Microbiological, Parasitological and Heavy Metals Assessment of selected Vegetables in Offa Metropolis

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Abstract: Vegetables are vital dietary components, supplying essential nutrients for human health. However, their safety can be compromised by various contaminants. This study assessed the microbiological, parasitological, and heavy metal contamination of ten commonly consumed vegetables—*Telfairiaoccidentalis*, *Amaranthus* spp., *Celosia argentea*, *Corchorusolitorius*, *Vernonia amygdalina*, *Solanumlycopersicum*, *Cucumissativus*, *Brassica oleracea*, *Daucuscarota*, and *Phaseolus vulgaris*—purchased from markets in Offa metropolis, Kwara State, Nigeria. Standard microbiological methods were employed for bacterial and fungal enumeration and isolation, while parasitological assessment utilized the sedimentation method. Heavy metal detection was performed using Atomic Absorption Spectrophotometry (Buck Scientific Model 230). Total bacterial counts ranged from 1.30×10^4 to 1.00×10^5cfu/g and fungal counts range from 4.20×10^6 to 1.24×10^7cfu/g. Key bacterial isolates included *Pseudomonas aeruginosa*, *Salmonella enterica*serovarTyphi (molecularly confirmed), *Staphylococcus aureus* (molecularly confirmed), and *Escherichia coli* (molecularly confirmed). Fungal isolates primarily comprised *Aspergillus niger*, *A. flavus*, and *Candida tropicalis*. Parasitological investigation revealed significant infestation by *Ascarislumbricoides* and *Balantidium coli*. Alarmingly, concentrations of lead (0.50±0.01 mg/kg) and manganese (2.47±0.06 mg/kg) in some vegetable samples exceeded permissible limits set by the World Health Organization (WHO). These findings highlight significant microbial, parasitic, and heavy metal contamination in selected vegetables from Offa metropolis, indicating their potential as sources of public health concern and necessitating enhanced food safety interventions.
Key word: Heavy metal concentration, Microbiological, Parasitology, Vegetables.

INTRODUCTION

Vegetables are a cornerstone of a healthy diet, celebrated for their low-fat, low-energy density, and rich content of vitamins, minerals, fiber, and other bioactive compounds (Rekhy and McConchie, 2022). High vegetable consumption is strongly linked to the prevention of various non-communicable diseases, including cardiovascular disease, cancer, diabetes, and osteoporosis (Wang *et al.,* 2022). These substantial health benefits have led to a considerable increase in global vegetable consumption in recent years, making them a significant economic commodity.

However, the safety of vegetables, especially those consumed raw or minimally processed, is a growing public health concern. Contamination can occur at multiple stages, from farm to fork (Wang *et al.,* 2022). Soil, for instance, is a primary source of contaminants, with agricultural lands often exposed to faecal matter from wild animals or through the application of untreated manure (Braudbury, 2022). This can introduce a range of pathogens to ready-to-eat vegetables, leading to foodborne illnesses (Beuchat, 2002). Indeed, epidemiological investigations have ranked raw vegetables as the second most common source of foodborne illness outbreaks (Callejon *et al.*, 2021). The consumption of improperly washed vegetables is also a well-established route for the transmission of parasitic diseases (Uga *et al.,* 2022).

Beyond microbial and parasitic threats, chemical contaminants, particularly heavy metals, pose significant risks. Unlike organic contaminants that degrade over time, heavy metals are natural components of the Earth's crust that are non-biodegradable and persist in the environment, accumulating in the food chain (Harrison *et al.*, 2021). Vegetables readily absorb these metals from contaminated soils and polluted environments. Elevated levels of heavy metals like lead (Pb) and cadmium (Cd) in food are associated with the etiology of serious health issues, including cardiovascular, kidney, neurological, and orthopedic diseases (Kabir *et al.,* 2022). The indiscriminate use of pesticides and failure to observe appropriate waiting periods

before harvest can further exacerbate chemical contamination, leading to harmful residues in vegetables (Aliu *et al*., 2012).

Given the critical role of vegetables in human nutrition and the multifaceted contamination risks vegetables face, assessing their safety is paramount, particularly in regions where consumption of raw or minimally processed produce is common. The external appearance of vegetables offers no guarantee of safety from microbial or chemical hazards. Therefore, a comprehensive evaluation of microbiological, parasitological, and heavy metal contamination in vegetables from the markets is crucial. This study aims to provide a baseline assessment of the safety of commonly consumed vegetables in Offa metropolis, Kwara State, Nigeria, by investigating their levels of microbial, parasitic, and heavy metals contamination.

MATERIALS AND METHODS

Study Area: This study was conducted in Offa metropolis, Kwara State, Nigeria, a densely populated area known for its various markets, including Owode and Oja-oba. Offa is geographically located at approximately 8.1500∘ N latitude and 4.7167∘ E longitude.

Samples Collection: Ten commonly consumed vegetables—*Amaranthus hybridus* (African spinach), *Celosia argentea* (Lagos spinach), *Telfairia occidentalis* (Fluted pumpkin), *Vernonia amygdalina* (Bitter leaf), *Corchorus olitorius* (Jute), *Daucus carota* (Carrot), *Brassica oleracea* (Cabbage), *Cucumis sativus* (Cucumber), *Solanum lycopersicum* (Tomato), and *Phaseolus vulgaris* (Green beans)—were purchased from Owode market in Offa metropolis, Kwara State, Nigeria. Samples collection occurred between November and December 2024. For each vegetable type, 25 g samples were collected from ten different points (A-J) within the market, totaling 250 g per vegetable type. All samples were immediately placed in sterile collection bags, transported to the Microbiology Laboratory early in the morning processing and analysis

Sample Preparation and Dilution: Upon arrival at the laboratory, representative portions of each vegetable samples were prepared. Each 25 g sample was aseptically introduced into 75 ml of sterile distilled water and soaked for 15 minutes, followed by thorough mixing using a vortex mixer. This resulted in an initial dilution. Subsequent serial dilutions were performed up to 10^{-5} by transferring 1ml of the initial suspension into 9 ml of sterile distilled water blanks.

Microbiological Analysis of Vegetable Samples

Total Viable Bacterial Counts: One milliliter from the 10^{n} dilution of each sample was pour-plated onto nutrient agar (NA). The NA medium was prepared according to the manufacturer's instructions, cooled to 45°C before pouring, and allowed to solidify. Plates were incubated in an inverted position at 37°C for 24 hours. Colonies that grew on the plates were then enumerated using a colony counter, and counts were expressed as colony-forming units per gram (cfu/g). Pure cultures of bacterial isolates were subjected to morphological and biochemical characterization tests for identification, referencing Bergey's Manual of Determinative Bacteriology (Cheesebrough, 2006).

Faecal Coliform Counts: Faecal coliforms were enumerated using the pour plate method. One milliliter from the 10^{-5} dilution was transferred into a sterile Petri dish, and Eosine Methylene Blue (EMB) agar (prepared per manufacturer's instructions and cooled to 45°C) was aseptically added. Plates were gently rocked to ensure even dispersion, allowed to set, and incubated at 45°C for 24 hours. Colonies were counted, and representative isolates were subjected to morphological and biochemical characterization (Cheesebrough, 2006).

Salmonella spp. Counts: The total *Salmonella* counts was determined by the pour plate method. One milliliter from the

10^{-5} dilution was transferred into a sterile Petri dish, and *Salmonella Shigella* agar (SSA) (prepared per manufacturer's instructions and cooled to 45°C) was aseptically added. Plates were gently rocked, allowed to set, and incubated at 37°C for 24 hours. Colonies were enumerated, and representative isolates were subjected to morphological and biochemical characterization (Cheesebrough, 2006).

Staphylococcus spp. Counts: Total *Staphylococcus* counts was determined by the pour plate method. One milliliter from the10^{-5} of solution was transferred into a sterile Petri dish, and Mannitol Salt Agar (MSA) (prepared per manufacturer's instructions and cooled to 45°C) was aseptically added. Plates were gently rocked, allowed to set, and incubated at 37°C for 24 hours. Colonies were enumerated, and representative isolates were subjected to morphological and biochemical characterization (Cheesebrough, 2006).

Molecular Identification of Bacterial Isolates: Molecular identification of three selected highly prevalent bacteria isolates (as identified by phenotypic methods) was performed at Inqaba Biotec, Ibadan, Oyo State, Nigeria. Sequence data were analyzed using the Basic Local Alignment Search Tool (BLAST) against the National Center for Biotechnology Information (NCBI) database for definitive identification. (CLSI, 2018).

Fungal Analysis: Fungal counts were performed similarly to bacterial counts. Twenty-five grams of each vegetable sample were aseptically introduced into 75mldistilled water, soaked for 15 minutes, and vortexed. Serial dilutions were prepared. One milliliter from the dilution was pour-plated onto potato dextrose agar (PDA) supplemented with streptomycin to inhibit bacterial growth. Plates were gently rotated, allowed to solidify, and incubated at room temperature (28°C) for 3-7 days. Discrete colonies were enumerated and expressed as spore forming unit per gram (sfu/g) Pure cultures were obtained by subculturing, and phenotypic characterization involved colonial and microscopic observations using Lacto phenol cotton blue staining method (Awe *et al.,* 2015)

Parasitological Analysis: Parasitological assessment was conducted using the sedimentation method. Fifty to one hundred grams of each vegetable sample were weighed and discretely soaked in 500 ml of normal saline contained in sterile beakers for 45 minutes. The mixtures were vortexed three times at intervals during this period to facilitate the detachment of parasitic stages (ova, larvae, cysts, and oocysts) of helminths and protozoans (Kayser *et al*., 2005; Versalovic *et al*., 2011). Following soaking, the supernatant was decanted and sieved through a clean, sterile muslin cloth to remove large debris. The filtrate was left for overnight sedimentation. The next morning, the sediment was concentrated by centrifugation at 5,000 rpm for 5 minutes. The supernatant was carefully discarded into a disinfectant jar, and the remaining sediment was examined under a light microscope (OLYMPUS) using x10 and x40 objectives. Observed parasitic stages were identified by comparing their morphological features with published descriptions (Kayser *et al.,* 2005).

Heavy Metal Analysis of Selected vegetable samples: Vegetable samples were first washed with distilled water to remove surface particles. Leafy stalks were removed, and the edible portions were sliced and dried to eliminate excess moisture. The dried samples were then carefully blended with blender into a fine powder. For digestion, one gram of the powdered vegetable sample was accurately weighed into a 250 ml Kjeldahl distillation flask. Wet digestion was performed using 20 ml of a 2:1 (v/v) HNO_3-$HClO_4$ acid solution on a hot digestion system until a clear, colourless Solution was obtained. After digestion, the solution was quantitatively transferred to a 100 ml volumetric flask and diluted to the mark with distilled water. The concentrations of heavy metals were then determined using a flame Atomic

Absorption Spectrophotometer (VARIAN model AA240FS, United States).

Statistical Analysis: All statistical analyses were performed using Statistical Package for Social Science (SPSS, version 20). Differences between samples for each parameter were assessed using One-Way Analysis of Variance (ANOVA). When ANOVA indicated a significant difference ($P < 0.05$), post-hoc analysis was conducted using New Duncan's Multiple Range Test to identify specific group differences. A significance level of $P < 0.05$ was used throughout the study.

RESULTS

Total Heterotrophic (Viable) Counts from Vegetable Samples. The results obtained showed the mean total viable count of the various assessed vegetable samples ranging from 100.00±16.97 CFU/g, (ugwu) to 13.00±4.24 ($\times 10^3$) CFU/g (Bitterleaf). The mean *E. coli* count ranged from 95.00±15.56 CFU/g (ugwu) to 13.00±4.2 ($\times 10^3$) CFU/g (green bean). The mean *Staphylococcus aureus* range from 46.00±11.31 c to 5.5±3.54 CFU/g g (green bean), and the mean *Salmonella typhymurium* ranged from 35.50±6.36 CFU/g (ugwu) to 2.00±2.83 CFU/g (carrot). The Fungi count ranges from 123.50±9.19 CFU/g (carrot) to 42.00±9.19 CFU/g (green bean) as shown in Table 1. Percentage Occurrence of Bacterial Isolates from Selected Vegetable Samples. The percentage occurrence of bacteria isolates from vegetable samples showed that *Escherichia coli* and *Staphylococcus aureus* have highest occurrence (15 %) and *Pseudomonas aeruginosa* was 7 % which was the lowest shown in Figure 1. Identification of Bacterial Isolates from the Selected Vegetable Samples. The identification of bacteria isolate of the selected vegetable samples were shown in Table 2 macroscopic, microscopic and biochemical characteristics of the different bacterial isolates identified from the examined vegetables include: *Escherichia coli, Staphylococcus aureus, Proteus* sp., *Salmonella* sp., *Pseudomonas* sp., *Lactobacillus bulgaricus, Bacillus subtilis, Micrococcus luteus, and Enterobacter aerogenes*. The *E. coli* isolated was susceptible to pefloxacin but resistant to ceftazidime, *Staphylococcus aureus* was susceptible to ciprofloxacin and pefloxacin but resist amoxicillin and *Salmonella* sp. was susceptible to ciprofloxacin, and no resistance to all antibiotics as shown in Table 3. Blasting of the bacteria genomic DNA sequence confirm the bacteria isolates to be *E. coli* O157H7, *Staphylococcus rostra* and *Salmonella typhimurium* as shown in Table 4. Distribution of the Fungi isolate of the selected vegetable samples showed that *Aspergillus niger* was the highest followed by *Candida tropicalis* and *Aspergillus nidulan* was the lowest in Figure 2. The characterization and identification of fungi isolates from the selected vegetables sample were *Aspergillus niger*, *Aspergillus flavus Aspergillus nidulan, Fusarium sp., Saccharomyces* sp., *Candida tropicalis, Rhizopus stolonifer* and *Penicillium notatum* in Table 5. The calculated prevalence of the parasite are shown in Table 6 which has the highest occurrence to be *Ascaris lumbricoides Balantidium coli* (17.5 %) and the lowest is *Isospora belli*. Table 7 shows the heavy metals detected in the samples [mg/kg], there is significance difference across all the samples at significant level of 0.05. The Lead content of the vegetable samples ranged from 0.00-0.50 mg/kg. The cadmium content in the vegetable samples ranged from 0.00-0.18 mg/kg. The manganese ranged from 0.10 -2.47 mg/kg. The Nickel ranged from 0.02 – 18.03 mg/kg. The zinc content ranged from 0.11-80.13 mg/kg.

Table 1: Total heterotrophic viable microbial counts of vegetable samples

Samples	Total viable count (TVC)×10^5 cfu/g	Fungal count ×10^4 cfu/g	*Staphylococcus aureus*×10^2 cfu/g	*Salmonella Shigella* ×10^2 cfu/g	Faecal coliform × 10^3 cfu/g
Ugwu	100.00±16.97^{a}	68.00±8.49^{bc}	46.00±11.31^{a}	35.50±6.36^{a}	95.00±15.56^{a}
Amaranthus	40.00±5.66^{bcd}	48.00±9.90^{bc}	40.00±9.90^{ab}	19.00±4.24^{b}	56.50±12.02^{b}
Jute	60.00±4.24^{b}	116.00±25.46^{a}	27.50±10.61^{abc}	9.00±2.83^{bc}	50.00±12.73^{bc}
Lagos Spinach	45.50±6.36^{bc}	54.00±8.49^{bc}	25.00±4.24^{abc}	21.00±4.24^{b}	46.00±4.24^{bcd}
Bitter Leaf	13.00±4.24^{e}	46.00±2.83^{bc}	7.50±2.12^{c}	8.00±5.66^{bc}	31.50±4.95^{cde}
Cucumber	33.50±6.36^{cde}	42.00±12.73^{c}	16.00±2.83^{bc}	13.00±9.90^{bc}	28.50±10.61^{cde}
Cabbage	27.50±10.61^{cde}	60.50±10.61^{bc}	7.50±2.12^{c}	10.00±2.83^{bc}	26.00±5.66^{de}
Carrot	17.50±19.09^{de}	123.50±9.19^{a}	20.00±28.28^{abc}	2.00±2.83^{c}	58.50±12.02^{b}
Tomato	14.00±2.83^{e}	75.00±9.90^{b}	18.50±4.95^{bc}	14.50±6.36^{bc}	67.00±8.49^{b}
Green Bean	27.00±8.49^{cde}	42.00±9.19^{c}	5.50±3.54^{c}	11.50±2.12^{bc}	13.00±4.24^{e}

Key: Values are mean of duplicate determinations. Data with different superscript across the same column are significantly different at $p < 0$.

Table 2: Colonial morphology of bacterial isolates of the selected vegetable samples

Bacteria Isolate	Elevation	Surface	Margin	Colour	Growth	Nature	Shape/form	Optical characteristics	Gram staining	Capsule Staining	Spore Staining	Shape	Indole	Methyl Red	Voges-Proskauer	Citrate	Catalase	Oxygen Relationship	Sugar Fermentation: Glucose	Sucrose	Lactose	Maltose	Probable Organisms
Proteus spp	Raised	Moist	Irregular	Pale yellow	Moderate	Mucoid	Swarming	Opaque	-	-	-	Rods	+	+	-	+	+	FA	AG	A	NA	AG	*Proteus mirabilis*
Pseudomonas spp	Flat	Smooth	Undulate	Greenish	Strong	Slimy	Irregular	Translucent	-	-	-	Rods	-	-	-	+	+	Ae	NA	NA	NA	NA	*Pseudomonas aeruginosa*
Escherichia coli	Convex	Moist	Entire	Creamy white	Strong	Mucoid	Circular	Opaque	-	-	-	Rods	+	+	-	-	+	FA	AG	AG	AG	AG	*Escherichia coli*
Staphyloccu aureuss	Convex	Smooth	Entire	Golden yellow	Strong	Butery	Circular	Opaque	+	-	-	Cocci	-	-	-	-	+	FA	A	A	NA	A	*Staphyloccus aureus*
Enterobacteraerogenes	Raised	Mucoid	Entire	Pink (MacConkey agar)	Moderate	Slimy	Circular	Translucent	-	+	-	Rods	-	-	+	+	+	FA	AG	AG	AG	AG	*Enterobacteraerogenes*
Salmonella spp	Raised	Smooth	Entire	Black-centre on SSA	Strong	Moist	Circular	Opaque	-	-	-	Rod	-	+	-	+	+	FA	AG	NA	NA	AG	*Salmonella typhi*
Lactobacillus spp	Flat	Smooth	Entire	White	Moderate	Butery	Circular	Translucent	+	-	-	Rods	-	-	-	-	-	Ae	A	A	A	A	*Lactobacillus bulgaricus*
Bacillus spp	Flat	Dry	Irregular	White	Strong	Powdery	Irregular	Opaque	+	-	+	Rods	-	+	-	+	+	Ae	AG	AG	NA	AG	*Bacillus subtilis*
Micrococcus spp	Convex	Smooth	Entire	Yellow	Moderate	Butery	Circular	Opaque	+	-	-	Cocci	-	-	-	-	+	Ae	NA	NA	NA	NA	*Micrococcus luteus*
Enterococcus spp	Raised	Mucoid	Entire	Grayish	Moderate	Mucoid	Circular	Translucent	+	+	-	Cocci	-	-	-	-	-	FA	A	A	A	A	*Enterococcus sp.*

Keys: + = positive, - = negative, A = acid production, Ae = aerobe, FA = facultative anaerobe, AG = acid and gas production, NA = no acid and gas production

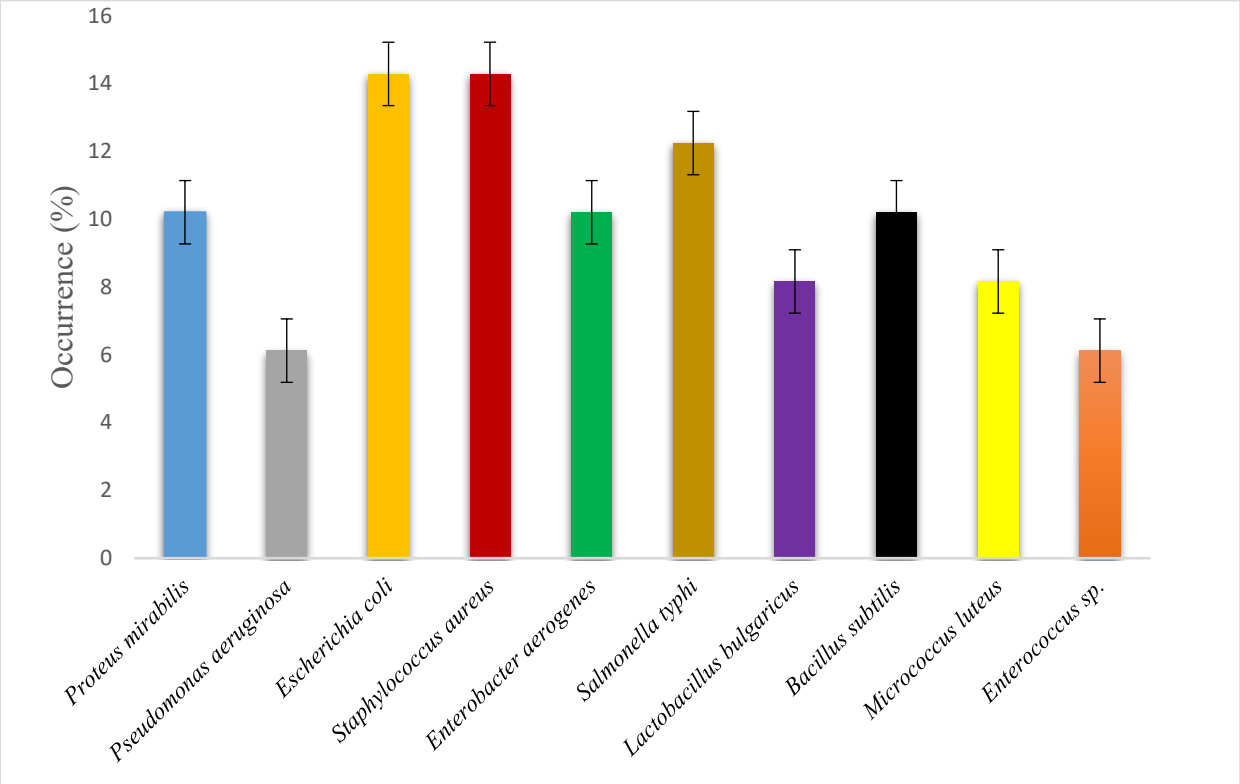

Figure 1: Percentage occurrence of bacterial isolates from sampled vegetables

Table 3: Antibiotics susceptibility pattern of selected vegetable samples (zones of inhibition in mm)

	Antibiotic Discs for the Gram Negative Bacteria									
Samples/ Antibiotic	CN	PEF	OFX	S	SXP	CH	SP	CPX	AM	AV
Proteus	8.5±0.71^{cd}	13.0±1.41^{b}	13.0±1.41^{a}	7.0±0.00^{a}	8.5±2.12^{abc}	6.5±2.12^{bc}	5.0±1.41^{d}	14.0±1.41^{a}	0.0±0.00^{c}	0.0±0.00^{f}
Pseudomonas	10.0±0.00^{bc}	14.0±1.41^{b}	14.0±5.66^{a}	8.5±0.71^{a}	7.0±0.00^{c}	8.0±0.00^{abc}	7.5±0.71^{c}	13.0±2.83^{a}	0.0±0.00^{c}	6.5±0.71^{c}
Escherichia	7.0±1.41^{de}	18.0±2.83^{a}	13.0±1.41^{a}	7.0±1.41^{a}	8.0±0.71^{bc}	8.5±0.71^{ab}	0.0±0.00^{e}	14.0±1.41^{a}	5.5±0.71^{b}	0.0±0.00^{f}
Enterobacter	8.0±1.41^{cd}	13.5±0.71^{b}	13.0±0.00^{a}	6.5±2.12^{a}	6.0±1.41^{c}	11.0±1.41^{a}	10.0±0.00^{ab}	15.0±0.00^{a}	6.0±0.00^{b}	10.0±0.00^{a}
Salmonella	9.0±1.41^{bcd}	14.5±0.71^{ab}	13.0±1.41^{a}	8.5±0.71^{a}	10.5±0.00^{ab}	10.0±0.00^{a}	9.0±1.41^{bc}	15.0±0.00^{a}	6.0±1.41^{b}	6.0±0.00^{cd}
Lactobcillus	5.0±0.00^{e}	13.0±1.41^{b}	12.0±1.41^{a}	0.0±0.00^{a}	8.0±0.00^{bc}	5.0±0.00^{c}	0.0±0.00^{e}	13.5±2.12^{a}	0.0±0.00^{c}	5.0±0.00^{e}
	Antibiotic Discs for the Gram Positive Bacteria									
	CPX	NB	CN	AMX	S	RD	E	CH	APX	LEV
Enterococcus	12.5±2.12^{a}	9±1.41^{abc}	12±2.83^{ab}	6±0.00^{c}	8±1.41^{bc}	9±2.83^{abc}	11±1.41^{ab}	8.5±0.71^{abc}	9.5±0.71^{abc}	11.5±2.12^{ab}
Staphylococcus	9.0±0.00^{bcd}	15.0±0.00^{ab}	14.0±1.41^{a}	7.0±0.00^{a}	6.5±2.12^{c}	9.5±2.12^{ab}	7.0±0.00^{c}	15.0±0.00^{a}	0.0±0.00^{c}	5.0±0.00^{e}
Bacillus	11.5±2.12^{ab}	14.5±0.71^{ab}	15.0±0.00^{a}	8.0±1.41^{a}	11.5±1.41^{a}	11.0±1.41^{a}	11.0±1.41^{ab}	15.0±0.00^{a}	8.0±0.00^{a}	5.5±0.71^{de}
Micrococcus	13.0±0.00^{a}	13.5±2.12^{b}	15.0±0.00^{a}	9.0±1.41^{a}	11.0±2.12^{ab}	10.5±2.12^{a}	12.0±0.00^{a}	15.0±0.00^{a}	9.0±1.41^{a}	8.0±0.00^{b}

Keys: Values are mean ± standard deviation of duplicate determination. Data with different superscript across the same column are significantly different at $p < 0.05$.

Table 4: Molecular identities of most occurring bacterial isolates

Scientific Name	Description	Total Score	Query cover	Per- identity (1%)	Accession No
Escherichia coli	*Escherichia coli* O157H7	13324	100 %	100	NC_002685_2
Staphylococcus aureus	*Staphylococcus rostri*	2200	96 %	100	NZ_AP018587
Salmonella enteric	*Salmonella tyhimurium*	1962	100 %	100	NC_003197_2

Table 5: Colonial morphology of fungal isolates of selected vegetables

Fungal Isolates	Colonial Morphology and Microscopy	Probable Organisms
F1	Yellow-green, flat, granular, and radially grooved colonies on potato dextrose agar; its conidial heads are radiate with hyaline and slightly rough conidiophores. Its conidia are globose and light green in color	*Aspergillus flavus*
F2	Relatively fast-growing mould with a characteristic black color; the conidial head is large, black and spherical. Smooth and colorless conidiophores, connects spherical vesicles which are covered by two layers of phialides all over their surface.	*Aspergillus niger*
F3	Green colonies on potato dextrose agar with a reverse grey colour. They possess short conidia heads and are biserate; conidia are globose and rough-walled while conidiophores are smooth-walled.	*Aspergillus nidulans*
F4	Glistering discrete cream colonies on potato dextrose agar resembling bacterial colonies. They are smooth, moist, and convex colonies with yellowish appearance	*Saccharomyces* sp.
F5	Rapidly growing colonies with cottony yellowish taint growth covering the agar surface densely; identical microscopic view to plants' roots was observed.	*Rhizopus stolonifer*
F6	Fast-growing sooth colonies that initially appear white but gradually develop a grayish-green surface with slightly yellow base on reverse.	*Penicilliumnotatum*
F7	The colonies were initially smooth, creamy, and moist in texture, but as they aged the surface became slightly wrinkled in appearance.	*Candida tropicalis*
F8	Fast-growing, cottony colonies that were initially white, but gradually develop a reddish	*Fusarium* sp.

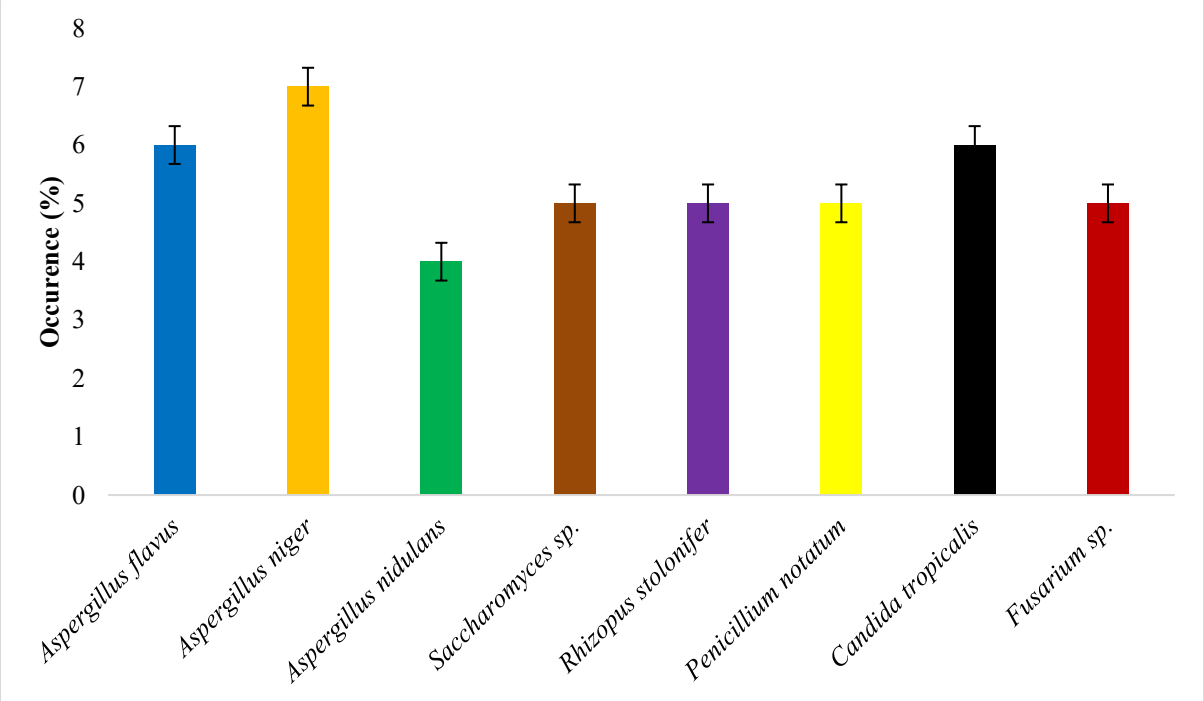

Figure 2: Percentage occurrence of fungal isolates from sampled vegetables

Table 6: Occurrence of parasites from the vegetable samples

Identified Parasites	Occurrence	Percentage (%)
Ascaris lumbricoides	7	17.5
Hymenolepis nana	5	12.5
Isospora belli	4	10
Toxocara sp.	6	15
Balantidium coli	7	17.5
Giardia intestinalis	5	12.5
Entamoeba histolytica	6	15
Parasites' Total Occurrence	40	100
Prevalence	571.4286	

Table 7: Heavy metal detected in vegetable samples

Samples	Lead (mg/kg)	Cadmium (mg/kg)	Manganese (mg/kg)	Nickel (mg/kg)	Zinc (mg/kg)
Ugwu	0.28±0.01^{e}	0.09±0.01^{e}	1.20±0.10^{e}	9.80±0.10^{e}	55.57±0.06^{e}
Amaranthus	0.50±0.01^{a}	0.18±0.00^{a}	2.47±0.06^{a}	18.03±0.06^{a}	80.13±0.06^{a}
Jute	0.25±0.01^{f}	0.12±0.01^{c}	1.47±0.06^{d}	10.47±0.06^{d}	60.43±0.06^{d}
Lagos Spinach	0.31±0.01^{d}	0.10±0.01^{d}	1.77±0.06^{c}	12.20±0.10^{c}	62.47±0.06^{c}
Bitter Leaf	0.40±0.01^{c}	0.15±0.00^{b}	2.03±0.06^{b}	15.17±0.06^{b}	65.20±0.00^{b}
Cucumber	0.47±0.06^{b}	0.00±0.00^{h}	0.10±0.00^{f}	0.03±0.00^{f}	0.82±0.01^{g}
Cabbage	0.03±0.01^{g}	0.02±0.01^{f}	0.04±0.01^{f}	0.02±0.01^{f}	0.11±0.01^{h}
Carrot	0.00±0.00^{g}	0.01±0.00^{g}	0.02±0.01^{f}	0.03±0.01^{f}	0.16±0.01^{h}
Tomato	0.03±0.00^{g}	0.00±0.00^{h}	0.02±0.01^{f}	0.05±0.01^{f}	3.01±0.00^{f}
Green Bean	0.01±0.01^{g}	0.00±0.00^{h}	0.02±0.00^{f}	0.02±0.01^{f}	0.88±0.01^{g}
WHO/FAO Standard	0.3	0.2	1.5	60	500

Keys: Values are mean ± standard deviation of triplicate determinations. Data with different superscripts across the same column are significantly different at $p < 0.05$

DISCUSSION

The comprehensive assessment of vegetables from Offa metropolis revealed widespread contamination with microorganisms, intestinal parasites, and heavy metals, underscoring significant public health risks. These findings align broadly with numerous studies conducted across Nigeria and other sub-Saharan African regions, consistently highlighting persistent challenges associated with poor agricultural practices, inadequate sanitation, and environmental pollution in local food supply chains.

The isolation of *Escherichia coli*, *Salmonella enterica* serovar Typhi, and *Staphylococcus aureus* from the vegetable samples is a critical indicator of faecal contamination and substandard hygiene practices throughout the production and handling chain. This observation corroborates findings by Ifeanyi *et al*. (2018), who reported similar bacterial species in vegetables from Enugu markets, attributing contamination to poor hygiene during harvesting and post-harvest handling. Similarly, Adegunloye *et al.* (2011) linked pathogenic bacterial presence in Akure vegetables to the use of animal manure and polluted irrigation water. The detection of *E. coli* specifically serves as a robust indicator of fecal pollution, strongly suggesting irrigation with untreated wastewater or unhygienic practices by vendors and

handlers. *Salmonella* spp. are well-known enteric pathogens capable of causing severe gastrointestinal infections, while the presence of *S. aureus* often points to human handling contamination.

The high total bacterial and fungal counts, frequently exceeding World Health Organization (WHO) permissible limits, are consistent with reports from Eniola *et al.* (2015) in Ilorin, who also noted elevated coliform levels in leafy vegetables. Findings of this study support the understanding that leafy vegetables, such as *Amaranthus* spp. and *Telfairia occidentalis*, are particularly susceptible to microbial contamination due to their larger surface areas and structural features, which provide ample sites for microbial adhesion. Beyond the high counts, the specific pathogenic and opportunistic bacterial isolates, including *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Staphylococcus aureus*, and *Escherichia coli*, are all of significant public health concern. *Escherichia coli* O157:H7 strains) is a known cause of diarrheal diseases (Nester *et al.,* 2007). *Enterobacter aerogenes*, while sometimes found in vegetation, can act as an opportunistic pathogen. *Staphylococcus aureus* produce enterotoxins, and *P. aeruginosa* is implicated in various human infections, including eye, skin, and ear infections.

The mycological examination further revealed the presence of various fungal species, notably *Aspergillus niger*, *A. flavus*, and *Penicillium* spp**.** These fungi thrive in warm, humid conditions typical of post-harvest storage and handling in tropical environments. The presence of *A. flavus* is particularly concerning due to its capacity to produce aflatoxins, potent mycotoxins with carcinogenic properties. Such findings raise significant alarms regarding potential exposure to fungal spores and mycotoxins, especially when vegetables are consumed raw or inadequately cooked (Nester *et al.,* 2007).

The detection of a range of intestinal parasites, including helminths like *Ascaris lumbricoides*, *Hymenolepis nana*, *Toxocara* spp., and protozoans such as *Balantidium coli*, *Entamoeba histolytica*, and *Giardia intestinalis*, unequivocally points to faecal contamination of the vegetables. This aligns with studies by Ogbolu *et al.* (2013), who found similar parasitic profiles in vegetables irrigated with untreated wastewater in Ibadan, Nigeria. Also, Olaoluwa *et al*. (2014), documented high parasitic loads in Lagos, leafy vegetables especially in produce cultivated near open drains and refuse dumps. This results further corroborate Damen *et al.* (2007), assertion that leafy vegetables grown close to the ground are more prone to accumulating parasite eggs and cysts. The higher levels of contamination observed in vegetables like *Amaranthus* spp. underscore their vulnerability to contaminated soil, water, and unhygienic handling practices.

The presence of heavy metals, specifically lead (Pb) and manganese (Mn), exceeding WHO permissible limits in some vegetable samples, is a serious concern for long-term consumer health. This finding is consistent with earlier reports by Orisakwe *et al*. (2012), who found elevated Pb and cadmium (Cd) in vegetables from roadside farms in Onitsha, and Olowoyo *et al*. (2014), who observed significant Pb and arsenic (As) contamination in urban vegetables from South Africa, primarily attributing it to industrial and vehicular emissions.

The accumulation of heavy metals in leafy vegetables, as noted in this study, reinforces the observations of Akan *et al.* (2013), who documented higher metal uptake in greens due to increased foliar exposure and transpiration. Ekpo *et al.* (2015) further highlighted that agricultural inputs, such as phosphate fertilizers and pesticides, could contribute to metal accumulation in edible crops. These comparative insights collectively underscore that vegetable contamination with heavy metals is a pervasive issue, not confined to Offa, but rather a recurring challenge across various urban and peri-urban agricultural systems, necessitating broader regulatory and monitoring efforts.

CONCLUSION

The These findings highlight significant microbial, parasitic, and heavy metal contamination in selected vegetables from Offa metropolis, indicating their potential as sources of public health concern and necessitating enhanced food safety interventions.

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