Microbial Density and Diversity and Lead Loads in Selected Street- Hawked Foods in Akure Metropolis, Nigeria

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Abstract
Microbial density and diversity, as well as concentrations of lead, in selected street-hawked foods in Akure metropolis were investigated in this study. Street-vended/hawked foods offer numerous advantages to food security; nevertheless, the safety of street foods has been an issue of serious concern, mainly because of the poor sanitary standards and inadvertent contaminants. Seven selected food samples (zobo drink, rice and stew, African star apple, meat pie, cucumber, white pap, smoked fish) were purchased from vendors at three busy road intersections within the city. Metals were analyzed with atomic absorption spectrophotometry. The metals determined are Ca, Cu, Pb, Fe, Zn. The concentrations of the metals in mg/kg were in the range of Ca (24.50±0.10 to 32.00±0.10), Cu (0.75±0.03 to 1.12±0.01), Pb (0.05±0.01 to 0.30±0.01), Fe (0.19±0.01 to 0.31±0.01), and Zn (1.40±0.01 to 2.33±0.01). Lead values were well above permissible limits set by WHO, EU and USEPA, indicating a significant health risk. On the other hand, the presence of high concentration of calcium in the street hawked food indicates that the food is of good value. The microbial density of the street-hawked foods were as follows: zobo, rice and stew, African star apple and meat pie (52 x 10⁸, 54 x 10⁸, 2 x 10⁹, 25 x 10⁷ cfu/g), respectively. Only zobo drink had viable fungal counts (34 x 10⁷ cfu/g) on Potato Dextrose Agar. The microbial isolates observed in this study were Escherichia coli, Klebsiella pneumonia, Shigella spp, Staphylococcus aureus, Bacillus cereus, in varied proportions which could be attributed to the utensils, exposure of food product environment and other related factors. Among the various microorganisms isolated from the street-hawked foods, Escherichia coli, Staphylococcus aureus and Bacillus cereus were quite prevalent. Some of the hawked street foods in Akure metropolis were of poor microbial quality, which is of public health concern.

Keywords: Street-hawked foods, lead contamination, microbial loads, identified isolates, hazards

Introduction
Food has long been recognised as one of the basic needs of life due to its ability to provide nourishment, health and vitality to the consumer (Nwiyi and Elechi, 2022). Despite the importance attached to food, it is now common knowledge that there is increase in the outbreak of food-borne illness as a result of both risky food preparation and eating behaviour. The issue of food security goes beyond increasing production, supply and consumption of food as the production, distribution and consumption capacities are being threatened by poor food safety and quality (Nwiyi and Elechi, 2022). Safe food is now seen as one of the fundamental human rights because of its potential to promote sound health and improve productivity for poverty alleviation as veritable platforms for sustainable development (Enujiugha, 2017). Lack of access to safe food leads to nutrition insecurity which in turn results in the double burden of malnutrition and food-borne diseases. Unfortunately, more research efforts are focused on value-addition to diversify uses and increase production (Enujiugha, 2000) without considerable attention being paid to the safety of such foods.

Food safety can be seen as the level of assurance that the consumers repose on the food being eaten to provide the intended vital nourishment without any form of health hazard or risk (Enujiugha et al., 2023). The issue of food safety has generated global attention considering the reported cases of global, regional and national food-borne disease outbreaks and huge numbers of food recalls which have eroded consumer confidence in the safety of food supply and agrifood production and trade in recent times (Enujiugha et al., 2023). The World Health Organisation reported estimate of 600 million people in the world falling sick after eating

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contaminated food as a result of which, more than 420 thousand die every year (WHO, 2015). This figure could be worse in the developing world if all the food borne outbreaks and deaths associated with food- borne diseases are reported and properly documented.

In Nigeria, the majority of street food vendors are very poor and uneducated, with no knowledge of food hygiene or safety implications to human health. These vendors sell at relatively low prices thereby attracting the majority of low-income workers, shoppers, travelers on the highway and school children in an attempt to earn a living. The consumers seem to be more interested in aesthetics and quantity of serving sizes than in the safety and health implications of the street-hawked foods (Makanjuola and Enujiugha, 2015). Pepple (2017) identified poor cooking methods and sanitary conditions of cooking environment, while Ezirigwe (2018) observed personal hygiene of food handlers as potential sources of food borne diseases in Nigeria. Street foods are regarded as one of the potential sources of food borne illness because of their high susceptibility to microbial spoilage (Yamine and Karam, 2020) due to poor storage temperature and mal-handling practices that expose them to contamination and cross-contamination (Kigigha et al., 2017). With the global rise in foodborne disease outbreaks and with the increasing demand for safe food by respective governmental regulatory and monitoring agencies, coupled with the rising trend of rural-urban migration in developing countries of the sub-Saharan Africa, there is a need to evaluate the quality, safety, and keeping quality of street hawked foods in a typical urban neighbourhood such as Akure metropolis in Nigeria.

Materials and Methods

Sources of Materials
Cucumber, orange, pap, cooked rice, garden egg, zobo, meat pie and smoked fish were purchased from three (3) busy road intersections as well as Oba market all in Akure, Ondo state. All reagents used in the study were of analytical grade.

Mineral Determination
For each sampled food type, one gram (1 g) of sample was placed in a crucible and ashed in a muffle furnace at 550 °C for 5 h and transferred into a desiccator to cool. The ashed sample was used by dissolving it with a mixture of 1 mL nitric acid and 1 mL HCl and made up to 100 mL. This was used to analyze for Na, K, Ca, Cu, Fe, Zn and Pb. The atomic absorption spectrophotometer Model-210 VGP (Buck Scientific, USA) was used to determine Ca, Cu, Fe, Zn and Pb in all the samples. The flame emission photometer (FES 902 Friedrich Ebert Stiftung) was used to measure the values of Na and K in all the samples.

Microbiological analysis
A 1-g quantity of each sample was aseptically weighed into 9 mL sterile water in a McCartney bottle and the content was shaken vigorously. Microbial count was estimated by the pour plate method (0.1 mL inoculum in 10-15 mL warm medium) using serial dilution technique. Subsequent decimal dilutions (10⁻², 10⁻³, 10⁻⁴, etc) were made from 1ml of solution of preceding concentration in 9 mL of diluent. Plates were inverted and incubated at 30 °C for 24 h in a Gallenkamp incubator. At the end of the incubation period, only plates showing between 30 and 300 colonies were counted. Colony counts were expressed as colony-forming units (cfu) per gram of sample, and all analyses were carried out in triplicates with average values reported. Identification and characterization of the isolates were carried out using appropriate morphological and biochemical tests, as outlined in previous research works (Enujiugha et al., 2008; Enujiugha, 2009).

Statistical analysis
All the data collected, especially for the mineral analysis, were evaluated using analysis of variance (ANOVA) and analysed using SPSS statistical package (Version 17.0). Duncan’s New multiple range test was used to determine significant differences among the mean values for the samples at p < 0.05. All values were expressed as mean ± SD.

Results and Discussion

Table 1 shows the results of the minerals composition of the selected street-hawked foods in Akure Metropolis. Heavy metals are considered as one of the most important constituents of food contamination from the environment due to their ability to persist, accumulate, and become toxic to living organisms through consumption along the food chain. Lead was detected in all the street food samples, with all the street food samples seen to be higher than 0.01 mg/kg which is the maximum permissible limit set by WHO/FAO and also the maximum allowable concentration of 0.02 mg/kg by EU and 0.05 mg/kg limit set by USEPA. The high percentage of food samples which were in violation of the maximum permissible limits of Pb set by WHO, EU, and US EPA is a cause for public health concern considering the frequency of exposure (Enujiugha and Nwanna, 2004). The consumption of food contaminated with Pb is the major source of exposure to Pb in a general population. The values obtained in this study were higher than the Pb values reported by Bordajandi et al. (2014) in food samples from Huelva (Spain). It is known that Nigerian gasoline is commonly infused with Pb to prevent or reduce engine knocking; and this is released into the environment through vehicular emissions. It is therefore not surprising that foods hawked within major city intersections will have high Pb loads. High concentration burden of Pb in the body can cause irreversible brain damage (encephalopathy), anemia, coma, and death if not treated immediately (Vijayakumar, 2012). Long-term system exposure can cause damage to the kidneys and reproductive and immune system. Children are more vulnerable than adults to the toxic effects of Pb, and they also absorb Pb easily.

The calcium content ranged from 24.50±0.10 mg/100g to 32.00±0.10 mg/100g for smoked fish and African star apple. Calcium helps in the regulation of muscle contractions and transmission of nerve impulses as well as bone and teeth development (Adelekan et al., 2013). The increases in the content of the minerals recorded in all the samples could therefore be of nutritional advantage to consumers of the products. Ca is crucial in promoting the deposition of hydroxyapatite in bone and serves the mechanical roles of strengthening bones and teeth, it supports the functions of excitable tissues, including nerves and heart muscles, as well as blood clotting (Aspray, 2017).
Copper is an essential metal and serves as anti-oxidant and help the body to remove free radicals, prevent cell structure damage (Salama and Radwan, 2005). It also plays an important role in bone formation and skeletal mineralization (Mariam et al., 2005). The concentration of Cu in all the samples ranged between 0.75±0.03 to 1.16±0.01 mg/kg with meat pie having the highest concentration and cucumber having the least concentration. The limit for Cu in food is 10 mg kg⁻¹ (European Commission, 2006). The results from this study is below the standard limit for Cu. The values of Cu reported in the present study were lower than the values reported in the literature for snacks samples (Cabrera et al., 2003; Salaman and Radwan, 2005). However, similar Cu levels have been recorded in confectionaries and other foods; for example, Ojo and Enujiugha (2018) reported high Cu levels for fermented ground bean.

Iron is a mineral essential for life and for our diets (Enujiugha and Olagundoye, 2001). Results of Fe concentrations for all analysed street food samples are in the ranges of 0.19±0.01 mg kg⁻¹ to 0.31±0.01 mg kg⁻¹. The maximum level of Fe was observed in white pap while the minimum level was observed in meat pie. These food items are potential sources of Fe for both children and adults. Deficiency of Fe in the body could result into anaemia. It is known that adequate iron in a diet is very important for increased work capacity, impaired mental function, and lowered immunity. However, iron loading due to primary or secondary hemochromatosis can produce liver damage, leading to fibrosis, cirrhosis, and an increased risk of hepatic cancer (Wessling-Resnick, 2017). The mean concentrations of Zn in the analyzed samples are in the range of 1.40±0.01 to 2.33±0.01 mg kg⁻¹. The highest mean level of Zn was observed in Zobo while the lowest mean level of Zn was observed in African star apple. Stadlmayr et al. (2012) reported mean Zn concentration in groundnut paste as 0.42 mg kg⁻¹. Sanusi et al. (2017) reported mean Zn concentration ranging from 6.8 to 15.3 mg kg⁻¹ in popcorn and some other cereal-based snacks. Zn has been established as essential for health and nutrition especially in growth enhancement and enzyme-substrate interaction respectively.

### Table 1: Composition of selected minerals in the street-hawked foods in Akure Metropolis (mg/100g)

<table>
<thead>
<tr>
<th>Samples/Elements</th>
<th>Ca</th>
<th>Cu</th>
<th>Fe</th>
<th>Pb</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>29.5±0.10e</td>
<td>1.12±0.01b</td>
<td>0.26±0.01c</td>
<td>0.05±0.01c</td>
<td>2.33±0.01a</td>
</tr>
<tr>
<td>B</td>
<td>26.50±0.01e</td>
<td>1.09±0.01c</td>
<td>0.28±0.01b</td>
<td>0.07±0.01b</td>
<td>1.64±0.01f</td>
</tr>
<tr>
<td>C</td>
<td>32.00±0.10a</td>
<td>1.05±0.01k</td>
<td>0.20±0.01c</td>
<td>0.10±0.01c</td>
<td>1.40±0.01h</td>
</tr>
<tr>
<td>D</td>
<td>25.00±0.05f</td>
<td>1.16±0.01a</td>
<td>0.31±0.01b</td>
<td>0.05±0.01c</td>
<td>2.03±0.01i</td>
</tr>
<tr>
<td>E</td>
<td>27.00±0.05d</td>
<td>0.75±0.03g</td>
<td>0.26±0.02c</td>
<td>0.30±0.01d</td>
<td>2.16±0.01b</td>
</tr>
<tr>
<td>F</td>
<td>30.00±0.10b</td>
<td>0.84±0.01f</td>
<td>0.19±0.01f</td>
<td>0.05±0.01c</td>
<td>1.95±0.01d</td>
</tr>
<tr>
<td>G</td>
<td>24.50±0.10e</td>
<td>1.03±0.01c</td>
<td>0.21±0.01f</td>
<td>0.07±0.01b</td>
<td>1.75±0.02c</td>
</tr>
</tbody>
</table>

Mean ± standard deviation. Values with the same superscript alphabet in the same row is not significantly different at p>0.05

A: Zobo Drink; B: Rice and stew; C: African star apple; D: Meat pie; E: Cucumber; F: White pap; G: Smoked fish

### Microbial counts and distribution in the street food samples

The results of the microbial analysis of the street hawked food in Akure metropolis are presented in Tables 2 and 3. The microbial result of the street hawked food samples revealed varying degree of microbial contamination with some samples showing acceptable level of microbial load of < 10² CFU/g. The total viable count (TVC) is an indicator of quality, not safety, and cannot directly contribute towards a safety assessment of food but can be used as part of a general quality assessment including that of extended shelf-life of foods. Presence of bacteria in street-hawked food in this study could pose health risk to the consumers. Poor food preparation handling, water, exposure to environment, inadequate washing of hands and utensils may have contributed to the presence of the various species of microorganism.

### Table 2: Bacterial and fungal counts in the samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>Bacterial (CFU/g)</th>
<th>Fungal (CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>52 x 10⁴</td>
<td>34 x 10²</td>
</tr>
<tr>
<td>B</td>
<td>54 x 10²</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>2 x 10²</td>
<td>0</td>
</tr>
<tr>
<td>D</td>
<td>25 x 10²</td>
<td>0</td>
</tr>
<tr>
<td>E</td>
<td>6 x 10²</td>
<td>0</td>
</tr>
<tr>
<td>F</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G</td>
<td>1 x 10²</td>
<td>0</td>
</tr>
</tbody>
</table>

A: Zobo; B: Cooked rice with stew; C: African star apple; D: Meat pie; E: Cucumber; F: white Pap; G: Smoked Fish

### Table 3: Bacterial and fungal isolates

<table>
<thead>
<tr>
<th>S/N</th>
<th>Samples</th>
<th>Bacterial isolates</th>
<th>Fungi isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A Escherichia coli,</td>
<td>Klebsiella pneumonia,</td>
<td>Saccharomyces cerevisiae</td>
</tr>
<tr>
<td></td>
<td>Shigella spp,</td>
<td>Staphylococcus aureus,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Escherichia coli,</td>
<td>Staphylococcus aureus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bacillus cereus,</td>
<td>Staphylococcus aureus</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>Escherichia coli,</td>
<td>Bacillus cereus,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Staphylococcus aureus</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Escherichia coli</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>Staphylococcus aureus</td>
<td>Bacillus cereus,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Staphylococcus aureus</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>4</td>
<td>D</td>
<td>Escherichia coli</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Staphylococcus aureus</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>E</td>
<td>Escherichia coli</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Staphylococcus aureus</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>Escherichia coli</td>
<td>Bacillus cereus,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Staphylococcus aureus</td>
<td></td>
</tr>
</tbody>
</table>

A: Zobo; B: Rice with stew; C: African star apple; D: Meat pie; E: Cucumber; F: white Pap; G: Smoked Fish
Microbial criteria regulation varies across countries. ICMSF (2017) Considers TVC in the range of 0-10^3 cfu/g, 10^3-10^5 cfu/g and >10^6 cfu/g as acceptable, marginally acceptable (tolerable), or unacceptable, respectively. The results of the microbial analysis from the TVC of the street hawked food were significantly visible in the microbial load of Zobo, (52 x 10^2 cfu/g), rice and stew (54 x 10^2 cfu/g), African star apple (2 x 10^2 cfu/g) and meat pie (25 x 10^2 cfu/g) respectively. The results are between the acceptable limit for street hawked food. This may be attributed to their low water content which reduces microbial population. On the other hand, rice and stew and zobo samples which had the highest water content were the most contaminated as they favour conditions for microbial growth. Cucumber, white pap and smoked fish were observed to be free of microbial contaminant. Conversely, the zobo drink showed the microbial load of (34 x 10^2 cfu/g) on Potato Dextrose Agar.

The morphological and biochemical characterization of the raw milk sample showed varied potential pathogens which can initiate food poisoning when present in high dose. The organisms were: Escherichia coli, Klebsiella pneumonia, Shigella spp, Staphylococcus aureus, Bacillus cereus, in varied proportion which could be attributed to the utensils, exposure of food product environment and other related factors (Downes and Ito, 2011).

Also, organisms isolated in this study might have been introduced into these foods from feacally polluted water used for washing utensils (e.g. knives, trays, and pans), wrapping materials and the exposure of these products to low temperature (Buchanan and Gibbons, 2014). It may also be as a result of the failure of food handlers to observe basic sanitary rules (Daniyan and Ajibo, 2011).

It may also be as a result of the failure of food handlers to observe basic sanitary rules (Buchanan and Gibbons, 2014). Contamination of the food samples from this agent may have resulted from talking and tasting during food preparation as well as through sharing of cutleries. Its presence also indicates careless handling of food after cooking.

Among the various microorganisms isolated from the street hawked food, Escherichia coli, Staphylococcus aureus and Bacillus cereus are predominating. Other bacterial species such as Klebsiella pneumonia, Shigella spp, were isolated. However, a study by Ezeh et al. (2017) isolated high percentage of Escherichia coli, while Bacillus cereus was the least for meat pie samples sold within Ochana Main Market. The Zobo, rice and stew, African star apple and meat-pie, likewise the white pap cucumber and smoked fish samples were considered fit for human consumption since the distributions of the bacteria isolates were below standard threshold limit as found in this study.

Besides the bacterial species, species of fungi such Saccharomyces cerevisae of food spoilage significant were equally isolated. Other researchers (Ezeh et al., 2017; Kidigha et al., 2017; Obande et al., 2017) isolated similar microorganisms on meat pies and other meat products. The presence of these microbial isolates in the street hawked food and meat pies are due to various factors such as their opportunistic nature and ability to thrive under harsh environment (Ezeh et al., 2017), use of dirty processing equipment/materials, contaminated water, poor hygiene and food safety practices of the food processors. Most of these isolates are both medical and public health significant due to their pathogenic nature. This agrees with Madueke et al (2014) who reported that laboratory analysis of samples of certain hawked food had shown high levels of coliform and pathogenic bacteria to include Salmonella sp, Staphylococcus aureus, Clostridium perfringens, and Vibrio cholera.

Bacillus species are environmental contaminants found in the air, water and withstanding harsh weather condition thereby contaminating “vended snacks as they are vending activities take place in busy-crowded environment” (Ike et al., 2015). Being mesophilic bacteria, Bacillus species produce heat-resistant endospores which on injection produces heat-labile toxic that causes diarrheal illness accompanied with abdominal pain. The most favourable factor that promote the growth of bacillus and subsequent food-borne infection and intoxication is wrong hot processing and holding temperatures of prepared food items. Meat pies fillings are minimally processed with the internal temperature not reaching the recommended core temperature of 145 °F and the street vended/hawked meat-pies are without any form of hot holding device that could maintain the holding temperature of 140 °F of the products. This keeps the meat pies within the “temperature danger zone” of 40 °F to 45 °F leading to proliferation of Bacillus and other co opportunistic bacteria.

Bacillus cereus is normally associated with rice, its presence in the rice and stew may be due to the fact that a spore former and the spores can withstand high temperature during frying of foods. Staphylococcus spp has the ability to form spores which are heat resistant. Achinewhu and Amadi (2016) reported the presence of B. cereus in some street foods in Port Harcourt.

Staphylococcus exists as part of normal skin flora of animals and humans and their presence in all the street hawked food samples suggest poor hygienic practices of both the processors and vendors such as the use of dirty hands, clothing and the practices of mouth blowing of air into packaging materials in an attempt to open them (Ezeh et al., 2017). Staphylococcus bacteria in foods are considered worrisome as they are known to tolerate high concentration of sodium chloride and secretion of thermal stable enterotoxins which causes diarrhea and vomiting on ingestion (Aleruchi et al., 2016)

Equally, E. coli is a normal intestinal micro flora with the enteropathogenic strains implicated for causing travellers' diarrheal and hemorrhagic colitis (Aleruchi et al., 2016). Although, the percentage of E. coli in zobo, rice and stew and smoked fish samples in this study was not significant, its presence shows serious contamination by human or animal faecal matter from water sources utilized during the preparation, sorting and handling.

**Conclusion**

There is no doubt that street food trade is very important to the socio-economy of developing countries. However, informal nature of the trade gives room for unwholesome activities which could pose serious hazards to the health and safety of the practitioners along the chain. The results revealed that the street foods contained lead in levels higher than the WHO permissible concentration, and this presents a real hazard to the

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Research article
consumers. This study also revealed the poor sanitation among street foods hawkers especially as regards feacal contamination of the foods, as well as the high loads of pathogens encountered. Proper management of the trade by all the stakeholders (farmers, vendors, consumers, governments, food and health professionals in academics and development partners) would ensure safe practices and engender safer and healthier society.

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