

Assessment of Levels of Heavy Metals in Selected Canned Lager and Native Beer (*burukutu*) Sold in Kugiya Market, Jos - Nigeria

ABSTRACT

Write the abstract. It is the summary of introduction, results and conclusion.

1. INTRODUCTION

Alcohols are of various types; some are home-made (locally produced) whereas others are prepared in breweries. The most conventionally consumed are the branded factory-based lager beers including Guilder®, Harp®, Star® and Guinness®. African traditional native beers such as *burukutu* and *pito* are popularly consumed. Foods become hazardous when contaminated by biological agents such as bacteria, fungi and their toxins or by metals from the walls of vessels used for brewing. Contaminants have been reported in foods including native beers [1].

Expatriate on the introduction. This is too short.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Apparatus/Reagents

Crucibles, plastic sample containers, glass rod stirrer, 10ml pipette, filter paper, spatula, and beaker, distill water.

2.1.2 Equipment

Analytical weighing balance; name-Adventurer ohaus (item no. ARC 120, max cap: 3100g, readability: 01g, power requirement: 8-14.5V 50/60 H₂6VA)., Atomic absorption spectrophotometer (AAS PG 940).

2.2 Methods

2.2.1 Collection of samples

Comment [UABY1]: delete

28 10 samples of *burukutu* were obtained from different sellers within Kugiya, market Jos South Local
29 Government Area, Nigeria. 10 samples **each** of branded canned lager beers were purchased from the
30 market and they included Guilder®, Harp®, Star®, and Guinness®.

31 2.2.2 Preparation of samples

32 Samples were poured into properly labeled crucibles and placed in an oven set at 42°C initially
33 and later increased to 60° to dry completely. The samples were then weighed using analytical
34 weighing balance. Thereafter, **the** samples were transferred into appropriately labeled set of
35 crucibles and placed in furnace set at 7000°C for one hour thereby burning off all organic
36 components in all the samples. The furnace was allowed to cool for an hour before the samples
37 were removed. 5ml of distilled water was added to each crucible and the contents stirred using
38 glass rod. The resulting mixture was then transferred into labeled set of plastic containers.
39 Thereafter, 5ml of distilled water was again added to each of the plastic sample containers
40 giving a final volume of 10ml. The samples were then mixed by shaking vigorously to allow the
41 solute dissolve. **(You could not have digested the samples without an oxidizing acid (HNO₃,
42 H₂SO₄ and or HClO₄).** The samples were then analyzed using atomic absorption
43 spectrophotometer (AAS). For each metal, the automated equipment generated standard
44 curves at maximum wavelengths thus: Zn = 214nm, Fe = 248nm, Cd = 229nm, Pb = 283nm. |

Comment [UABY2]: delete

Comment [UABY3]: 7000degC????

Comment [UABY4]: delete

Comment [UABY5]: delete

46 2.4 Statistical analysis

47 Results obtained were analysed using One Way ANOVA (Turkey-Kramer multiple comparisons
48 test). $P = 0.05$ was considered significant.

50 3. RESULTS AND DISCUSSION

51 **3.1 Results:** From table (1), levels of Fe and Zn appear higher compared to Cd and Pb in all the
52 samples. Comparing iron and zinc, concentration of iron was higher than zinc in the beers. Iron
53 level was highest in Star, followed by Guinness, Harp and then Guilder. As for zinc, the trend in
54 descending order was: Guilder, Harp, Star and Guinness. The level of Pb in Star® lager beer

was not detected but was quantified in the other brands. Guinness had the highest level of lead, followed by Guilder, then Harp. In the case of cadmium, all the brands contained it over a range of values in descending order thus: Guilder, Star, Harp and Guinness. Concentration of cadmium in canned Guilder[®] was statistically significant (p=.05) compared with its levels in Harp[®], Star[®] and Guinness[®]. Level of cadmium compared to zinc in all brands of canned beers showed significant (p=.05) increase. Generally, cadmium levels in all the canned beer brands were above the safe limits (p=.05). Comparing level of Cd in Guilder[®] with all other metals, there was significant (p=.05) relationship except Cd in Harp[®] versus Guinness[®], Cd in Harp[®] versus Zn in Guinness[®], Cd in Harp[®] versus Pb in Guinness[®], Cd in Guinness[®] and Pb in Guilder[®], Zn in Guinness[®] and Pb in Guilder[®], Fe in Guilder[®] and Fe in Star[®] (p>.05). Similar trends were obtained with iron, zinc and lead in all the brands of the canned beers and with the reference values.

Comment [UABY6]: significant what??

Table (1): Levels of Pb, Cd, Fe, and Zn (mg/L, ppm) in branded **canned** factory-based lager beer samples

Brand	Cd	Zn	Pb	Fe
Guilder[®]	0.866±0.24	3.527±0.12	0.254±0.63	2.037±0.56
Harp[®]	0.441±0.32	1.885±0.11	0.073±0.14	2.143±0.26
Star[®]	0.804±0.51	1.421±0.41	-0.896±0.22	5.035±0.16
Guinness[®]	0.311±1.12	0.355±0.31	0.350±0.43	4.266±0.34

Comment [UABY7]: this is impossible mean < s.d?

*Values are means of triple determinations (±SEM); n = 10. You stated that the results are mean of triplicate yet you give the value of n = 10?

Table (2) bears the results of *burukutu* samples where the level of Fe was highest in all the samples thus: Fe>Zn>Cd. Pb was not detected by the AAS machine. Mean level of cadmium in

76 *burukutu* is lower than in canned beers. Similarly, mean concentration of zinc in canned beers is
 77 above that in *burukutu*; this trend includes the level of iron as well.
 78 Table (2): Levels of Pb, Cd, Fe, and Zn (mg/L, ppm) in samples *Burukutu*.

BKT	Cd	Zn	Pb	Fe
1	0.363±0.002	0.814±0.001	-0.100±0.005	3.829±0.001
2	0.417±0.003	0.826±0.001	-0.112±0.004	3.843±0.001
3	0.385±0.002	0.818±0.001	-0.100±0.005	3.963±0.002
4	0.402±0.003	0.834±0.001	-0.126±0.004	3.832±0.001

Comment [UABY8]: How could you get negative values of concentration?

Comment [UABY9]: negative values not possible

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 80 **Take the mean values of the metals in *burukutu*, find SEM and add up the result into Table 1.**
 81 **3.2: DISCUSSION:** This work sought to determine the levels of Cd, Pb, Fe, and Zn in canned
 82 branded lager beers and in *burukutu* (native beer) from Kugiya area of Bukuru town, Nigeria.
 83 Generally, metals (mineral elements) are harmful to health when ingested at amount higher than
 84 required by the body. Heavy metals such as cadmium and lead do not serve any biological
 85 functions in living systems. The metals exert their toxicity by forming adducts/and ligands with
 86 organic compounds thereby losing their biological functions which lead to tissue and cellular
 87 necrosis. Metals could inhibit the catalytic role of certain enzyme families.
 88 The presence of metals in alcoholic beverages results from the containers, materials or
 89 ingredients used in its production [2]; [1]; [3]. [4] reported that sources of potential toxic
 90 chemicals in human foods and drinks include food utensils and packaging materials, food
 91 spoilage microbes, agrochemicals, industrial chemical wastes and domestic water). *Burukutu* is
 92 a fermented native drink prepared using clay or metallic (iron, aluminum or galvanized)
 93 containers as fermentation vessels while branded lager beers are made from malted cereal
 94 grains, hops and water at breweries.

96 Although metallic vessels are used in breweries, they are generally of higher quality than the
97 ones used in making *burukutu*.

98 Cadmium accumulates in the human kidney for a relatively long time, 20 to 30 years, and at
99 high doses, produces health effects on the respiratory system and bone disease. It has no
100 known necessary function in the body. It acts as a catalyst in forming Reactive Oxygen Species
101 [5] [6]. It increases lipid peroxidation just as it depletes glutathione and protein-bound
102 sulphhydryl groups and promotes the production of inflammatory cytokines. [7] reported that the
103 most important metabolic parameter for cadmium uptake is a person's possible lack of iron;
104 people with low iron supplies showed a 6% higher uptake of cadmium than those with a
105 balanced iron stock.

106 Cadmium accumulates in biologic systems and has a long half-life. [8] reported that cadmium
107 content in factory based lager beers was low; however, when the consumption of large amounts
108 of certain beverages causes the dietary intake of cadmium to reach the maximum allowable
109 levels, it may be physiologically significant. Consumption of both lager beers and *burukutu* is
110 common in the study area. Cadmium is known to be better absorbed in presence of alcohol
111 albeit the concentration or quantity of the alcohol is not defined [9].

112 Drinkers stand the risk of cadmium toxicity more so that it is used as coating for copper, iron
113 and steel vessels which are used in breweries and homes in preparing alcoholic beverages.
114 Mean levels (ppm) of cadmium in canned Guider®, Harp®, Star® and Guinness® were
115 0.866±0.84, 0.441±0.79, 0.804±0.64, and 0.311±0.93 respectively. These values are above
116 safe limits of the WHO, 0.003ppm and NAFDAC, 0.01ppm (P<0.05). The level of cadmium in
117 *burukutu* samples ranged from 0.363 to 0.417 which is lower than that in canned lager beers. [1]
118 have reported the pH of *burukutu* at 3.2. The pH of some selected branded lager beers was
119 reported to be 5.3 [7]. Therefore, *burukutu* is a 'stronger acid' relative branded canned beers.
120 Acidic pH will favour leaching of walls of containers thereby dissolving metals into the drink.
121 Hence, branded canned beers should be less contaminated with metals from the walls than
122 *burukutu*. But that is not the case in this work.

123 Alcoholic beverage prepared in galvanized metallic containers coated and plated with cadmium
124 caused gastric upset traced cadmium toxicity [10]. Compared to their levels in **bottled**
125 Guider®, Harp®, and Star® as reported by [7], levels of Cd, Fe, and Zn in **canned** containers
126 were higher. However, level of Pb in bottled Guider® [7] was highest relative all other brands of
127 bottled beers as well as reference safe limits for drinking water (p=0.001). Lead is a component
128 of glass as lead trioxosilicate (iv), [PbSiO₃] and its reduction by hydrogen peroxide has been

Comment [UABY10]: reference?

129 reported to cause formation of metallic lead granules [11]. It might be that Pb was leached from
130 the wall of the container

131

132 (glass) into the brew. Lager beers and fermented drinks have been reported to contribute to Pb
133 burden in foods [12]. Daily exposure ($\mu\text{g/kg bw/day}$) to Cd, Pb and Zn through beers in Ethiopia
134 was 0.0006, 0.0024, and 0.676 respectively [13]. The levels may be low but Cd and Pb are
135 particularly toxic even at very low concentrations more so that they are not easily excreted and
136 could accumulate over time and exert their deleterious effects.

137 Lead is also a toxic heavy metal widely distributed in the environment. The entry of lead into the
138 food chain is a major concern, because of its toxicity. Lead has strong effects on haem
139 biosynthesis and erythropoiesis [14]. It can injure the kidney and cause symptoms of chronic
140 toxicity, including hepatic dysfunction, reduced intelligence quotient [15], slow growth,
141 behavioral abnormalities, hearing difficulties and cognitive functions in human. Lead interferes
142 with the activity of three enzymes; indirectly stimulates the mitochondrial enzyme δ -
143 aminolevulinic acid synthetase; directly inhibits the activity of the cytoplasmic δ -aminolevulinic
144 acid dehydratase and interferes with the normal functioning of intra- mitochondrial
145 ferrochelatase, which is responsible for the insertion of iron (II) into the protoporphyrin ring.
146 Anaemia is a frequent outcome of chronic Pb intoxication [16].

147 Zinc is an essential component of several enzymes participating in the synthesis and
148 degradation of carbohydrates, lipids, proteins and nucleic acid as well as in the metabolism of
149 other nutrients. Furthermore, zinc has an essential role in polynucleotide transcription and thus
150 in the process of genetic expression. Its involvement in such fundamental activities probably
151 accounts for all life forms [17].

152 [3] have reported that zinc and manganese were contaminants of local alcoholic beverages
153 (*burukutu* and *pito*). These metals were reported to emanate from either the substrates or water
154 used or from the walls of the brewing vessels used. Levels of Zn obtained in this work were
155 higher than levels in drinking water ($p=0.05$); this is similar to the report of [18]. Zinc levels
156 observed in beers produced in Ethiopia are higher than the values found in beers from other
157 origins [13].

158 Iron is a component of haemoglobin, the oxygen-carrying component of the blood; it is also a
159 component of myoglobin, which helps muscle cells store oxygen. Without iron, ATP cannot be

properly synthesized. Although iron is a component of the antioxidant enzyme, catalase, it is not generally considered an antioxidant because too much iron causes oxidative damage [19].

The safe limits of levels of iron in lager beers as stated by NAFDAC and Standards Organisation, SON, are 0.030ppm and 0.300 respectively. Drinkers of native beers are at risk of iron accumulation although that would be dependent on absorption of iron from the gastrointestinal tract,

gastric emptying, and level of fibre in both the native beers drank and the foods consumed (bioavailability). Excess intracellular iron results in formation and deposition of haemosiderin which can lead to cellular dysfunction and damage [20]. Iron solubility gradually increases during beer making process of germination and fermentation [21]. Iron toxicity can be classified as corrosive or cellular. Ingested iron can have an extremely corrosive effect on the gastrointestinal (GI) mucosa which can manifest as nausea, vomiting, abdominal pain, hematemesis, and diarrhea; patients may become hypovolemic because of significant fluid and blood loss. Haemochromatosis, a disorder of iron metabolism is characterised by excessive iron absorption, saturation of iron-binding proteins and deposition of haemosiderin in the tissues is of the numerous problems associated with iron metabolism; the primary tissues affected are liver, skin and pancreas. Iron deposition in liver leads to cirrhosis, incapacitating liver's numerous biochemical functions, and in the pancreas causes diabetes.

The biochemical significance of iron in alcohol toxicity in drinkers of *burukutu* remains a matter worthy of consideration.

Both branded lager beers and *burukutu* samples analysed contained metals whose levels are above the safe limits for drinking water except Pb in *burukutu* samples that was not detected. Although branded beers are brewed by different brewing industries, it would be reasonable to assume that the quality of water and substrate used are of better grade relative the ones used in preparing *burukutu*. Also, regulatory agencies such as SON and NAFDAC (in Nigeria) do monitor the quality of

187 products produced and sold to drinkers. However, except for Fe whose level is similar in both
188 branded and *burukutu* beer samples, the mean levels of Zn and Cd in the branded beers are
189 higher than in *burukutu* samples.

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191 It is therefore concluded that branded canned beers and *burukutu* are contaminated with
192 respect to Fe, Zn, Cd leached from the wall of the containers. Contamination with the metals is
193 greater in branded canned lager beers than in *burukutu* implying cans used are sources of
194 metal contaminants. Quality of cans to be used in storing lager beers should be of high
195 standards to check metal contamination of lager beers.

196

197 Competing Interest

198 We wish to state here that there are no competing interests in this work.

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Comment [UABY11]: were

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