.

Long X. X., Yang, X. E., Ni, W. Z., Ye, Z. Q., He, Z. L., Calvert, D. V. and Stoffella, J. P. 2003. Assessing zinc thresholds for phytotoxicity and potential dietary toxicity in selected vegetable crops. Communication in Soil Science Plant Analysis, 34 (9 &10): 1421-1434

Mackie, T. J. and McCartney, J. E. 1999. Medical microbilology: a guide to the laboratory diagnosis and control of infection. Edited by Collee, J., Fraser, A. G., Marmion, B. P. and Simmons, A. E & S. Livingstone Limited.

Mapanda, F., Mangwayana, E.N., Nyamangara, J. and Giller, K.E. 2005. The effect of long-term irrigation using wastewater on heavy metal contents of soils under vegetables in Harare, Zimbabwe. Agriculture, Ecosysstem and Environment, 107: 151-165.

Mohammed, M. I. and Sharif, N. 2011. Mineral composition of some leafy vegetables consumed in Kano, Nigeria. Nigerian Journal of Basic and Applied Science, 19: 208-212.

Muhammad, S., Shehu, K. and Amusa, N.A. 2004.Survey of the market diseases and Aflatoxin contamination of Tomato (Lycopersicon esculentum mill) fruits in Sokoto North-Western Nigeria. Nutritional and Food Science, 34: 72-76.

Muller, M. and Anke, M. 1994. Distribution of

cadmium in the food chain (soil-plant-human) of a cadmium exposed area and the Health Risks of the General Population. Science of the total environment, 156: 151–158.

Nwajei, G. E. 2009. Trace elements in soils and vegetation's in the vicinity of shell Petroleum Development Company Operating Area in Ughelli, Delta State of Nigeria. American Eurasian Journal of Sustainable Agriculture, 3: 574-578.

Olutiola, P.O., Famurewa, O. and Sonntag, H.G. 2000. Introduction to general microbiology: A Practical Approach. 2nd Edition, Bolabay Publications, Ikeja, Nigeria.

Onuorah, S.I, Adesiyun, A.A. and Adekeye, J.O. 1987. Occurrence of Staphylococcus and coliforms in "Kununyazaki" and utensils used in its Preparations in Samaru, Zaria. Journal of Food Agriculture, 1: 31-34.

Otitoju, O., Akpanabiatu, M.I., Otitoju, G.T.O., Ndem, J.I., Uwah, A.F., Akpanyung, E.O. and Ekanem, J. T. 2012. Heavy metal contamination of green leafy vegetable gardens in Itamroad construction site in Uyo, Nigeria. Research Journal of Environmental and Earth Science, 4: 371-375.

Ramadan, M. A. E. and Al-Ashkar, E. A. 2007. The effect of different fertilizers on the heavy metals in soil and tomato plant. Australian Journal of Basic Applied Science, 1: 300-306.

Salau, I. A., Shehu, K. and Maishanu, M.A. 2014. A preliminary study on microbial contamination of leafy vegetables in Sokoto Metropolis, Nigeria. Aceh International Journal of Science and Technology, 3: 140-144.

Sharma, N., Kalra, K. L., Oberoi, H. S. and Bansal, S. 2007. Optimization of fermentation parameters for production of ethanol from kinnow waste and banana peels by simultaneous saccharification and fermentation. Indian Journal of Microbiology, 47 (4): 310-316.

Sobukola, O. P., Adeniran, O. M., Odedairo, A. A. and Kajihausa, O. E. 2010. Heavy metal levels of some fruits and leafy vegetables from selected markets in Lagos, Nigeria. African Journal of Food Science, 4: 389 – 393.

Sobukola, O. P., Awonorin, S. O., Idowu, M. A. and Bamiro, F. O. 2008. Chemical and physical hazard profile of 'robo' processing – a street vended melon snack. International Journal of Food Science and Technolog, 43: 237-242.

Sobukola, O. P., Dairo, O. U., Sanni, L. O., Odunewu, A. V. and Fafiolu, B. O. 2007. Thin layer drying process of some leafy vegetables under open sun. International Journal of Food Science and Technology, 13: 35-40.

Tembo, D.B., Sichilongo, K. and Cernak, J. 2006. Distribution of copper, lead, cadmium and zinc in soils around Kabwe Town in Zambia. Chemosphere, 63: 497-501.

Xiangwu, N. and Yaguang, L. 2010. Whole-leaf improves chlorine efficacy for microbial reduction and prevents pathogen cross-contamination during fresh-cut lettuce processing. Journal of Food Science, 75: 283-290.