

Evaluation of Selected Metal Contents in Selected Locally Produced Alcoholic Herbal Bitters Sold in the Ashanti Region of Ghana

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Abstract

This work assessed levels of selected metals in Herbal Alcoholic Drinks (AHB and CGL) produced in Ghana. Three hundred (N = 300) consumers were interviewed to determine drinks frequently consumed. Consumption was CGL (70%) (n = 210) > AHB (20%) (n = 60) > AGB (5%) (n = 15) and (5%) (n = 15) (AGB = JTB). These facts were the basis for selecting AHB and CGL for this work. Herbal Alcoholic Drinks were digested at 180°C with aqua regia and analysed using AAS (Zeeman Varian spectra 220Z) for Pb, Cr and Cu. Recoveries ranged from 97.67% (Cr) - 99.80% (Pb) whilst selected metals contents ranged from $0.28 \pm 80.4E-04$ mg/mL (Pb) - $0.66 \pm 25.1E-01$ mg/mL Cr (AHB) and $5.83 \pm 42.7E-03$ mg/mL (Pb) - $80.6 \pm 14.1E-03$ mg/mL Cr (CGL). Total Pb, Cr and Cu was 12.34 mg/mL (AHB) < 311.06 mg/mL (CGL) and individual metals exceeded reported levels in similar studies, 1.5 µg/mL (WHO) and 5.0 µg/mL (Organisation International De La Vigne et Du Vin). These levels are health threats to consumers and authorities must institute requisite monitoring schemes to protect consumers from risks associated with these metals.

Keywords

Herbal Alcoholic Drinks, Adonko Herbal Bitters, Oligopeptide Complex, Charlie Ginger Liquor, Tukey Post-Hoc Multiple Evaluation

1. Introduction

Bitters is a generic terminology for alcohol-based plant extracts of either barks, leaves, roots or flowers of plants [1]. In certain instances, bitters are a blend of two or more of several parts of plants renowned for curative properties [2]. Plants have

been used since antiquity to treat and manage diverse health conditions [3]. Presently, there appears to be a certain twist to the way Ghanaians perceive alcohol-based plant(s) extracts of medicinal significance. Ghana has reached a phase where nearly all ingestible alcohols introduced into the alcoholic beverage marketplace have been advertised to have aphrodisiac properties, cure impotency, treat stomach disorders, cure fibroid, treat sexual weakness among other human health conditions [4]-[6]. Producers assert that drinks they produce contain extracts from potent medicinal plants. Producers also claim products have the aptitude to trigger taste receptors on the tongue to secrete saliva and other digestive juices and hence are good appetite boosters [7] [8]. These claims seem to be a strategy engineered to bait patrons to consume more. A check from website of Ghana Food and Drugs Authority (GFDA) suggest that sufficient investigations are not done on bitters to establish the truth or otherwise of these claims. It is likely patrons would be exposed to potentially dangerous levels of some xenobiotics such as pesticides and heavy metals that may be present in the raw materials (alcohol and the plant parts) used in the formulation of the bitters. These unavoidable additives (xenobiotics) could initiate detrimental health scenarios that could add to the health burden of consumers.

1.1. Heavy Metals

Subject to the area of interest, heavy metals have many characterisations [9]. Therefore, there appear to be no agreed definition for them [10]. For instance, in metallurgy, characterisation of a metal as a heavy metal is a function of its density [11]. Whilst in chemistry, atomic number is the criterion used to characterise a metal as a heavy metal [12]. However, in the conventional characterisation of a metal as a heavy metal, the defining factor is density [13]. Hence, chemical elements with densities ranging from $3.5 \text{ g}\cdot\text{cm}^{-3}$ to $7 \text{ g}\cdot\text{cm}^{-3}$ are categorised as heavy metals [14]. Examples include Hg, Pb, and Cd and metalloids such as Sb and As [15]. Heavy metals are notoriously harmful [16]. Cd, Hg, Pb and As are listed by the WHO to be among the top ten chemicals of serious public health concern [17]. Toxicities of heavy metals are due to their non-degradable nature [18]. These metals are harmful to humans, animals, and the environment, and have the potential to bioaccumulate in the food chain [18]. In humans, they are not metabolised and are stored in various tissues [19]. They are known or suspected carcinogens or teratogens [20]. Their abilities to bind to structural proteins, enzymes and nucleic acids thereby inhibiting their functioning is a serious public health issue [21]. The degree of health injuries exerted by these metals is a function of the form in which they exist, the scope of exposure and the quantities to which an individual is exposed [22]. Long-term contact with substantial quantities of heavy metals could cause carcinomatous conditions to the central and peripheral nervous and circulatory systems [23]. Anthropogenic activities such as mining, indecorous industrial waste disposal, and fertilizer and pesticides applications have progressively contributed to the significantly high levels of these metals and their compounds

in humans, animals and in the global environment in general [24].

1.2. Lead

Its chemical symbol is Pb, atomic number 82 and a mass number 208. Its electronic configuration is $_{54}[\text{Xe}] 4f^{14}5d^{10}6s^26p^2$. It is in group IV and period VI of the periodic table. In Latin, it is called *Plumbum*, hence its symbol Pb [25]. Pb occurs naturally in the Earth's crust. Pb is soft; malleable, has a shiny exterior when cut new and very abundant in the environment [26]. Its levels in the earth crust have been determined to be around 15 to 20 mg/kg [27]. Although ubiquitous, it has no biological reputation, and combines with other elements such as S and O to form PbS and PbSO₄ [28]. When compared to Al and Fe, Pb is an uncommon metal [29]. Pb seldom occurs in its Pb⁰ state, rather it exists as Pb²⁺ in ores such as Galena (PbS), Anglesite (PbSO₄), and Cerussite (PbCO₃) in the earth crust [30]. Pb concentrations not confined in minerals deposits have progressively reached elevated levels over the past three centuries due to anthropogenic activities [31]. Pb has excellent properties and through that, human exposure to it is usually attributable to its myriad usages. Currently, the principal use of Pb is for the manufacture of Pb batteries used in the automobile industry [32]. Alloys used in soldering, x-ray machines shielding devices, and deterioration and acid resistant materials used in the construction works are also made of Pb [33]. Widespread distribution of Pb in the environment originated from its former applications as additive in petrol, and as pigment in interior and exterior paints contributed to human contact with the metal [34]. Though Pb is no more used as additives and pigments, human exposure to it remains due to its non-degradable nature [35]. Leaded paints are still widespread in countless older homes worldwide [36] and shedding of these paints continues to contribute to indoor and outdoor dust Pb levels globally [37]. Because Pb does not degrade and is strongly absorbed onto soil, it released from past applications persist in soil [38]. From the period leaded fuel was phased out until now, there has been an estimated 93% drop in its levels in the atmosphere [39]. Although Pb levels in the atmosphere have dropped, significant quantities exist in areas near Pb smelters [40] [41]. In distant areas such as the Antarctica, Pb levels in air range from 7.6E-05 to a little above 10 µg·m⁻³ [42]. Whilst Pb levels in the environment are progressively decreasing, the quantities available in some areas of the world are above the USEPA air quality standard of 1.5 µg·m⁻³ for Pb [41]. Pb toxicity is of serious worry to environmentalists and public health experts [42]. Due to the low solubility of most of its compounds, Pb tends to precipitate out of complex solutions and increases the possibility of its exposure to both humans and animals [43]. Pb is noxious and affects a wide range of physiological, biochemical and behavioural functions in humans [44]. Pb is not biodegradable and occurs in insoluble form that poses several serious human health issues [45]. Pb may enter the human body via ingesting Pb contaminated foods, water or via prolonged dermal contact with Pb containing objects [46]. Children are at high risk from Pb poisoning than adults due to their hand-to-mouth

activity [47]. Children exposed to Pb often suffer retardation in physical growth, have inferior intellect, reduced attention span, hyperactive and cognitive deterioration and in some scenarios suffer complete brain harm [47]. These are because children have their vital organs at the developing stage and cannot stand the harsh effects of Pb even at sub lethal concentrations [48]. In adults, Pb may affect the gastrointestinal tract, kidney, and the central nervous system [49]. and has the propensity to reduce hemoglobin synthesis, affect normal functioning of the kidney, and damage the central and peripheral nervous systems [50].

1.3. Chromium

Chromium with chemical symbol Cr is the first element in group (VI) of the periodic table, it is transition metal and has atomic number 24 [51]. This metal derived its name from the Greek word *chroma*, which means colour and most of its compounds have intense colour [52]. Cr is the first element whose ground state electron configuration violates the Aufbau Principle [53]. It is the fourth transition element and has electronic configuration of $18[\text{Ar}] 3d^5 4s^1$. It has three naturally occurring stable isotopes (^{52}Cr , ^{53}Cr and ^{54}Cr) with ^{52}Cr being the most abundant [54]. Crocosite (PbCrO_4) was the first mineral that found to hold small amounts of Cr [55]. However, Cr occur in chromite (FeCr_2O_4), the ore from which it is extracted [56]. Cr is the 13th most abundant element in the earth crust and is present at almost $100 \text{ mg}\cdot\text{kg}^{-1}$ [57] [58]. High levels of Cr in the environment is partly a product of weathering of rocks with substantial amounts of Cr content and partly from volcanic outbursts [59]. In air, Cr produces oxygen resistant oxide layer [60]. This layer shields fresh chromium surface underneath it from coming into contact with air thereby protecting Cr from deteriorative oxidation [61]. Cr boils and melts at 2672°C and 1907°C respectively, and at 20°C , it has atomic mass and density of $51.99 \text{ g}\cdot\text{mol}^{-1}$ and $7.2 \text{ g}\cdot\text{cm}^{-3}$ respectively [62]. Cr is used in several ways. For instance, the metal is used to formulate alloys such as stainless steel [63]. Other uses of Cr include but not limited to Cr plating to produce a corrosion free silvery exterior for automobile parts [64]. It also has properties which allow it to be used as catalyst [65]. In addition, Cr compounds exhibit diverse colourations due to their stable nature, and that allow them to be used as pigments [66]. Toxicity of Cr also offers it a preservative property used in the timber trade to preserve wood to safeguard wood decay due to fungi and other wood destructive pests [67]. Cr has been used in the manufacture of insulin, a hormone that is critical for the metabolism and storage of carbohydrates, fats and protein among others [68]. Notwithstanding the numerous benefits of Cr and its compounds, it has some damaging human health effects that include skin rashes, respiratory problems, kidney and liver damage, modification of genetic materials and lung cancer [69]. Although Cr^{3+} is an essential ion, exposure to high levels via inhalation, ingestion or dermal absorption have adverse health conditions [70]. These include immunological, neurological, reproductive, developmental, genotoxic and carcinogenic effects and even death [17].

1.4. Copper

Copper occurs in ores such as Chalcopyrite (CuFeS_2), Chalcocite (Cu_2S), Cuprite (Cu_2O), and Tennantite ($\text{Cu}_{12}\text{As}_4\text{S}_{13}$) among others and has symbol Cu [71]. Cu is also released into the environment via anthropogenic activities such as mining, agriculture and industrial activities [72]. Cu is used extensively in the textiles, anti-fouling paints, electrical conductors, plumbing fixtures, pipes, coins, cooking utensils, wood preservatives, pesticides, fungicides, and in fertilizers production [73].

Cu movement in soil is influenced by the pH of the soil, organic matter contents of the soil, the cation exchange capacity of the soil and other minerals in the soil which could interact with copper [74]. Cu has low mobility in plants compared to other elements [75]. Its chemical forms, the quantity exposed to, extent of exposure, and its interaction with other minerals influence lethal effects of Cu [76]. High levels of Cu could cause nausea, abdominal discomfort, diarrhoea, haemoglobinuria and/or haematuria, jaundice, oliguria/anuria, hypotension, coma and even death [77]. Histopathological effects of copper have been observed in the gastrointestinal tract, liver and kidney of humans [78] [79]. Although limited information on chronic effects of Cu exists, the metal is known to be mutagenic, carcinogenic, affect reproductive health and wield other chronic effect such as Wilson's disease [80] [81].

Lately, herbal alcoholic beverages have subjugated the Ghanaian alcoholic drink marketplace. These drinks are predisposed to contaminants including metals as the raw materials (herbs and plants) used in their formulations have their habits exposed to different contaminants. Though metals are likely to occur at high levels in these beverages, people are oblivious of them and their associated conceivable health effects such as liver and kidney damage. The public is consuming these drinks daily in larger quantities and they could suffer some health issues. In Ghana, data on safety of bitters in reference to hazardous metals are scanty, it is imperative that people become aware of them and their associated possible health matters. Thus, this study seeks to establish the levels of Cu, Cr and Pb in the most consumed herbal alcoholic beverages within the Mampong-Ashanti Municipality of the Ashanti Region of Ghana and its immediate surrounding towns. It also aims to compare metal levels to the WHO permitted levels and predict possibility of health issues from the selected heavy metals in these drinks consumed daily by the Ghanaian public.

2. Methodology

2.1. Materials and Chemicals

Locally produced Alcoholic Herbal Bitters (AHB and CGL), 250 mL digestive flasks, 250 mL beakers, measuring cylinder (100 mL), pipette (50 mL), Whatman No.1 filter paper, deionized water, 36% Hydrochloric Acid (Philip Harris, UK), 68% Trioxonitrate (V) Acid (Sigma-Aldrich, USA), 98% Chromic Acid, Merck KGaA, Germany and Deion -5 Deionized Water, Chemiphase, UK.

2.2. Study Areas and Design of the Study

The study areas were Ashanti-Mampong Municipality, Nsuta (Sekyere Central District) and Agona (Sekyere South District) in the Ashanti Region of Ghana. Prior to sample collection, a preliminary study was done to establish the most patronised locally produced herbal drinks in the study areas. The purpose of the study was explained in the native Twi language to enable participants appreciate the study. Identities of participants were kept confidential, and were informed that they could withdraw at any time in the course of the study if any participant perceives the need to do so. Without coercion, participants who consented fully were included in the study. This was done in compliance with the protocol approved by the ethical board of Akenten Appiah-Minka University of Skills Training and Entrepreneurial Development (AAMUSTED). Three hundred fully consented consumers of locally produced herbal drinks were interviewed and the responses were analysed using Excel component of the Microsoft Office Suite 2016. Drinks included in the study were selected based on the statistics obtained from the preliminary investigations. One hundred and thirty samples (65 of each drink type) in plastic bottles were purchased and transported in brown paper boxes to the Chemistry Laboratory at AAMUSTED, Ashanti-Mampong, Ghana, for heavy metals extraction and then analysed at the Department of Chemistry, University of Cape Coast, Cape Coast, Ghana.

2.3. Quality Control

Deion -5 Deionized Water (Chemiphase, UK) was used throughout the sample preparation and analysis. A strict quality control scheme was followed to ensure that data produced were reliable. Glass wares were soaked in 98% chromic acid for 48 hours after which they were thoroughly and rinsed thrice, each with copious quantities of deionised water. The samples (blank samples, Certified Reference Standard Solutions and herbal drinks) were handled carefully to avoid cross contamination.

2.4. Preparation of Herbal Drink Samples

One milliliter of each drink type was put into a different 250 mL digestion flasks, 50 mL aqua regia added, contents of flasks were swirled gently for 2 minutes and then put into a heating mantle at 180 °C. The mixtures were boiled at 180 °C until total digestion was achieved. Digestion was completed when the thick brown nitrogen dioxide gas evolved from the boiling stopped. Boiling was further sustained at 180 °C until contents of the flasks were dried. The flasks were removed from the heat source, allowed to cool to room temperature after which 10 mL of deionized water was added to the contents of each flask and swirled gently for 3 minutes to reconstitute the mixtures. The reconstituted mixtures were filtered under gravity through Whatman No.1 filter paper, filtrates were topped to 50 mL with deionized water and then analysed for the selected metals using Zeeman Atomic Absorption Spectrometer (Varian Spectra 220Z).

2.5. Preparation of Certified Reference Materials (CRMs) for Process Validation

Efficiency of the method used was assessed using 1000 mg/L mixed standard solution IV (CRM 111355, Certipur^(R)). It contained Pb (0.39 ± 0.05 mg/L), Cr (1.89 ± 0.17 mg/L) and Cu (15.50 ± 0.63 mg/L) in 0.1M HNO₃. Two (2), 4, 6, 8, 10 and 12 µg/mL working solutions were prepared by diluting 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mL respectively of the mixed standard solution IV to 50 mL. One milliliter of each working solution was put into different 250 mL digestion flasks, 50 mL aqua regia was added and then digested using the procedure described for herbal drinks. These were prepared simultaneously with herbal drink samples and analysed in triplicates.

2.6. Preparation of Blank Samples

Blank samples were prepared and analysed along with the drink samples to monitor contamination that may result from chemicals, filter paper and glassware used. One milliliter of deionized water was put into three different 250 mL digestion flasks. Fifty milliliters of aqua regia were added to the content of the flasks. Deionised-aqua regia mixtures (blank samples) were treated synchronously with drink and quality control samples and then digested as described for herbal drinks.

2.7. Results and Discussion

Preliminary assessment of herbal drinks was done via interview to determine patronization pattern among consumers. This introductory study included 300 individuals and of these, 70% (n = 210) patronised CGL, 20% (N = 60) AHB, 5% (n = 15) AGB and 5% (N = 15) JTB. Responses of patrons indicated patronage to be CGL > AHB > AGB = JTB. Hence, results presented (Figure 1) formed basis for examining CGL and AHB for the selected metals.

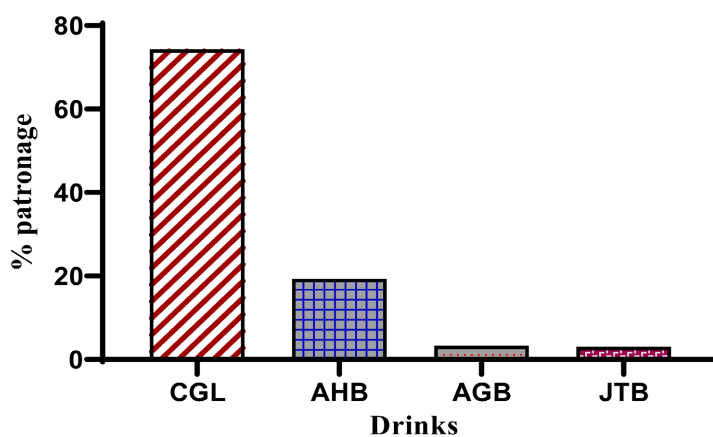


Figure 1. A graph of drink types against percentage patronage.

2.8. Quality Assessment of Experimental Method

Efficiency of method used was evaluated by repeated analysis of 2, 4, 6, 8, 10, and

12 µg/L working solutions prepared from 1000 mg/L mixed standard solution IV (CRM 111355, Certipur^(R)). Mean levels of Pb, Cr and Cu recovered from working solutions, associated Standard Error of Measurements (SEM) and Expanded Uncertainties (U) were computed and presented (**Table 1**).

Quality control data obtained (**Table 1**) indicate that metal extraction protocol used has satisfactory accuracy and precision. Efficiency of the metal extraction method used accuracy ranged from 97.67% (Cr) - 99.80% (Cu) and error margin (0.7% (Pb) - 4.4% (Cr)) below acceptable 5.0%. Thus, experimental method used herein was appropriate for the purpose for which it was adopted.

2.9. Pb Level in AHB

Pb levels in AHB purchased from Mampong, Nsuta and Agona were analysed in triplicates, and data reported as Mean ± SEM. The data were compared with those recorded in analogous studies and WHO acceptable limits. Mean level of Pb (**Table 2**) exceeded 3.50E-02 µg/mL in Brazilian Cachaça [81], 25.0E-02 µg/mL in Spirits [82] and 8.0E-03 - 31.3E-02 µg/mL in Spanish Brandy [83]. Mean level of Pb (0.28 - 0.45 mg/mL) in AHB (**Table 2**) was also above 2.00E-01 µg/mL in some Alcoholic Drinks sold in Brazil [84], 5.0E-01 µg/mL in some Alcoholic Drinks sold in some European countries and 2.0E-02 µg/mL in Cream Liquor [85]. Mean Pb levels in AHB similarly exceeded 24.0E-02 µg/mL in Punch, 15.0E-02 ± 18.0E-02 µg/mL in Aromatic Schnapps, 22.0E-02 ± 18.0E-02 µg/mL in Whisky, 17.0E ± 9.0E-02 µg/mL in Vodka, 11.0E-02 ± 6.0E-02 µg/mL in Brukutu sold in Nigeria [86] and 5.0E-06 µg/mL WHO and the European Union permissible limits [87]. This result is worrying since regular AHB ingestion could cause Pb to build-up in the blood, liver, kidney, lungs, brain, muscles and heart

Table 1. Mean metal levels (mg/L) recovered from certified reference material.

Metal	Certified Value	Recovered Value	Recovery (%)	SEM	Expanded Uncertainty (µg/L)
Pb	0.39 ± 0.05	0.383 ± 1.52E-03	98.20	8.81E-03	7.0E-03 ± 25.0E-03
Cr	1.89 ± 0.17	1.846 ± 8.50E-03	97.67	4.91E-03	44.0E-03 ± 14.0E-03
Cu	15.50 ± 0.63	15.47 ± 18.00E-03	99.80	10.68E-03	3.0E-02 ± 31.1E-03

Table 2. Mean metal levels (mg/mL) in AHB.

Metal	Mampong	SE	Nsuta	SEM	Agona	SE
Pb	0.28 ± 80.4E-04	17.9E-03	0.45 ± 130.0E-04	5.8E-03	0.31 ± 81.8E-04	3.6E-03
Cr	0.66 ± 25.1E-04	1.2.0E-04	0.66 ± 12.9E-04	6.0E-04	0.66 ± 27.0E-04	1.3E-03
Cu	2.92 ± 35.8E-03	17.0E-03	3.18 ± 68.5E-03	3.4E-02	3.22 ± 10.0E-03	5.0E-03

of consumers and then employ its effects on consumers. So, AHB producers and the Ghana food and authority must employ rigorous and efficient monitoring protocols to ensure that acceptable Pb levels are achieved in AHB in order to safeguard wellbeing of consumers.

2.10. Cr Level in AHB

Cr in AHB ranged from 0.66 - 0.67 mg/mL in Mampong, 0.64 - 0.66 mg/mL in Nsuta and 0.65 - 0.66 mg/mL in Agona, whilst the mean level was $0.66 \pm 25.1E-04$ in Mampong, $0.66 \pm 12.9E-04$ in Nsuta and $0.66 \pm 27.0E-04$ mg/mL in Agona (Table 2). These results were at variance with 1.13 µg/L in Canned Pale Lager, 4.32 µg/L in some low-alcoholic beer sold in Italy and 0.05 µg/L WHO tolerable limit for alcoholic drinks [88]. Mean Cr in AHB (Table 2) was similarly above 37.40 µg/L in some commercial brandies, 4.36 µg/L in natural wine spirit and 3.73 µg/L in Pot Still Spirit [89]. Further, Cr in AHB were above 10.50 - 36.00, 7.30 - 14.70, 6.60 - 43.90 and 7.00 - 9.00 µg/L in Champagne, Rose Wine, White Wine and Red Wine respectively [90]. Cr was equally above 0.15 mg/L in Brukutu, an indigenous alcoholic drink in Nigeria, 0.05 mg/L in Raphia Wine [89], 0.012 - 0.06 mg/L in Brazilian Cachaça [91], 12.86 - 13.30 mg/L in Croatian Blackberry Wine [86], 0.09 mg/L in Red Wine [92], 0.01 - 0.41 mg/L in some German Wines [93] and 0.11 mg/L in some wines sold in Brazil, Portugal and Chile [91]. High Cr in AHB may have arisen from the raw materials used in its production [94]. Due to this, continuous AHB consumption could cause Cr to accumulate in the blood, tissues and organs of consumers. Cr causes innumerable health problems and exposure to the level in AHB could cause severe skin and distressed stomach conditions, ulcers, kidney failure and liver impairment in the lifetime of AHB consumers. Thus, it's imperative that food safety establishments in Ghana work to shield consumers from health matters which could originate from constant AHB consumption due to the high Cr levels in the drink.

2.11. Cu Level in AHB

Cu level in AHB (Table 2) ranged from 2.88 - 2.96 mg/mL in Mampong, 3.08 - 3.22 mg/mL in Nsuta and 3.20 - 3.22 mg/mL in Agona whilst mean level was $2.92 \pm 35.8E-03$ mg/mL in Mampong, $3.18 \pm 68.5E-03$ mg/mL in Nsuta and $3.22 \pm 10.0E-03$ mg/mL in Agona. Cu level in AHB exceeded 1.64 - 4.40 µg/mL in Brazilian cachaça and spirits sold in Brazil [91], 0.10 - 8.01 µg/mL in Whisky, Gin, Rum and Liquor [95], 0.10 - 0.77 µg/mL in Cognac alcoholic drink sold in Nigeria [86], 0.10 - 8.70 µg/mL in Gin, Rum and Whisky [96] and 5.0 µg/mL in some alcoholic drinks [97]. It is acknowledged that Cu deficit in humans could affect brain health, damage the immune system among others, its level in AHB was high when compared with those stated in analogous works and WHO permissible limit, it's obvious that AHB when frequently consumed could cause Cu to build up in tissues, bones and other organs of the body. These build ups have harmful outcomes such as oxidative stress, nausea, vomiting and liver damage among others

on humans. Therefore, it's vital that producers of the drink and authorised food monitoring outlets collaborate to implement strict and effective quality control protocols which would cutback Cu in AHB to level protective of health.

Table 3. Mean levels of metals (mg/mL) in CGL.

Metal	Mampong	SEM	Nsuta	SEM	Agona	SEM
Pb	5.83 ± 42.7E-03	21.3E-03	5.6 ± 14.1E-03	7.0E-03	6.5 ± 22.1E-03	11.0E-03
Cr	74.6 ± 25.0E-03	12.5E-03	75.3 ± 50.5E-02	25.2E-02	80.6 ± 14.1E-03	7.0E-03
Cu	34.8 ± 1E-02	5.0E-03	53.3 ± 21.0E-01	105.0E-01	42.3 ± 26.4E-03	13.2E-03

2.12. Pb Level in CGL

Pb in CGL ranged from 5.60 - 6.56 mg /mL. Minimum level 5.60 mg /mL occurred in CGL from Nsuta, whilst maximum 6.56 mg/mL occurred in CGL from Agona. Mean Pb level 5.83 ± 42.7E-03 mg/mL occurred in Mampong whilst mean level in Nsuta and Agona were 5.6 ± 14.1E-03 mg /mL and 6.5 ± 22.1E-03 mg /mL respectively. These exceeded 0.02 µg/mL in Punch, 0.24 µg/mL in Cream Liquor sold in Nigeria [86], 0.035 µg/mL in Brazilian Cachaça [91] and 0.25 µg/mL in Spirits [82]. Similarly, Pb level exceeded 0.008 - 0.313 µg/mL in Spanish Brandy [83], 0.20 µg/mL permitted by some EU countries [84] and 0.50 µg/mL allowed by Brazilian Authorities [98]. These high Pb levels in CGL could affect the gastrointestinal tract, normal functioning of the kidney, reduce hemoglobin synthesis and damage the central and peripheral nervous systems when CGL. This indicates that CGL consumers are risk of the unwanted health outcomes of Pb due to the high levels in the drink.

2.13. Cr Level in CGL

Cr in CGL ranged from 74.64 mg/m/L in Mampong to 80.62 mg/m/L in Agona. Mean level ranged from 74.61 ± 25.0E-03 mg/mL in Mampong to 80.60 ± 14.14 mg/mL in Agona (Table 3). These values exceeded 0.15 mg/mL in Brukutu sold in Nigeria [86], 0.05 mg/mL in Raphia Wine [89] and 12.86 - 13.30 mg/L in Croatian Blackberry Wine sold in Croatia [99]. Additionally, Cr content in CGL likewise exceeded 0.011 µg/mL in Aromatic Schnapps, 0.28µg/mL in Cream Liquor [86] and 3.70, 4.36 and 3.73 µg/L in Natural Wine, Sprits and Pot Still Sprits respectively [89]. Also, Cr in CGL exceeded 10.50 – 36.00 µg/L in Champagne, 7.30 - 14.70 µg/L in Rose Wine, 6.60 - 43.90 µg/L in White Wine and 7.0 - 9.0 µg/L in Red Wine [100]. So, frequent CGL consumption may accumulate Cr in tissues and organs of the human body from where it would execute its' effects on consumers.

2.14. Cu Level in CGL

Cu in CGL ranged from 34.79 mg/mL in Mampong to 84.81 mg/mL in Nsuta

(Table 3). Mean Cu similarly ranged from $34.81 \pm 10.0E-03$ mg/mL in Mampong - 53.31 ± 21.0 mg/mL in Nsuta. Mean Cu level in CGL was Nsuta > Agona > Mampong and this exceeded 1.64 - 4.40 μ g/Lin Brazilian cachaça [91], 0.10 - 8.0 μ g/mL in Whisky, Gin, Rum and Liquor [95] and 0.10 - 0.77 μ g/mL in Cognac [86]. Also, Cu in CGL was above 0.10 - 8.70 μ g/mL in Gin, Rum and Brandy [96], 1.50 μ g/mL in Whisky [101] and 5.0 μ g/mL allowed for alcoholic drinks [88]. This result indicates that regular CGL customers may pile up substantial amounts of Cu in their body fluids, tissues and organs among others. This could prompt heart diseases, brain damage, gastrointestinal irritation and necrotic changes in liver and kidney. Thus, regular CGL consumers are at risk of facing these unwanted effects at some point in their lives.

2.15. Tukey Post-Hoc Multiple Evaluation of Pb, Cr and Cu in AHB and CGL

Tukey post-hoc multiple assessment of Pb, Cr and Cu levels in AHB and CGL indicated that though same company produced AHB, Pb content varied among AHB purchased from the study areas. For instance, mean Pb level in AHB from Mampong and Agona varied significantly at $\alpha = 0.05$ by $48.34E-02 \pm 63.41E-04$ mg/mL, those from Mampong and Nsuta varied by $17.5E-02 \pm 63.4E-04$ mg/mL whilst Agona and Nsuta also varied by $12.7E-03 \pm 63.4E-04$ mg/mL at $\alpha = 0.05$. This was unexpected because same factory produced the AHB drinks. These disparities may have emerged due to Pb leakage from machine parts into drinks during production and apparent lack or abandonment of rigorous and effective quality control arrangement by the producers of AHB. Adulteration of AHB with ample quantities of cheap alcoholic product(s) by bar owners in attempt at maximizing profit could also be a contributory factor to the variations in Pb levels in AHB. Local distillers use Pb soldered brass tubes as condensers in distillation set-ups. Thus, Pb may have leaked from soldered joints of the piping system into distilled alcohols which bar owners used to contaminate AHB. Though Cr level varied by $5.0E-04 \pm 15.9E-04$ mg/ mL in drinks purchased Mampong and Agona, $10.0E-04 \pm 15.9E-04$ mg/mL in drinks from Mampong and Nsuta and $5.0E-04 \pm 5.0E-04$ mg/mL in those from Agona and Nsuta, variations were insignificant at $\alpha = 0.05$. Cu level similarly varied among Mampong-Nsuta ($0.29 \pm 31.8E-03$ mg/mL) and Mampong to Agona ($0.26 \pm 31.8E-03$ mg/mL). These Tukey's post-hoc approximations confirm that AHB drinks sold during this study were contaminated with substandard alcohol produced by local distillers.

Pb level equally varied significantly at $\alpha = 0.05$ among CGL drinks procured in the study areas. Least significant variation ($19.2E-02 \pm 20.4E-03$ mg /mL) occurred in CGL drinks purchased in Mampong and Agona, $78.5E-02 \pm 20.4E-03$ mg /mL in those purchased in Mampong and Nsuta and $97.7E-02 \pm 20.4E-03$ mg/mL in CGL drinks obtained in Agona and Nsuta. Similarly, the disparities were not expected because CGL drinks were produced by same company. These observations could have resulted due to non-adherence to quality control schemes

during production which would have protected consumers from lethal impacts of Pb. Pb leakage from machine parts and drinks contamination with cheap alcoholic product(s) produced using brass tube as condensers by distillers native to the study areas could also account for high Pb level in CGL. Similarly, Cr level varied amongst CGL drinks purchased in the study areas. For example, $0.74 \pm 20.6E-02$ mg/mL variation occurred in CGL drinks purchased in Mampong and Nsuta, $5.98 \pm 20.6E-02$ mg/mL in drinks purchased in Mampong and Agona and $5.24 \pm 20.6E-02$ mg/mL in drinks purchased in Agona and were significant at $\alpha = 0.05$. Although 18.51 ± 8.57 mg/mL variance was observed in CGL drinks purchased in Mampong and Nsuta, 7.58 ± 8.57 mg/mL in those procured in Mampong and Agona and 10.93 ± 8.57 mg/mL in those procured in Nsuta and Agona. However, disparities that occurred in Cu level in CGL were statistically insignificant at $\alpha = 0.05$.

2.16. Total Pb, Cr and Cu Burden in AHB and CGL

Total Pb, Cr and Cu burden in AHB was 4.29 mg/mL in Nsuta > 4.19 mg/mL Agona > 3.86 mg/mL Mampong respectively. Pb contributed 7.25% < Cr (17.09%) and Cu (75.66%) to metal burden in drinks procured in Mampong, 10.48% (Pb) < 15.38% (Cr) < 74.14% (Cu) in drinks procured in Nsuta and 7.39% (Pb) < 15.75% (Cr) < 76.86% (Cu) in those procured in Agona whilst total metal burden in CGL was 129.40 mg/mL (5.05%) Pb > 115.23 mg/mL (64.23%) Cr > 66.43 mg/mL (30.72%) Cu in CGL purchased in Mampong, 8.42% (Pb) < 11.35% (Cr) < 80.23% Cu in those purchase in Nsuta and 5.02% (Pb) < 32.70% Cu < 62.28% (Cr) in drinks purchased in Agona. These values far exceeded WHO permitted limits. These suggest that depending on frequency of consumption and rate of accumulation, such high levels of Pb, Cr and Cu could progressively degenerate muscular and neurological health of consumers. So, it is appropriate producers and regulatory agencies implement schemes to monitor metal contents of AHB and CGL in order to safeguard health of consumers.

3. Conclusion

Preliminary assessment was executed to determine types of herbal bitters regularly consumed in Ghana. Three hundred consumers (N = 300) included in the study were interviewed via face-to-face method. Of these, 70% (n = 210) preferred CGL, 20% (n = 60) preferred AHB, 5% (n = 15) AGB and 5% (N = 15) JTB. Consumption was CGL > AHB > AGB = JTB, and these outcomes were used as a basis to select AHB and CGL for the study. AHB and CGL were purchased in Mampong, Nsuta and Agona, digested with aqua regia and analysed for Pb, Cr and Cu contents. Prior to sample preparation, method recovery study was done by analysing 2, 4, 6, 8, 10 and 12 µg/L working solutions prepared using 1000 mg/L mixed elemental standard solution IV (CRM 111355, Certipur^(R)). Recoveries were 98.20% (Pb), 97.67% (Cr) and 99.80% (Cu) with Standard Error of $8.89E-03$ mg/mL (Pb), $4.91E-03$ mg/mL (Cr) and $10.68E-03$ mg/mL (Cu). Pb, Cr and Cu contents of

AHB were $0.28 \pm 80.4E-04$ mg/mL, $0.66 \pm 25.1E-04$ mg/mL and $2.92 \pm 35.8E-03$ mg/mL (Mampong), $0.43 \pm 13.00E-04$ mg/mL, $0.66 \pm 12.90E-04$ mg/mL and $3.18 \pm 68.5E-03$ mg/mL (Nsuta) and $0.31 \pm 81.8E-04$ mg/mL, $0.66 \pm 27.0E-04$ mg/mL and $3.22 \pm 10.0E-03$ (Agona) respectively. Similarly, Pb, Cr and Cu contents of CGL were $5.83 \pm 42.7E-03$ mg/mL, $74.6 \pm 25.0E-03$ mg/mL and $34.8 \pm 1.0E-02$ mg/mL respectively in drinks purchased in Mampong, $5.6 \pm 14.1E-03$ mg/mL, $75.3 \pm 50.00E-02$ mg/mL and $53.30 \pm 25.0E-01$ mg/mL respectively in drinks purchased in Nsuta and $6.5 \pm 22.1E-03$ mg/mL, $80.6 \pm 14.1E-03$ mg/mL and $42.3 \pm 26.4E-03$ mg/mL in those purchased in Agona respectively. Total Pb, Cr and Cu level in AHB was Nsuta (4.29 mg/mL) > Agona (4.19 mg/mL) > Mampong (3.86 mg/mL) whilst their percentage contribution to metal burden in AHB ranged from 7.39% (Pb) Mampong to 76.86% Cu (Agona). Total Pb, Cr and Cu burden in CGL was Agona (129.40 mg/mL) > Mampong (115.23 mg/mL) > Nsuta (66.43 mg/mL) whilst their percentage to metal burden in CGL ranged from 5.05% (Pb) Mampong to 80.23% Cu (Nsuta). Though same company produced AHB drinks, variations ($48.34 \pm 63.41E-04$ mg/mL (Pb), $17.5E-02 \pm 63.4E-04$ mg/mL (Cr) and $12.7E-03 \pm 63.4E-04$ mg/mL (Cu)) observed in metal levels were significant at $\alpha = 0.05$ in AHB drinks purchased in Mampong and Agona, Mampong and Nsuta and Agona and Nsuta respectively. Similarly, variations ($19.2E-02 \pm 20.4E-03$ mg/mL (Pb), $78.5E-02 \pm 20.4E-03$ mg/mL (Cr) and $97.7E-02 \pm 20.4E-03$ mg/mL (Cu)) occurred in metal levels in CGL purchased in Mampong and Agona, Mampong and Nsuta and Agona and Nsuta respectively were significant at $\alpha = 0.05$. Pb, Cr and Cu levels in both AHB and CGL drinks exceeded those reported in similar works. The high Pb, Cr and Cu levels in the drinks were attributed to leakage of the metals from machine parts during production, contamination of the drinks with cheap alcohols produced by local distillers and geological formations of the areas where raw plant materials were obtained for production. Incidence of high Pb, Cr and Cu levels in the drinks are of serious public health issues and consumers are at risk of grave threats associated with them. So, it's important that producers of drinks and Ghana Food and Drugs Authority collaborate to establish and implement monitoring regimes which would ensure that drinks are examined in order to shield consumers from effects of the metals.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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