Determination of Potentially Toxic Metals in Over-the-Counter Pediatric Drugs and Commonly Consumed Beverages in Nigeria: A Health Concern

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Abstract

This study investigated the levels of Cd, Cu, Pb, Mn and Zn in over-the-counter (OTC) pediatric drugs and commonly consumed beverages in Nigeria. This was done to evaluate the possible health implications of these metals in humans who regularly consume the products. Seven commonly administered OTC pediatric syrups and eight brands of beverages were randomly purchased from various pharmaceutical shops and stores located within Ile-Ife, Southwestern Nigeria. Metal concentrations in the acid digested samples were profiled using Flame Atomic Absorption Spectrophotometer (FAAS). The mean concentrations (µg/mL) of the metals in the OTC pediatric drugs ranged from Below Detection Limit (BDL) in Cd to 6.75 ± 0.06 in Pb, while for the beverages the mean concentrations ranged between Below Detection Limit (BDL) in Cd and 8.79 ± 0.23 in Zn. The Pb levels in the sampled drugs were generally above the threshold levels set by the European Food Safety Authority for children's foods and significantly higher than the values of Pb in the beverages. Caution should be exercised to avoid indiscriminate administration of the OTC drugs and beverages to infants and children to reduce the prevalence of toxic metals-induced short or long term effects among them.

Keywords: Pediatric drugs; Beverages; Metals; Health

Abbreviations: FAAS: Flame Atomic Absorption Spectrophotometer; BDL: Below Detection Limit; OTC: Over the Counter; CV: Coefficient of Variation.

Introduction

Monitoring the levels of potentially toxic metals in pediatric drugs and beverages is important because...
potentially toxic metals, such as Cd, Cu, Pb, Mn and Zn, have far reaching health implications at elevated levels in humans [1-5]. Besides, children and youths are the predominant population group that consumes the classes of consumable items under investigation in this study. Apart from constituting a large percentage of current population, this group also constitutes the future work force of any nation. Also, such monitoring will call the attention of both the producers and consumers of the products to safety levels of the items produced and consumed so as to take necessary precautionary health-promoting measures. It has been noted that periodic surveillance of contaminants, such as potentially toxic metals in food related items, is advisable because build-up of such contaminants in human systems can result in unpleasant health challenges [6].

The aggressive attention-catching adverts of various soft drinks and beverages on media outlets and cutting-edge marketing strategies coupled with the often elevated temperatures associated with the sub-Saharan Africa, such as Nigeria, has led to an increase in sales and consumption of beverages by a large percentage of Nigerians after a hectic day of work. Similarly, administering drugs to ailing infants is a common phenomenon in Nigeria. This is partly because the most vulnerable time in human life is the first five years of infancy, and it is during this time that between 70 and 80% of the toxicants that have long resident time in the body are absorbed by humans [7]. One of the most neglected pediatric diseases in Sub-Saharan Africa is metal poisoning, which sadly, still persists in most African countries as a result of the existence of numerous potential sources [8]. In a biomonitoring survey of potentially toxic metal levels in children aged 2 - 6 years in Nigeria, many metals such as Cr, Ni, Mn, Pb and Cd and even radioactive elements were detected [9].

It is almost impossible to specifically attribute metal presence in a matrix to a particular source. However, drinking local water supplies, consumption of chicken eggs, beverages (canned and uncanned), vegetables and other food items, use of lead-containing eye cosmetics and herbal concoctions have been identified as significant contributors to children's daily sources of metal exposure [10-12].

On many occasions, drugs are purchased for use from patent stores without prescription by a competent practitioner. Thus, most of these medications are administered before an ailing child is even taken to the hospital. Low literacy level, unemployment, poverty, poor accessibility to proper healthcare and medication are some of the factors responsible for the patronage of patent medicine dealers. A study by Orisakwe, et al. showed that about 99% of children aged 1-6 were found to have received medication before seeing a doctor and the usual drugs administered ranged from pain relievers to worm expellers, vitamins, anti-diarrheas and anti malaria drugs [9].

The present study evaluated levels of Cu, Pb, Mn, Zn and Cd in popularly consumed beverages and commonly administered over-the-counter (OTC) pediatric drugs sold in Nigeria.

Materials and Methods

Sample Categories and Size

Two categories of substances, commonly consumed by infants and children, namely: beverages and pediatric drugs were used for the present study. Eight beverage types (coded B1 to B8) and seven different types of pediatric drugs (coded D1 to D7) were purchased from retailers within Ile-Ife between January and February, 2015.

Pretreatment and Sterilization of Apparatus

The sample bottles, volumetric flask, Teflon beakers, watch glass and measuring cylinder were first washed with liquid detergent and rinsed with distilled water. These were further soaked in 10% HNO₃ for 48 hours and then finally rinsed with distilled water. The sample bottles, Teflon beakers and watch glass were left to dry in an oven at 105°C, while the volumetric flask and measuring cylinder were allowed to dry at ambient temperatures under conditions that reduced cross contamination to the barest minimum.

Reagents Used and their Sources

The reagents used were of analytical grade and they included nitric acid (Riedel-deHaën, Germany), perchloric acid (Riedel-deHaën, Germany). The water used for reagent preparation was triply distilled.

Sample Digestion

The method described by APHA, et al. [13] was adopted in which case 20 mL of the properly homogenized beverage or drug sample was added into a Teflon beaker. Accurately measured 5 mL of 70% HNO₃ was added and the beaker was covered with a watch glass. This was placed on a thermostatically controlled hot plate operated at 105°C.
at 120°C for about 45 minutes until the mixture became clear. Replenishing of the acid content was done at intervals to avoid bringing the content to total dryness. The beaker was taken off the hot plate and allowed to simmer. Additional 5 mL concentrated HNO₃ and 5 mL HClO₄ were added and the beaker was replaced on the hot plate operated at a digestion temperature of 150°C, and digested further for about 25 minutes after which the digestion was considered complete. The beaker was removed from the hot plate, allowed to simmer and the watch glass was rinsed into the beaker. The content of the beaker was then quantitatively transferred into a volumetric flask and made up with distilled water to the 25 mL mark. The resulting solutions were analyzed for their trace metal concentration using Atomic Absorption Spectrophotometer (AAS) available at Bowen University, Iwo, Osun State, Nigeria. The AAS was operated as per the manufacturer’s manual.

**Quantification of Potentially Toxic Metals in the Sample**

The actual metal concentration in each sample was calculated from the relationship:

$$[M]_a = [M]_i \times \text{d.f.}$$

Where $[M]_a$ = actual metal concentration in sample; $[M]_i$ = instrumental metal concentration obtained; and d.f. = dilution factor.

**Quality Control Measures Adopted**

The quality control measures adopted were blank determination and spike recovery.

**Blank Determination**

Blank determination is an analysis of a sample without the analyte or sample. This was accomplished by measuring out all the reagents used in the sample digestion in the same proportion and digesting them minus the beverage or drug samples. The values of metals obtained were subtracted from the ones of the digested samples.

**Recovery Analysis**

For recovery experiments, a given well homogenized beverage or drug sample was divided into two equal portions A and B. Portion A was spiked with a known concentration (C) of the metal of interest while portion B was left unspiked. Both samples were carried through the digestion stages enumerated earlier. Percentage recovery (%R) was calculated from the relationship:

$$%R = \frac{A - B}{C} \times 100$$

**Statistical Analysis of Results**

The statistical method employed in the trace metal analysis included determination of mean, standard deviation and coefficient of variation.

**Evaluation of Analytical Procedures Used**

The reliability of the analytical procedures used in this study was tested in terms of percentage recovery (%R) and linearity of calibration ($r^2$). From their values for each metal determined (Table 1), a quantitative agreement was observed with recovery values of potentially toxic metals in the samples ranging from 92.9 ± 1.3% of Cu to 96.7 ± 2.7% of Mn. The %R values obtained were within the 70-110% range for recovery as stipulated by the EU Guidelines for evaluating accuracy and precision of a method [14]. For the FAAS used, the standard calibration curves obtained showed high level of linearity with values of $r^2$ ranging from 0.9789 for Pb to 0.9993 for Zn. These values were adjudged reliable to give accurate metal concentrations contained in the samples.

**Table 1: Calibration Parameters of FAAS and Percent Recovery (%R) of Metals.**

<table>
<thead>
<tr>
<th>Metal</th>
<th>Current (mA)</th>
<th>Wavelength (nm)</th>
<th>Calibration Curve ($r^2$)</th>
<th>%R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td>8</td>
<td>228.6</td>
<td>0.9982</td>
<td>95.6±3.5</td>
</tr>
<tr>
<td>Cu</td>
<td>6</td>
<td>325.1</td>
<td>0.9957</td>
<td>92.9±1.3</td>
</tr>
<tr>
<td>Mn</td>
<td>10</td>
<td>280</td>
<td>0.9981</td>
<td>96.7±2.7</td>
</tr>
<tr>
<td>Pb</td>
<td>10</td>
<td>282.9</td>
<td>0.9789</td>
<td>93.8±5.1</td>
</tr>
<tr>
<td>Zn</td>
<td>8</td>
<td>214.2</td>
<td>0.9993</td>
<td>95.2±2.3</td>
</tr>
</tbody>
</table>

**Levels of Potentially Toxic Metals in Pediatric Drugs**

The results of levels of potentially toxic metals in Over-The-Counter (OTC) pediatric drugs are summarized in Table 2. None of the OTC pediatric drugs sampled contained Cd at detectable levels indicating that all the drugs could be safely administered to all the children in the various age groups without fearing any cadmium related ill health consequences.
Copper was not detected (ND) in pediatric drugs coded D1, D2, D5 and D7, while Cu levels (µg/mL) varied between 0.02 ± 0.00 in D6 and 0.22 ± 0.02 in D4. Assuming the drugs are to be administered at 6 hours intervals of 10 mL per dosage, the amount of Cu consumed would be between 0.8 and 8.8 µg/mL per day. These values are quite lower than the Recommended Dietary Allowance and Adequate Intake (RDA/AI) limit of between 200 µg per day for infants in the age bracket of 0-6 months and 700 µg per day for children in the age bracket of 9-13 years. Recommended Dietary Allowance is the average daily dietary intake level sufficient enough to meet the nutrient requirement of about 97% - 98% healthy individuals in a group, while Adequate Intake is a value based on observed or experimentally determined approximations of nutrient intake by a group of health individuals; it is used when an RDA cannot be determined. Thus no adverse effects of copper induced health infarctions are expected as a result of consuming these drugs according to prescription by infants/children. The level of Cu found in the drugs might only serve as supplement of the daily Cu requirement for the consumers. The amount of Cu in the sampled drugs could also ensure the normal growth and development of the children in addition to preventing anemia and strengthening immunity against infections [15].

On the other hand, Mn was detected in all the drug samples at levels that ranged from 0.04 ± 0.00 in D4 to 2.39 ± 0.05 µg/mL in D7. If the drugs are to be administered at 10 mL per dosage on six hourly bases, Mn consumption would range from 1.6 to 95.6 µg per day. This dosage could be dangerous for infants in the age bracket of 0-6 months who require only 3 µg of Mn per day (Table 3) depending on the particular drug, while it is considered safe for children in the age bracket of 9-13 years who could consume up to 1,600 or 1,900 µg of Mn per day, depending on sex. The benefits of Mn as it occurred in the sampled OTC drugs could include facilitating normal skeletal growth in infants and children and quick healing of wounds [3]. Wound healing is a complex process that requires increased production of collagen. Manganese is required for the activation of prolidase, an enzyme that aids the production of collagen.
the amino acid, proline, used for collagen formation in human skin cells [3].

Lead levels in the various drugs, ranging from ND in D6 to 6.75 ± 0.06 µg/mL in D2, were all above the threshold set by EFSA [17]. Thus, only D6 could be regarded as Pb free. The other drugs contained Pb at more worrisome levels especially in D2 where the Pb level could raise fear of developing symptoms of Pb poisoning, such as encephalopathy, nausea, anemia, palsy and neuropathy [18], in infants/children on regular administration of D6. A regular and consistent administration of this drug in children could lead to a decreased IQ and cognitive measures, and increased incidence of attention-related behaviours [19].

Levels of Zn in all the drugs sampled were virtually less than 5.2 µg/mL. Thus, Zn levels were far below the RDA/AI ranges for all the children within 0-13 years range. The implication of the metal levels falling within the tolerable level in the sampled drugs is that it serves the children a whole lot of benefit. For instance, adequate intake of dietary zinc in infants and children reduces their susceptibility to diarrhea, it is estimated that diarrheal diseases result in the deaths of over 1.8 million children less than five years of age in developing countries annually [20]. A 2007 metal analysis of randomized controlled trials concluded that zinc supplementation reduces the frequency, severity, and duration of diarrheal episodes in children less than five years of age [21]. Zinc supplementation is also known to reduce the incidence of lower respiratory infections, such as pneumonia [22].

With the coefficient of variation (CV) values varying from 0.0 in Cd to 200.0 in Cu, it could be deduced that the potentially toxic metals in the drugs were from widely different sources that resulted in diverse amounts of the metals in the drugs analyzed. Total metal load (in µg/mL) of the drugs followed the order: D6 (1.81 ± 0.03) < D3 (3.20 ± 0.07) < D5 (3.21 ± 0.12) < D1 (4.57 ± 0.12) < D2 (6.97 ± 0.06) < D7 (6.99 ± 0.11). The results of total metal concentration in D2 and D7 were particularly worrisome, as Pb constituted the bulk of the metals present in these pediatric drugs, with minimal presence of essential metals present in them. Administering of these drugs to infants and children should either be avoided or done with utmost caution, as regular and continuous administration of such drugs could bring about serious lead induced ill-health consequences in the consumers of such drugs.

Levels of Potentially Toxic Metals in Beverages

Results of levels of potentially toxic metals in the beverages (coded B1-B8) investigated are presented in Table 4. None of the beverages showed any appreciable level of Cd, as it was not detected in any of the beverages; either there was no Cd at all or Cd in the samples occurred at levels below the instrumental detection limit. By implication, those who consume these beverages regularly might not experience health effects, such as liver and kidney damage, diarrhea, stomach pains, osteomalacia, fracture, reproductive failure, immune and central nervous systems damage, psychological disorders, lung cancer and DNA damage for which exposure to Cd has been implicated [23,24,18].

<table>
<thead>
<tr>
<th>Code</th>
<th>Cd</th>
<th>Cu</th>
<th>Mn</th>
<th>Pb</th>
<th>Zn</th>
<th>Total Metal</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>ND</td>
<td>0.31±0.06</td>
<td>0.77±0.04</td>
<td>ND</td>
<td>2.23±0.13</td>
<td>3.31±0.15</td>
</tr>
<tr>
<td>B2</td>
<td>ND</td>
<td>0.33±0.00</td>
<td>0.10±0.03</td>
<td>0.25±0.06</td>
<td>2.60±0.21</td>
<td>3.28±0.22</td>
</tr>
<tr>
<td>B3</td>
<td>ND</td>
<td>0.25±0.02</td>
<td>0.82±0.08</td>
<td>0.93±0.08</td>
<td>4.69±0.31</td>
<td>6.69±0.33</td>
</tr>
<tr>
<td>B4</td>
<td>ND</td>
<td>0.10±0.01</td>
<td>0.04±0.01</td>
<td>0.83±0.03</td>
<td>8.79±0.23</td>
<td>9.76±0.23</td>
</tr>
<tr>
<td>B5</td>
<td>ND</td>
<td>0.17±0.03</td>
<td>0.28±0.03</td>
<td>1.56±0.11</td>
<td>4.89±0.12</td>
<td>6.90±0.17</td>
</tr>
<tr>
<td>B6</td>
<td>ND</td>
<td>0.31±0.02</td>
<td>0.92±0.06</td>
<td>1.31±0.09</td>
<td>ND</td>
<td>2.54±0.11</td>
</tr>
<tr>
<td>B7</td>
<td>ND</td>
<td>0.12±0.02</td>
<td>1.42±0.20</td>
<td>0.52±0.10</td>
<td>2.06±0.22</td>
<td></td>
</tr>
<tr>
<td>B8</td>
<td>ND</td>
<td>0.01±0.00</td>
<td>6.39±0.11</td>
<td>2.84±0.15</td>
<td>1.28±0.07</td>
<td>10.52±0.20</td>
</tr>
<tr>
<td>Mean ± s.d.</td>
<td>ND</td>
<td>0.18 ± 0.14</td>
<td>1.18 ± 2.13</td>
<td>1.14 ± 0.88</td>
<td>3.12±2.89</td>
<td>5.62±6.04</td>
</tr>
<tr>
<td>CV</td>
<td>0</td>
<td>77.8</td>
<td>180.5</td>
<td>77.2</td>
<td>92.6</td>
<td>428.1</td>
</tr>
</tbody>
</table>

Table 4: Levels* of potentially toxic metals in beverages.

*Value = mean of triplicate determinations ± s.d.

Levels below the instrumental detection limit. By implication, those who consume these beverages regularly might not experience health effects, such as liver and kidney damage, diarrhea, stomach pains, osteomalacia, fracture, reproductive failure, immune and central nervous systems damage, psychological disorders, lung cancer and DNA damage for which exposure to Cd has been implicated [23,24,18].
Most of the beverages investigated come in packs of 750 mL. This is the amount served either at home or at ceremonies per attendee. Sometimes, this amount is served to two or three persons. In a situation where someone drinks a whole pack of 750 mL, with Cu range of ND in B7 to 0.33 µg/mL in B2 as shown in Table 4 the possible amount of Cu consumed along would fall within the range 0.0 to 247.5 µg Cu, depending on the type of the beverages consumed. With the RDA/AI values of 440-1300 µg/day for age groups of 4 years and above (Table 5), the quantity of beverages taken per time might not be sufficient to cater for the amount of Cu needed for normal functioning of the human systems; other sources of food have to be relied upon. Copper is a required component of many redox enzymes, including cytochrome oxidase and plays a crucial role in neurotransmission and in the strengthening and formation of connective tissues [25,26]. Relying on these beverages for the sole source of Cu needed in the body systems might lead to Cu deficiency manifesting in neutropenia (abnormally low level of white blood cells), a condition that may be accompanied by increased susceptibility to infections [27,25]. However, the copper present in the sampled beverages might help in energy production and assist in iron metabolism [26].

Manganese levels in the beverages ranged from 0.04 ± 0.01 µg/mL in B4 to 6.39 ± 0.11 µg/mL in B8. This range translates to 30.0-4,792.5 µg/mL in 750 mL pack of the beverages considered. The RDA/AI value for Mn is given as 1,500 – 2,600 µg/day for 4 year old individuals and above. Although Mn is an essential element in the human diet that is responsible for the regulation of reproduction, carbohydrate and lipid metabolism, its deficiency or accumulation of large concentrations in human systems can impair the CNS and disrupt homeostatic mechanisms, especially those involving iron metabolism [28]. Thus, beverages B1-B7 would serve as good Mn supplement sources, while regular consumption of B8 might lead to excessive concentrations of Mn and its attendant ill health manifestations in the body of consumers.

<table>
<thead>
<tr>
<th>Copper (µg/day)</th>
<th>Manganese (µg/day)</th>
<th>Zinc (µg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Life stage</td>
<td>Age</td>
<td>Male</td>
</tr>
<tr>
<td>Children</td>
<td>4-8 years</td>
<td>440</td>
</tr>
<tr>
<td>Children</td>
<td>9-13 years</td>
<td>700</td>
</tr>
<tr>
<td>Adolescents</td>
<td>14-18 years</td>
<td>890</td>
</tr>
<tr>
<td>Adults</td>
<td>19 years and older</td>
<td>900</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>All ages</td>
<td>1000</td>
</tr>
<tr>
<td>Breast feeding</td>
<td>All ages</td>
<td>1300</td>
</tr>
</tbody>
</table>

Table 5: RDA/AI for Zinc, Copper and Manganese in Beverages. Source: FNB/IOM, 2001 [16]

Levels of Pb (µg/mL) ranged from ND in B1 to 2.84 ± 0.15 µg/mL in B8, translating to 0.0-4,792.5 µg/mL in 750 mL pack of the beverages considered. Lead is a highly toxic naturally occurring metal that can cause serious health problems, especially for infants, children and pregnant women. Too much of Pb in human system can cause damage to the nervous systems, red blood cells, brain and kidneys, and lead to increased risk of hypertension and increased incidence of tremor [19]. Beverages, such as B1 and B2 could be suitable for occasional consumption by children as their Pb levels were below 0.5 µg/mL, but others, such as B3, B4, and B6 should be consumed sparingly, while B5 and B8 should be totally avoided by children. On the other hand, beverages such as B1, B2, B3, B4, B6 and B7 as occasional drinks could be considered safe for adults based on the tolerable Pb levels established by EFSA. The implication of the excessive consumption of B3-B8 is that consumers are predisposed to adverse health consequences of high Pb levels in their systems. In the same vein, it could be particularly devastating for a pregnant adult to indiscriminately consume these drinks as accumulation of Pb in the woman retards the growth of her foetus and exposes her developing baby to the hazards of Pb; Pb is released from her bones as maternal calcium which would be used in the formation of the foetus bones is transported to the foetus [29].

Zinc levels in the beverages ranged between ND in B6 and 8.79 ± 0.23 µg/mL in B4. Hence, a pack of 750 mL of the beverages could contain Zn level of up to 6,592.5 µg as seen in B4. Thus, Zn levels fell within the RDA/AI ranges.
of 5,000-8,000 µg Zn/day for children within 4-13 years range, in particular. Generally, values of Zn content in the beverages indicated that the beverages could serve as sources of Zn supplements for humans. It is known that Zn in humans performs catalytic, structural, and regulatory functions in the body [30]. In addition to reducing the susceptibility of infants and children to diarrhea [20], adequate intake of dietary zinc is needed for the immune system to function well, for cell division and growth, wound healing and carbohydrate metabolism; it is also needed for the senses of smell and taste to function properly; the body needs Zn to grow and develop properly during pregnancy, infancy and childhood, and enhances the action of insulin [31-33]. Zinc supplementation is also known to reduce the incidence of lower respiratory infections, such as pneumonia [22].

The coefficient of variation (CV) values of the metals in the beverages varied from 0.0 in Cd to 180.5 in Mn. The high coefficients of variation reflect a wide variation in the prospective sources of the metals in the commonly consumed beverages implying that the sources of these metals in the analyzed beverages cannot be authoritatively established. Total metal load (in µg/mL) of the beverages were of the order: B7 (2.06 ± 0.22) < B6 (2.54 ± 0.11) < B2 (3.28 ± 0.22) < B1 (3.31 ± 0.15) < B3 (6.69 ± 0.33) < B5 (6.90 ± 0.17) < B4 (9.76 ± 0.23) < B8 (10.52 ± 0.20). With no Cd and little Pb in the beverages, moderate consumption of these beverages might generally enhance the body daily requirement of essential micronutrients [34], such as Cu, Mn and Zn. Generally, caution should be exercised to avoid indiscriminate consumption of the beverages as some of them (e.g. B3, B4, B5 and B8) if consumed regularly and excessively could result in bioaccumulation of potentially toxic metals in the body and manifest some short or long term individual or synergistic effects, ranging from malfunction of the organs in the body, increased risk of hypertension to increased incidence of tremor [19].

Conclusion

This study clearly demonstrated that many of the mainstream commonly consumed beverages and OTC pediatric drugs consumed by individuals in all age groups and life stages, are a potential source of potentially toxic metals. In some cases, the extent of the lead levels found in some of the drinks as well as the drugs presents itself as a well established reason for concern, especially considering the fact that the sampled drugs are inadvertently administered to infants and growing children-who are at their most vulnerable stage of development and growth. The results of this study revealed that Zn and Mn exhibited highest levels in the beverages, while Cu and Cd came up with the lowest levels. In contrast, Pb and Mn levels were highest in the sampled pediatric drugs, while Cu and Cd had the lowest levels. The high concentration of Pb in some of the sampled OTC drugs and beverages suggested that the potentially toxic metal contamination might most likely be from anthropogenic sources. It was feared that long-term ingestion of the syrups and beverages containing elevated Pb levels might constitute a significant source of potentially toxic metal exposure to the populace and should therefore be consumed with caution.

References


