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Comparative Assessment of the Levels of Pathogenic Bacteria and Heavy Metals in Herbal Liquors, Roots and Teas Sold Within Awka and Enugu Metropolis

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Abstract

Studies were carried out to comparatively assess the levels of pathogenic bacteria and heavy metals in the herbal liquors, roots and teas sold within Awka and Enugu metropolis using relevant analytical procedures and instrumentation. Three pathogenic bacteria (Staphylococusspp, Klesiella pneumonae and Aspergillus niger) were identified and isolated in the herbal root, liquor and tea samples sold within Awka and Enugu metropolis. The mean Cfu counts of the pathogenic bacteria in the herbal products were within the microbial contamination limits for medicinal plant materials intended for internal use. The studied heavy metals (Cd, Cu, Pb, Cr, Zn and Hg) were found present at non-toxic levels in all the herbal products sold within Awka and Enugu Metropolis. Statistical analysis of the Cfu counts of the pathogenic bacteria and heavy metal levels in the herbal products showed significant difference at $p < 0.05$. The order of decrease of the mean Cfu counts and heavy metal contamination of the herbal samples sold within the two studied environments were, herbal liquor > herbal root > herbal tea.

Keywords: Heavy Metals; Pathogenic Bacteria; Herbal Roots; Herbal Teas; Herbal Liquors

Introduction

Herbal products encompass a variety of self-prescribed preparations of plant origin that may generally be categorized as food dietary supplements, cosmetics and herbal medicinal products [1]. The use of medicinal plants is perhaps the oldest method of coping with illnesses. Therefore, phytotheraphy has been integrated into all system of traditional medicine, often as the main source of health care in low and middle-income countries [2]. The popularity of herbal drugs increased in the past decade, probably due to rapid increase in aliphatic drugs prices and media report on their safety [3]. In Nigeria, people use herbal products especially in the rural areas because of availability, affordability and perceived safety. Herbal products such as teas or liquors are beverages made from the infusion or decoction of herbs, spices or other plant material in hot water [4]. Herbal products such as teas, liquors and roots have been imbibed as written history exists. They are usually infusion through the combination of boiling water and dried fruits, flowers or herbs [5]. According to [6] herbal products are made with green and dried flowers, leaves seeds and roots by pouring boiling water or any other polar solvent (ethanol) onto the plant parts and letting them steep for a specified period of time.

In recent decades, the use of herbal products has increased in the developed countries due in part to the wide spread assumption that "natural" implies harmless [1]. There is a general belief that herbal products are not validated according to recommended pharmaceutical guidelines, often herbal products contained toxic and lethal concentrations of heavy metals and other contaminants [7]. However, with their popularity and global market expansion, the safety of herbal products has become a major concern in public health [2]. Lack of regulation and loose distribution channels (including internet sales) may result in adverse reactions attributed to poor quality

of herbal products [8]. The most common causes of poor quality of herbal products are adulteration of herbal products with undeclared patent pharmaceutical substances, substitution or misidentification with toxic plant species, incorrect dosing interactions with conventional medicines and contamination with hazardous substances such as microbial metabolites, radioactive particles, heavy metals and agro chemical residues [8,9]. Heavy metals have bio-importance as trace elements but the bio toxic effects of many of them in human biochemistry are of great concern. They enter our bodies via food, drinking water and air [10]. Depending on the origin of the herbal products, heavy metal accumulation can be derived naturally by soil contamination, use of pesticides and fertilizers [11].

Plants take up heavy metals from the soil and under certain conditions high levels can be accumulated in those roots, leaves and other edible parts of the plant. In recent years, it has been discovered that some herbal products contains a considerable amounts of toxic metals such as arsenic, cadmium, lead and mercury [12]. According to [13] the heavy metal contaminant in traditional medicines may occur due to polluted environment in which the medicinal plants grow, polluted conditions in which the plants are dried and processed, the storage conditions or during manufacturing of the final dosage form. Equally [7,14] stated that there are three mechanism that have accounted for heavy metal contamination in medicinal herbal plants; contamination during cultivation, accidental cross contamination occurring during processing and/or the deliberate introduction of heavy metals and therapeutic ingredients. The genetic and epigenetic effect of dietary heavy metals such as cadmium, chromium, lead, arsenic and selenium in the human body are associated with an increased risk of different cancers [15]. Thus the harmful effects of medicinal plants can be linked to an indirect contamination of those plants by heavy metals and toxic or radioactive agrochemical residues [16]. Prolonged consumption of heavy metals from food can lead to their accumulation in the kidney and liver, causing disruption of numerous biochemical processes and potentially causing cardiovascular, nervous, kidney and bone diseases [17]. Although heavy metals are considered as the oldest known toxins harmful to human, heavy metal toxicity remains a very general subject due to the variety of symptoms caused by heavy metal poisoning [18]. Microbial contamination of herbs and/or products may result from improper handling during production and packaging [1]. The most likely sources of contamination are microbes from the ground and processing facilities (contaminated air, microbes of human origin) [2]. Stated that sources of biological contamination of herbal products could be from human excreta, animal manure and fertilizers.

Contamination of medicinal herbs and herbal products with bacterial strains resistant to known antibiotics poses a particular health risk [19,20]. Studied the prevalence of antibiotic resistant microbes in twenty-nine herbal supplements purchased from local stores in the USA. They isolated the following resistant species; *Bacillus spp., Staphylococcus spp., Ervnia spp., Enterobacter cloacae* and *Stenotrophomonas maltophilia* [21,22] reported that products contaminated with Clostridum spp. spores such as honey and medicinal herbs are not recommended for infants younger than one year of age. This is because the spores of *clostridium botulinium* could cause infant botulism. Research has shown that opportunistic microbial species (bacteria and moulds) in herbal products can cause infection and pose a threat to immune suppressed patients, especially those with AIDS [23]. The presence of large numbers of microbes can be harmful for human consumption. The risk of mycotoxin production especially aflatoxin should be considered in the process of the herbal preparation because of the proven mutagenic, carcinogenic, tetratogenic, neurotoxic, nephrotoxic and immunosuppressive activities of these microbes [24,22]. In Nigeria, herbal medicines are crude preparations of various kinds of medicinal plants, involving dried plants or any of its parts such as the leaf, stem, roots, flower, fruit or seed, which are used to treat minor to severe illnesses and disturbances. Thus, in order to ensure the safety of consumers, the fallacy that traditional medicinal plants are safe, pure and natural and pose no harm to users must be challenged. This informed the focus of this research to comparatively assess the levels of pathogenic bacteria and heavy metals in herbal liquors, roots and teas sold within Awka and Enugu Metropolis.

Materials and Methods

Sample Collection

10 samples each of herbal roots, liquors and teas were purchased in major markets/ outlets in Awka and Enugu metropolis. A total of 30 herbal samples were used in each of the two studied metropolis. The samples were packaged in clean polyethene bags and taken to the laboratory for analysis. All the reagent used were of annular grade.

Sample Preparation For Heavy Metal Analysis

The herbal roots were first ground to powdery form 2g each of finely powdered herbal roots and tea samples were dried in an oven at 105 oC. The samples were digested in 10ml mixture of nitric acid and perchloric acid (3:2) in polyethene plugged bottle. The mixture was then heated at 130 oC for 1hr until the sample dissolved. The samples were filtered with black filter paper and then the volume adjusted up to 25ml with deionized water. The samples were then kept in a refrigerator at 4 oC until analysis. The different digests of the herbal roots and tea samples were analyzed for heavy metal content using 1CP-OES. All the studied solutions (1000mg/l) for (Cd, Cu, Pb, Cr, Zn and Hg) were of spectroscopic grade. Also the samples of the herbal liquors from the infusion by the herbal drug peddlers were analyzed for Cd, Cu, Pb, Cr, Zn and Hg content using ICP - OES.

Microbiological/Biochemical Analysis and Identification of Bacteria Isolates

In order to identify bacteria species, the microbiological techniques employed included inoculation, gram staining, colony

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and morphological characterization for physical and structural features of organisms [17]. Pure cultures of the isolates were subjected to various biochemical tests to determine the identity of the bacteria. The result of each test was recorded and the probable identity of the isolates was determined by the use of Bergey's manual of determinative bacteriology [25]. Table 1 shows that Staphylococcus spp was identified in the samples and the mean concentration of the bacteria were, 1.6 x 10^3, 0.63 x 103 and 2.23 x 103Cfu for the herbal roots, teas and liquor samples respectively. The concentration of Staphylococcus spp in the herbal samples was statistically significant and within the recommended limits for medicinal plant materials [2]. The mean concentration of the isolated *Klebsiella pneumonia* in the herbal roots, teas, and liquor samples sold within Awka metropolis were, 0.86 x 10^2, 0.34 x 10^2 and 1.05 x 10^2 Cfu respectively. The mean concentration of the bacteria in the herbal samples were found to be statistically significant and within recommended permissible limits. *Aspergillus niger* was identified and isolated in the herbal sample sold within Awka metropolis as shown in Table 1. The mean concentration of the bacteria (*Aspergillus niger*) in the herbal roots, tea and liquor samples sold within Awka metropolis were, 1.14 x 10^3, 0.45 x 10^3 and 1.37 x 10^3Cfu respectively. The mean concentrations of the bacteria in the herbal samples were found to be statistically significant ($p < 0.05$) The results for *Aspergillus niger* in the herbal samples sold within Awka metropolis shows that the Cfu counts were within the limits acceptable for internal and tropical use. Table 2 shows that the mean Cfu for *staphylococcus spp* in the herbal roots, tea and liquor samples sold within Enugu metropolis were, 2.5 x 10^3, 1.08 x 10^3 and 2.84 x 10^3 respectively.

Table 1: Mean concentration of the isolated bacteria in the herbal roots, teas and liquors sold within Awka metropolis.

Isolated Bacteria	Mean Concentration in Samples				
	Herbal Roots (Cfu)	Herbal Teas (Cfu)	Herbal Liquors (Cfu)	F-Test	WHO STD
Staphylococcus Spp.	1.6 x 10^3	0.63 x 10^3	2.23 x 10^3	0.01	10^5 - 10^7
Klebsiella pneumonia	0.8 x 10^2	0.34 x 10^2	1.05 x 10^2	0.01	10^5 - 10^7
Aspergillus niger	1.14 x 10^3	0.45 x 10^3	1.37 x 10^3	0.01	10^5 - 10^7

Table 2: Mean concentration of the isolated bacteria in the herbal roots, teas and liquors sold within Enugu metropolis.

Isolated Bacteria	Mean Concentration in Samples				
	Herbal Roots (Cfu)	Herbal Teas (Cfu)	Herbal Liquors (Cfu)	F-Test	WHO STD
Staphylococcus Spp.	2.5 x 10^3	1.08 x 10^3	2.84 x 10^3	0.02	105 - 107
Klebsiella pneumonia	1.21 x 10^2	0.66 x 10^2	1.39 x 10^2	0.03	105 - 107
Aspergillus niger	1.62 x 10^3	0.87 x 10^3	1.96 x 10^3	0.02	105 - 107

The mean Cfu limits of the bacteria in the herbal samples were statistically significant and within established recommended limits. The mean Cfu for *Klebsiella pneumoniae* in the herbal root, tea and liquor samples were statistically significantly and within established recommended limits. The mean Cfu of *Klebsiella pneumoniae* in the root, tea and liquor samples sold within Enugu Metropolis were, 1.2 x 10^2, 0.66 x 102 and 1.39 x 10^2 respectively. The mean Cfu of the bacteria in the herbal samples were within the limits for medicinal plant materials intended for internal use. The mean concentrations of the bacteria in the herbal samples showed significant difference at $p < 0.05$. Table 2 shows that the mean Cfu for Aspergillus niger in the herbal roots, tea and liquor of samples were, 1.62 x 10^3, 0.87 x 10^3 and 1.96 x 10^3 respectively. The Cfu of the bacteria in the herbal samples were statistically significant ($p < 0.05$) and equally within the recommended limits for a consumable medicinal product. The trends of bacterial contamination of the herbal products were found to be the same in the two studied metropolis. The order decrease in Cfu of the isolated bacteria (Staphylococus spp, *Klebsiella pneumonia* and *Aspergillus niger*) in herbal samples sold within the two metropolis were, herbal liquors > herbal roots > herbal teas. In each of the herbal products within the studied environments, the Cfu of staphyloccous spp was the highest while least was *Aspergillus niger*. According to [26], the mode by which plants are conveyed to markets further increases the risk of microbial contamination. Once plants reach the markets, they are stored uncovered, exposed to air contaminants and hence is rendered susceptible to further adverse environmental conditions and microbial attack. [27] Stated that the risk of contamination of herbal products increases when harvesting is done in areas with high temperatures and humidity or when plants are collected during the rainy seasons. This assertion corroborates the findings in this research because most of the herbal products were unduely exposed to high temperatures in market place for long period of time. Humans are mainly the host of some of the organisms detected; therefore contamination of the herbal

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products could possibly have occurred at any production steps that involve human handling. Contamination of herbal medicines with bacteria and fungi has been found in studies conducted in China, Indonesia and Brazil [28,29]. Obtained 5.0 x 10^3 and 3.87 x 10^3Cfu for *Staphylococcus aureus* and *salmonella spp* in herbal products sold in Thailand. An infection with *Salmonella spp*, *Aspergillus niger* from contaminated herbal tea bags containing aniseed and caravaythat broke out among infants under the age of 13months in Germany from October 2002 to July 2005, put in the spotlight the need for rigid microbiological quality control of medicinal herbs and herbal preparations [30].

According to [31], to control microbial contamination of herbal products the following measures have to be taken; avoid harvesting or collecting in damp and cool weather, avoid contact between fresh herbs and soil, avoid outdoor drying processes, observe appropriate drying and storage procedures. The microbial contamination of the herbal samples sold within Enugu metropolis was found to be higher than those from Awka metropolis. This could be attributed to both differences in the geogenic, botanic and anthropogenic sources of contamination of the herbal products in the two studied environments.

Cadmium

Table 3 shows that the mean levels of cadmium in the herbal roots, liquors and tea samples sold within Awka metropolis were, 0.173, 0.226 and 0.08ppm respectively. The levels of the metal in the herbal samples were statistically significant ($p < 0.05$) and within the recommended limits in herbal products [2]. The mean levels of cadmium decreased in the herbal samples in the following order; herbal liquor > herbal root > herbal tea (Figure 1) Cadmium in the herbal roots, liquor and tea samples sold within Enugu metropolis were, 0.091, 0.210 and 0.06ppm respectively. The levels of cadmium in the herbal samples were found to have significant difference ($p < 0.05$) and generally within the permissible limits for a consumable herbal product. The decrease of cadmium in the analyzed herbal products sold within Enugu metropolis followed the same trend as that from Awka metropolis. The mean levels of cadmium were found to be highest in the herbal liquors samples sold in the two studied environments while herbal teas gave the least cadmium values [32]. Obtain cadmium values of 0 - 0.003ppm in herbal products sold in Port Harcourt, Rivers State, Nigeria and the result was lower than what was reported for the metal in this research. Anthropogenic processes and geo genic and botanical factors could have accounted for the higher levels of cadmium in the herbal products sold in Awka metropolis than in Enugu metropolis. Cadmium is one of the heavy metals toxic to human tissues even at low concentrations. Accumulation of cadmium in human bodies could create health problems like cardiovascular, kidney, nervous and bond diseases [33].

Copper

Copper is an essential element for human metabolic system. It regulates various biological processes inside the body like oxidation reduction reactions, energy production, connective tissue formation, iron metabolism and synthesis of neurotransmitter [34]. Table 3 shows that the mean levels of copper in the root, liquors and teas of the herbal samples sold within Awka metropolis were, 0.822, 2.017 and 0.514ppm respectively. Cu decreased in the samples in the following order; herbal liquor >herbal roots > herbal tea. The mean concentration of copper in the herbal samples were statistically significantly ($p < 0.05$) and within the WHO recommended permissible for a consumable herbal product. According to [35] the accumulation of heavy metals in medicinal plants is dependent on climatic factors, plant species, air pollution and other environmental factors.

Table 3: Mean levels of heavy metals in herbal liquors, roots and teas sold within Awka metropolis(ppm).

Metal	Herbal Roots (ppm)	Herbal Teas (ppm)	Herbal Liquors (ppm)	F-Test	WHO STD
Cd	0.091± 0.02	0.210± 0.03	0.06± 0.02	0.01	0.5
Cu	0.575± 0.06	1.68± 0.21	0.412± 0.05	0.02	300
Pb	0.422± 0.05	0.813± 0.06	0.231± 0.03	0.01	10
Cr	0.661± 0.03	1.491± 0.12	0.446± 0.02	0.02	10
Zn	1.870± 0.16	2.55± 0.43	0.58± 0.04	0.02	350
Hg	0.101± 0.05	0.183± 0.07	0.08± 0.12	0.1	0.2

Table 4 shows that the mean levels of copper in herbal root, liquor and tea samples sold within Enugu metropolis were, 0.575, 1.68 and 0.402ppm respectively. The concentration of the metal decreased in the following order in the herbal samples; liquor > root > tea (Figure 2) Copper levels were statistically significant in the samples and generally within the WHO permissible limits for a consumable herbal product [2,5]. Reported high mean level of 2.0 - 24mg/g in the herbal teas and leaves sold in Turkey than what was obtained in this study [18]. Stated that chronic exposure to high concentration of copper causes, irritation of nasal mucosa, vomiting nausea, diarrhea and liver and kidney damage.

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Lead

Lead is considered as a potential carcinogen and is associated with the cardiovascular, kidney, blood, nervous and bone diseases [28]. It is particularly toxic to children because it causes potentially permanent behaviour disorders in them. Table 3 shows that the mean levels of lead in the herbal root, liquor and teas samples sold within Awka metropolis were, 0.370, 0.650 and 0.171ppm respectively. The mean levels of lead in the herbal samples were statistically significant ($p < 0.05$) and within the permissible limits for a consumable herbal product. The order of decrease of the metal in the herbal samples were; liquor > root > tea (Figure 1).

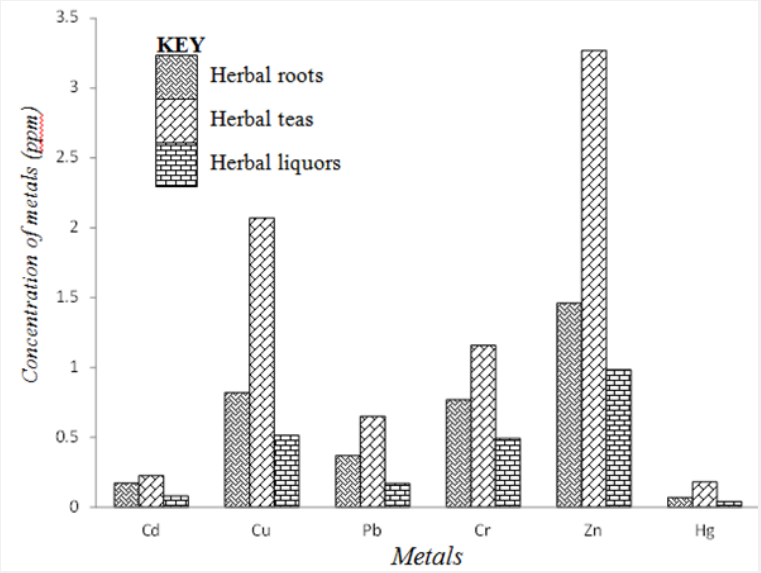

Figure 1: Bar chart representation of levels of heavy metals in herbal liquors, roots and tea sold within Enugu metropolis.

Table 4 shows that the mean levels of lead in the herbal root, liquor and tea samples sold within Enugu metropolis were, 0.422, 0.813 and 0.23ppm respectively. The levels of lead in the herbal samples were statistically significant and generally within established limits. The metal decreases in following order in the herbal samples; liquor > root > tea (Figure 2). The result of this study was lower than 0.942mg/g reported by [36] for lead in herbal teas sold in streets in Cotonou.

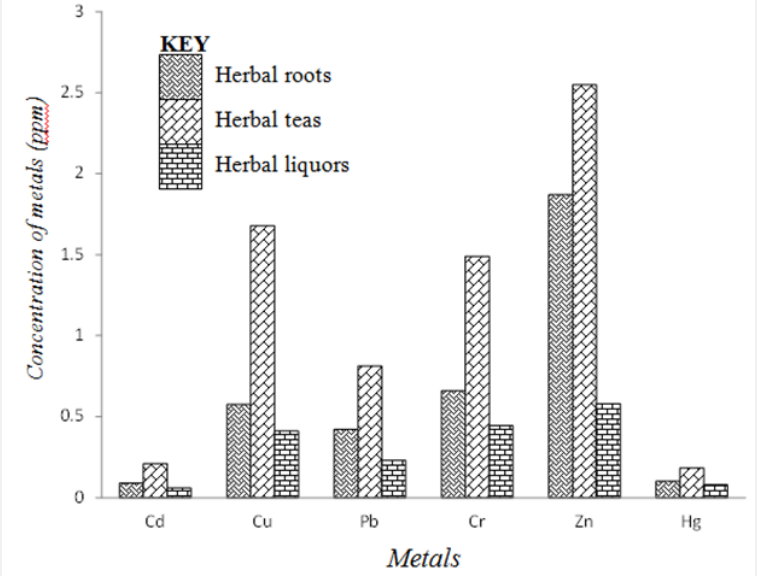

Figure 2: Bar chart representation of levels of heavy metals in herbal liquors, roots and tea sold within Enugu metropolis.

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Table 4: Mean levels of heavy metals in herbal liquors, roots and teas sold within Enugu metropolis (ppm).

Metal	Herbal Roots (ppm)	Herbal Teas (ppm)	Herbal Liquors (ppm)	F-Test	WHO STD
Cd	0.091± 0.02	0.210± 0.03	0.06± 0.02	0.01	0.5
Cu	0.575± 0.06	1.68± 0.21	0.412± 0.05	0.02	300
Pb	0.422± 0.05	0.813± 0.06	0.231± 0.03	0.01	10
Cr	0.661± 0.03	1.491± 0.12	0.446± 0.02	0.02	10
Zn	1.870± 0.16	2.55± 0.43	0.58± 0.04	0.02	350
Hg	0.101± 0.05	0.183± 0.07	0.08± 0.12	0.1	0.2

Chromium

Chromium is available in two ionic forms, that is trivalent and hexavalent. The trivalent chromium is present in food and is utilized by humans because of its safety [32]. Chromium participates in glucose metabolism. The hexavalent chromium has been reported to be carcinogenic and is highly detrimental to humans at even low concentrations [18]. Table 3 shows that the mean levels of chromium in the herbal root, liquor and tea samples within Awka metropolis were, 0.770, 1.16 and 0.493ppm respectively. The mean levels of the metal in herbal samples differ significantly at $p < 0.05$ and were within the recommended limit for a consumable herbal product. Chromium levels decreased in herbal samples in the following order; liquor > root > tea (Figure 1).

Table 4 shows that the mean levels of chromium in the herbal root, liquor and tea samples sold within Enugu metropolis were, 0.661, 1.491 and 0.446ppm respectively. The mean levels of chromium in the herbal samples sold within Enugu metropolis were found to have significantly difference at $p < 0.05$. The mean levels of chromium in the herbal samples were within the recommended permissible limit [2]. Chromium was not detected in herbal products sold in Port Harcourt, Rivers State as reported by [32].

Zinc

Zinc is one of the major essential elements required by the human system. Zinc plays several functions in the human body such as wound healing, blood clotting, and proper thyroid function, maintenance of good vision, fetal growth, cell growth and protein synthesis [37,38]. Table 3 shows that zinc levels were, 1.46, 3.27 and 0.984ppm respectively in the herbal roots, herbal liquors and herbal teas sold within Awka metropolis. Statistical analysis of the levels of zinc in the herbal samples shows significant difference ($p < 0.05$). The order of decrease of zinc levels in the herbal samples sold within Awka metropolis were, liquor > root > tea. Zinc was present at non-toxic levels in the herbal samples. Table 4 shows that the mean levels of zinc in the herbal root, liquor and tea samples sold within Enugu metropolis were, 0.870, 2.55 and 0.58ppm respectively. The mean levels of zinc in the herbal samples were statistically significant ($p < 0.05$) and within WHO recommended permissible limits [5]. Obtained a higher value of 14mg/g for zinc in the herbal teas sold in Turkey than reported in this research.

Mercury

Mercury is a major non-essential metal not needed in food. Mercury is a neuro development poison; it can cause problems in neuronal cell migration and division and can ultimately cause cell degeneration and death [33]. Table 3 shows that the mean levels of mercury in the herbal roots, liquor and tea samples sold within Awka metropolis were 0.07, 0.180 and 0.04ppm respectively. The mean levels of mercury in the herbal samples were statistically significant ($p < 0.05$) and within the permissible limits for a herbal product intended for internal body use. The metal decreased in the herbal samples in the following order, liquor > root > tea (Figure 1). Table 4 shows that the mean levels of mercury were, 0.101, 0.183 and 0.08ppm for herbal root, liquor and tea samples sold within Enugu metropolis respectively. The order of decrease of the levels of mercury in the herbal samples were liquor > root > tea. The mean levels of the metal in the herbal samples were statistically significant ($p < 0.05$) and within WHO recommended permissible limits [38,39].

Conclusion

Three pathogenic organisms (*Staphylococcus spp*, *Klebsiella pneumonia* and *Aspergillus niger*) were identified and isolated in herbal tea, liquor and root samples sold within Awka and Enugu metropolis. The mean Cfu counts of the three isolated pathogenic organisms were within the permissible limits for medicinal plant materials intended for internal use. The herbal liquor samples showed the highest contamination of the isolated bacteria while the least contaminated with the bacteria was herbal tea samples. The studied heavy metals (Cd, Cu, Pb, Cr, Zn and Hg) were found present at non-toxic levels in the herbal product sold within Awka and Enugu metropolis. The levels of the contamination of the isolated pathogenic bacteria and heavy metals in the herbal products were statistically significant ($p < 0.05$). The levels of microbial and heavy metal (except Cd) contamination of the herbal products sold in Enugu metropolis were found to be higher than those in Awka metropolis. Anthropogenic processes, botanical and geogenic factors could have been responsible for the varying contamination levels in the herbal products sold in the studied environments.

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