Toxicological evaluation of chicken-livers ingested around Mafikeng metropolitan city, South Africa

1,2Raymond Limen Njinga, 3Hilma Rantilla Amwele, 4Kalumbu Gideon Pendapala, 1Victor Makondelele Tshivhase, 5Motsei Lebogang and 4Kgabi Nnenesi Anna
1Centre for Applied Radiation Science and technology, North West University, Mafikeng, South Africa
2Department of Physics, Federal University Dutse, Jigawa State, Nigeria
3Department of Agriculture, Namibia University of Science and Technology, Windhoek, Namibia
4Department of Civil and Environmental Engineering, Namibia University of Science and Technology, Windhoek, Namibia
5Department of Animal Science, North West University, Mafikeng, South Africa

Corresponding author, E-mail: njingaraymond@yahoo.co.uk

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1 ABSTRACT
This study evaluates the health risk associated with consumption of chicken livers contaminated by heavy metals. One hundred and thirty five (135) packaging bags filled of chicken-livers, each bag weighing 0.5 kg were obtained from three supermarket shops around Mafikeng local government municipality and were transported to the laboratory for analysis. Forty five (45) packaging bags filled of chicken-livers with each bag weighing 0.5 kg were obtained from shops SRL3, SPL1 and FVL2 respectively at different periods of the year (2016). They were analysed for macro-metals, trace metals, and human carcinogens using Inductively coupled plasma mass spectrometry (ICP-MS). The evaluated concentrations of these metals were then used to calculate the health risk for adults and children. The hazard index (HI) values obtained for all the age groups were above one making the non-carcinogenic effects significant. Hence, the exposure may pose serious non-carcinogenic effect to the population living around Mafikeng metropolis. The average value of the carcinogenic risk due to Chromium (Cr) for all the age groups and the three shops was found to be 9.99 × 10⁻⁴ implying that 1 persons in every 1000 would be affected.

2 INTRODUCTION
The South Africa Legislation together with the increasing consumer’s demands entails the slaughterhouses’ owners and processors of poultry meat to provide high quality and hygienically safe products. A careful monitoring must be imposed on all factors from the slaughterhouses to the supermarkets. This affects the finished product from farm to consumers (Windal et al., 2010). Edible offal inspection including the liver is one of the basic steps of health impact monitoring. The liver being one of the major organs involved in metabolic processes is considered one of the most important witnesses of any disturbance in the body (Hsu et al., 2007). It is subjected to different types of etiologic attacks: infectious, toxic, metabolic, nutritional and traumatic (Doneley, 2004). Heavy metals from man-made pollution sources are
assumed that eating chicken liver may be bad for human consumption (Nwude et al. 2011). This is because the liver is the organ responsible for the detoxification of body waste products. Meat and liver products of birds and other animals are known to accumulate heavy metals. Studies have shown that some of these metals are essential for the body. These metals include Cu, Fe, Mg, Se and V even Ca and Na (USEPA, 2007). These metals are required for the electron transport system and all required for the electron transport system and all}

continually released into the surrounding waters and soils and may be ingested by the chickens. It is certain that some heavy metals may be ingested in the chicken feet recipes or from the surrounding waters/soils in the environments tend to accumulate in the chicken livers during their lifespan according to the European Food Safety Authority (EFSA, 2006) and the U.S. Environmental Protection Agency (USEPA, 2007). These accrued toxins are then passed on to humans who eat the livers. Indeed, poultry liver consumption needs special attention. The liver is considered an important source of nutrients, such as vitamins, macro elements and trace elements (Oana-Margarita et al. 2014). It is used in pregnant women diet and in nutritional disorders in some countries (Baars et al., 2004). Although studies in different countries were conducted regarding dioxins contents of different foods, very few reports which have been published refer to these contaminants level in offal and in particular in poultry liver (Fernandes et al. 2010; 2011). According to the U.S. Department of Agriculture, the chicken livers can also contain contaminants metals, which may be harmful for human consumption (Nwude et al. 2011). Since the liver filters toxins and other potentially harmful substances out of the blood, it is assumed that eating chicken liver may be bad for our health (Musante et al. 1993). Studies have shown that some metals are required for body structure, fluid balance, and protein structures, hence, they are the building blocks of our bodies (De Smet, 2012). Recently, science has shown that some metals are key for human system and that they function as co-factors, catalysts or inhibitors of all enzymes in the body (Paul et al. 2014). Cu and Fe along with other metals are required for the electron transport system and all cellular energy production. Metals needed in minuscule quantities are usually toxic in greater amounts such as Cu, Fe, Mg, Se and V even Ca and Na (USEPA, 2007). Heavy metals are a common occurrence in the environment and have resulted in human exposure for an immeasurable amount of time. Elevated levels of these contaminants in the environment have been recorded as result of anthropogenic activities (Ogwuegbu and Muhanga, 2005). The typical pathway for human exposure to environmental contaminants sites may include; ground water (ingestion from drinking, inhalation of volatiles, dermal absorption from bathing), surface water (ingestion from drinking, inhalation of volatiles, dermal absorption from bathing or swimming, ingestion during swimming), and soil ingestion, inhalation of particles, inhalation of volatiles, exposure to indoor air from soil gas, exposure to ground water contaminated by soil, ingestion via plants or dairy products and dermal absorption from gardening (HECE, 1995). It is well established that the people of Mafikeng metropolitan city consumed chicken liver commonly known as “sebete” almost on daily bases. They either consumed the livers with the following combinations; cooked spices spinach and mieliepap i.e. smooth maize meal porridge (Hugo & Simon 2012). Research works of this nature are needed to ascertain concentration levels in human due chicken liver consumption. The sources or places where the chicken livers are obtained, sold (shops) or packaged may contain some high levels of metals. The industrial activities and consumer products for chickens have led to the creation of over 70,000 chemicals (Bender, 1992). The rate at which these new chemicals are formulated outpaces the rate at which their safety can be evaluated. So there a lot of health hazard effects due to exposure to these chemicals (Akan et al, 2010). Carcinogenic and non-carcinogenic metals always cause a risk no matter how low the dose is (D’Mello, 2003). This study aims to analyse the contaminants in chicken livers causing, a threat to humans through the ingestion pathway. It focused on classifying quantitatively the identified metals into macro-metals, trace metals, possibly required trace metals, toxic metals and human carcinogens as obtained in the chicken livers grouped into 135 nylon bags with each bag weighing 0.5 kg. Forty five bags with each bag weighing 0.5 kg were obtained from shops SRL3, SPL1 and FVL2.
respectively at different periods of the year (2016). The chicken livers from each of the bags i.e., 45 bags from shop SRL3, 45 bag from shop SPL1 and 45 bags from shop FVL2 were used to evaluate the lifetime average daily dose (LADD) due to carcinogens for all age groups. The work further evaluated the cancer risks associated with the exposure to a measured dose of chemical contaminant evaluated using the incremental lifetime cancer risk. The non-carcinogenic risk assessment was performed based on the hazard quotient (HQ) which characterized the non-carcinogenic risks and is a unit less number. This number expresses the probability of an individual suffering an adverse effect. It is evaluated from the ratio of Lifetime Average Daily Dose (LADD) to the toxicity threshold value i.e. the chronic reference dose (RfD) in mg/kg/day of a specific heavy metal.

3 MATERIALS AND METHODS
3.1 The study site: The Mafikeng local municipality is the capital city of the North-West Province. It is located within the Ngaka Modiri Molema District and is next to the Botswana border. Mafikeng is the seat of the Provincial Legislature and the majority of the National State Departments regional offices and is just a three-hour drive from Johannesburg and about 294km from Pretoria. Mafikeng municipality has a rich and diverse history, which dates to 1852, when the town was founded, and 1899 to 1902 during the Anglo-Boer War, the Mafikeng Siege. The town is home to the Mafikeng Museum, with its antique steel ceiling, old town clock, Sol Plaatje’s history and display of rock species and covers a geographical area of 3,698 km².

3.2 Sample collections and Analysis Using the NexION 300Q ICP-MS Instrument: One hundred and thirty five (135 bags) filled of chicken-livers with each bag weighing 0.5 kg were obtained from three shops around Mafikeng local government municipality and were transported to the laboratory for analysis. Forty five of the bags filled of chicken-livers with each bag weighing 0.5 kg were obtained from shops SRL3, SPL1 and FVL2 respectively at different periods of the year (2016). From each of the shops, a group of 15 bags filled with chicken livers each weighing 0.5 kg were obtained in the months of January to April, 15 groups from May to August and 15 groups from September to December. The measurement was performed using the NexION 300ICP-MS instruments at the North West University, Centre for Applied Radiation Science and Technology. The instrument (Perkin Elmer, Waltham, Massachusetts, U.S.A.) used has been accredited according to ISO standard17025 (European Standard EN ISO/IEC17025:2000). A solution analytical method was used with internal multi-standard calibration method. In the laboratory, 0.5 kg of each of the groups of the chicken-liver sample was air-dried for two weeks. They were ground using an electric mill and ashed for 18 hr at 600 °C. 100 mg each, were digested with mineral acids (a mixture of 9 mL diluted HCl and 3 mL diluted HNO₃) using a MARS 5 microwave digester (CEM GmbH, Kamp-Lintfortstate, Germany). After digestion, the samples were evaporated to near dryness at 800 °C for another 18 hr. The dry ash weight of the chicken-liver samples was recorded. Finally, the residue was dissolved with 1 mL of 40% HNO₃ and diluted with deionized water. The digested samples (aqua regia method) were filtered through the whatman paper (number 42), diluted into 100 mL of deionized water. All the acids used were ultra-pure analytical grade (AA-100, Tama Chemicals Co, Kanagawa, Japan) Water (>18:1 MΩ) which was treated by a Milli-Q water system (Millipore Co., Massachusetts, USA). After diluting the acid solutions to a suitable concentration, metals in the chicken-liver samples were measured using ICP mass spectrometry. For accurate determination of elemental compositions in the samples, a Perkin Elmer Pure Plus NexION Dual Detector Calibration Solution as the Atomic Spectrometric Standard, with the specifications: 200 micro-
grams per liter of Al, Ba, Ce, Co, Cu, In, Li, Mg, Mn, Ni, Pb, Tb, U and Zn were used.

4 RESULTS AND DISCUSSION

The results of metals analysed in the liver samples from the three shops were classified into five groups as shown in Tables 1-5.

Table 1: Macro-metals (needed in large quantity)

<table>
<thead>
<tr>
<th>Metal</th>
<th>SPL1 Conc. ppm Mean*</th>
<th>Range*</th>
<th>FVL2 Conc. ppm Mean*</th>
<th>Range*</th>
<th>SRL3 Conc. ppm Mean*</th>
<th>Range*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium (Na)</td>
<td>126.405</td>
<td>98.675-129.561</td>
<td>59.229</td>
<td>40.561-65.291</td>
<td>157.284</td>
<td>124-165.768</td>
</tr>
<tr>
<td>Phosphorus (P)</td>
<td>206.696</td>
<td>194.671-215.213</td>
<td>105.796</td>
<td>98.452-115.061</td>
<td>263.824</td>
<td>198.578-289.901</td>
</tr>
<tr>
<td>Sulphur (S)</td>
<td>3.139</td>
<td>3.159-5.254</td>
<td>0</td>
<td>ND</td>
<td>4.882</td>
<td>3.417-6.452</td>
</tr>
<tr>
<td>Iron (Fe)</td>
<td>1.3</td>
<td>0.167-3.056</td>
<td>0.542</td>
<td>0.352-0.781</td>
<td>1.022</td>
<td>0.985-1.871</td>
</tr>
<tr>
<td>Copper (Cu)</td>
<td>0.022</td>
<td>0.001-0.045</td>
<td>0.01</td>
<td>0.001-0.051</td>
<td>0.023</td>
<td>0.015-0.056</td>
</tr>
<tr>
<td>Zinc (Zn)</td>
<td>0.149</td>
<td>0.057-0.356</td>
<td>0.094</td>
<td>0.001-0.158</td>
<td>0.156</td>
<td>0.067-0.276</td>
</tr>
</tbody>
</table>

ND = Not Detected, * = 15 liver samples

Table 1 shows the macro elements identified in this study. Macro-metals are the natural elements which the body needs more often and are more important than any other minerals. Among the macro-minerals measured Na (98.675 to 129.561 ppm from shop SPL1), (40.561 to 65.291 ppm, from shop FVL2) and (124 to 165.768 ppm, from shop SRL3), P (194.671 to 215.213 ppm, from shop SPL1), (98.452 to 115.061 ppm, from shop FVL2) and (198.578 to 289.901 ppm, from shop SRL3), Mg (19.815 to 39.067 ppm, from shop SPL1), (12.679 to 25.532 ppm, from shop FVL2) and (30.567 to 52.679 ppm, from shop SRL3) and Ca (2.091 to 8.765 ppm, from shop SPL1), (3.547 to 9.746 ppm, from shop FVL2) and (4.856 to 9.776 ppm, from shop SRL3) were obtained in large quantity. The obtained Na, Mg and Ca serve as principal cations while P serve as principal anion in the human body. Macro-minerals such as Na is a good electrolyte and the body uses electrolytes to maintain acid-base balance and fluid balance (homeostasis) for normal neurological, myocardial, nerve, and muscle function. Electrolyte activity usually activate the neurons and muscles and the macro elements such as Ca and Mg have been associated with impaired insulin release, insulin resistance, and glucose intolerance in experimental animals and humans.

Table 2: Trace metals (required in trace quantity)

<table>
<thead>
<tr>
<th>Metal</th>
<th>SPL1 Conc. ppm Mean*</th>
<th>Range*</th>
<th>FVL2 Conc. ppm Mean*</th>
<th>Range*</th>
<th>SRL3 Conc. ppm Mean*</th>
<th>Range*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lithium (Li)</td>
<td>0.074</td>
<td>0.054-0.095</td>
<td>0.424</td>
<td>0.257-0.769</td>
<td>0.286</td>
<td>0.057-0.586</td>
</tr>
<tr>
<td>Molybdenum (Mo)</td>
<td>0.004</td>
<td>0.002-0.007</td>
<td>0.003</td>
<td>0.001-0.005</td>
<td>0.005</td>
<td>0.001-0.008</td>
</tr>
<tr>
<td>Bromine (Br)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Chromium (Cr)</td>
<td>0.001</td>
<td>0.000-0.003</td>
<td>0.002</td>
<td>0.001-0.004</td>
<td>0.002</td>
<td>0.000-0.004</td>
</tr>
<tr>
<td>Manganese (Mn)</td>
<td>0.028</td>
<td>0.001-0.058</td>
<td>0.013</td>
<td>0.004-0.024</td>
<td>0.027</td>
<td>0.003-0.056</td>
</tr>
<tr>
<td>Silicon (Si)</td>
<td>0.111</td>
<td>0.045-0.252</td>
<td>0.089</td>
<td>0.025-0.135</td>
<td>0.757</td>
<td>0.35-0.972</td>
</tr>
<tr>
<td>Boron (B)</td>
<td>0.041</td>
<td>0.029-0.079</td>
<td>0.029</td>
<td>0.005-0.045</td>
<td>0.073</td>
<td>0.046-0.097</td>
</tr>
</tbody>
</table>

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Although required in very small amounts (Table 2), trace elements such as Li, Mo, Br, Cr, Mn, Si, B and Sc are vital for maintaining health. These trace elements are part of enzymes, hormones and cells in the body. Out of the three shops, Br was only obtained from the liver samples analysed from shop SRL3. The element Br is essential for tissue architecture in humans and all other animals. Also, Sc was obtained from liver samples obtained from shop SPL1. The element Sc can be a threat to the human liver when it accumulates in the body. At a certain amount, Sc becomes dangerous in the working environment. This is because damps and gasses can be inhaled with air and cause lung embolisms, especially during long-term exposure.

Table 3: Possibly required trace metals

<table>
<thead>
<tr>
<th>Metal</th>
<th>SPL1 (Conc. in ppm)</th>
<th>FVL2 (Conc. in ppm)</th>
<th>SRL3 (Conc. in ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean*</td>
<td>Range*</td>
<td>Mean*</td>
</tr>
<tr>
<td>Nickel (Ni)</td>
<td>ND</td>
<td>ND</td>
<td>0.001</td>
</tr>
<tr>
<td>Rubidium (Rb)</td>
<td>0.049</td>
<td>0.024-0.065</td>
<td>0.016</td>
</tr>
<tr>
<td>Strontium (Sr)</td>
<td>0.001</td>
<td>0-0.043</td>
<td>0.002</td>
</tr>
</tbody>
</table>

ND = Not Detected, * = 15 liver samples

Elements are present in different forms in the nature, and these elements are essential for the body to perform different functions. Trace elements (Table 2) are important for cell functions. These elements act as cofactors for many enzymes and serve as centres for stabilizing structures of enzymes and proteins (Osredkar and Sustar, 2011). Some of the trace elements (Table 2) and possibly required trace elements (Table 3) bind to molecules on the receptor site of cell membrane or alternate the structure of membrane to prevent entry of specific molecules into the cell (WCRC/AICR, 2007). The functions of trace elements have a double role; (1) in normal levels, they are important for stabilization of the cellular structures, (2) in deficiency states, they may stimulate alternate pathways and cause diseases. Trace elements have clinical significance and in large quantities may be harmful to the body. As revealed in Table 3, Rb was found in the samples from the three shops (SPL1, FVL2 and SRL3) and ranged from 1.014 ppm to 1.945 ppm with a mean value of 1.417 ppm in samples from shop SPL3. The toxicity of Rb is usually a consequence of the anion and the ingestion of large quantities should always be avoided. In the body, Rb substitutes for K and too much can be dangerous since large amounts can cause hyperirritability and spasms (Lingamaneni et al, 2015).

Table 4: Toxic metals

<table>
<thead>
<tr>
<th>Metal</th>
<th>SPL1 (Conc. in ppm)</th>
<th>FVL2 (Conc. in ppm)</th>
<th>SRL3 (Conc. in ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean*</td>
<td>Range*</td>
<td>Mean*</td>
</tr>
<tr>
<td>Barium (Ba)</td>
<td>0.001</td>
<td>0-0.004</td>
<td>0.001</td>
</tr>
<tr>
<td>Bismuth (Bi)</td>
<td>0.001</td>
<td>0-0.003</td>
<td>ND</td>
</tr>
<tr>
<td>Titanium (Ti)</td>
<td>0.100</td>
<td>0.025-0.145</td>
<td>0.048</td>
</tr>
<tr>
<td>Chromium (Cr)</td>
<td>0.001</td>
<td>0-0.002</td>
<td>0.002</td>
</tr>
<tr>
<td>Aluminium (Al)</td>
<td>0.07</td>
<td>0.025-0.095</td>
<td>0.98</td>
</tr>
</tbody>
</table>

ND = Not Detected, * = 15 liver samples
As revealed in Table 4, the toxic metals such as Ti, Cr, Ba, and Bi are directly toxic to cells and demonstrate hepatotoxicity in vitro (Landrigan et al., 2002). At little concentrations in the diet, these elements are safe. Indeed at trace concentration (Table 4), some of these elements are included in homeopathic medications and in dietary supplements advertised as being effective in enhancing vitality or improving immune function (De Smet, 2012). In higher amounts, many of the elements have been linked to instances of acute or chronic liver injury in human. However, in this study, they were far below the recommended maximum acceptable levels proposed by the Joint FAO/WHO and EC Committees (WHO, 1993 and EC, 2001).

Table 5: Human carcinogens

<table>
<thead>
<tr>
<th>Metal</th>
<th>SPL1 (Conc. in ppm)</th>
<th>FVL2 (Conc. in ppm)</th>
<th>SRL3 (Conc. in ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean*</td>
<td>Range*</td>
<td>Mean*</td>
</tr>
<tr>
<td>Chromium (Cr)</td>
<td>0.001</td>
<td>0.0002</td>
<td>0.002</td>
</tr>
<tr>
<td>Nickel (Ni)</td>
<td>ND</td>
<td>ND</td>
<td>0.001</td>
</tr>
</tbody>
</table>

ND = Not Detected; * = 15 liver samples

Human health risk assessment is a process used to estimate the health effects that might result from exposure to carcinogenic and non-carcinogenic chemicals (Khan et al., 2013). Table 5 shows the spatial distribution of the identified human carcinogens. In shop (SPL1), the concentration of Cr ranged from 0 to 0.002 ppm with mean value of 0.001 ppm while Ni was not detected. In shop (FVL2), both Cr and Ni were detected and their concentrations ranged from 0.001 to 0.005 ppm and 0 to 0.003 ppm with average values of 0.002 ppm and 0.001 ppm respectively. In shop SRL3, only Cr was identified with the concentration range from 0.001 to 0.004 ppm with the mean value of 0.002 ppm.

4.1 Lifetime Average Daily Dose (LADD): The LADD for each of the age groups between 0 - 0.5 years to 20.0 > years were analysed based on the five classified groups;
1. Macro-metals (needed in large quantity),
2. Trace metals (required in trace quantity),
3. Possibly required trace metals,
4. Toxic metals,
5. Human carcinogens.
The LADD of the above five classified contaminant in the liver were calculated based on the relation;

\[
L_{\text{ADD}} = \frac{CF \times CR \times EF}{BW}
\]

where \(CF\) = The concentration of the contaminant in the liver is expressed in milligrams of contaminant per kilogram of liver (mg/kg),
\(CR\) = The amount of liver eaten per day (see Table 6),
\(BW\) = The average body weight for groups (see Table 6),
\(EF\) = The exposure factor that indicates how often the individual has eaten the contaminated liver in a lifetime. The exposure factors for each of the age groups were evaluated based on equation 2 and results presented in Table 6.

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Based on the data in Table 6, the Lifetime Average Daily Dose (LADD) for each of the age groups were plotted for macro-metals, trace metal, possibly trace metals, toxic metals and human carcinogens in the experimented liver samples from three shops as shown in Figures 1-5.

Table 6: Ingestion dose evaluation parameters.

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>BW (kg)</th>
<th>CR (mg/day)</th>
<th>EF</th>
<th>Eating exposure (yrs)</th>
<th>Exposure per week</th>
<th>Eating exposure in a yr per lifetime</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 0.5</td>
<td>7</td>
<td>37</td>
<td>0.16</td>
<td>0.2</td>
<td>3</td>
<td>0.5</td>
</tr>
<tr>
<td>0.5 - 5.0</td>
<td>13</td>
<td>90</td>
<td>0.27</td>
<td>2.5</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>5.0 - 12.0</td>
<td>27</td>
<td>120</td>
<td>0.34</td>
<td>6</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>12.0 - 20.0</td>
<td>57</td>
<td>169</td>
<td>0.34</td>
<td>10</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>20.0 above</td>
<td>70</td>
<td>183</td>
<td>0.29</td>
<td>30</td>
<td>5</td>
<td>70</td>
</tr>
</tbody>
</table>

NB It is assumed that 100% of the contaminant eaten with the liver is absorbed into the body.

Based on the data in Table 6, the Lifetime Average Daily Dose (LADD) for each of the age groups were plotted for macro-metals, trace metal, possibly trace metals, toxic metals and human carcinogens in the experimented liver samples from three shops as shown in Figures 1-5.

**Figure 1:** Lifetime Average Daily Dose of macro-metals in the experimented liver samples from three shops for different age groups

The LADD of macro-metals in the experimented liver samples from three shops for different age groups are presented in Figure 1. The LADD of P, Na and Mg were high compared to the other metals in the liver samples from the three shops.

The LADD of P, Na and Mg were high in shop SRL3 with values of $4.93 \times 10^3$ mg/kg/day, $2.94 \times 10^2$ mg/kg/day and $7.51 \times 10^1$ mg/kg/day respectively for the age group 0.5 – 5.0 years.
Figure 2: The LADD of trace metals in the liver samples from three shops for different age groups.

The LADD of the trace elements in the liver samples from three shops for different age groups are presented in Figure 2. The LADD was high with values of 1.42 mg/kg/day (age 0.5 – 5.0 years), 1.14 mg/kg/day (age 5.0 – 12.0 years), 6.40 x 10⁻¹ mg/kg/day (age 0 – 0.5 years), 7.63 x 10⁻¹ mg/kg/day (age 12 – 20 years) and 5.74 x 10⁻¹ mg/kg/day (age 20 years above) for Si in shop SRL3. For Li, the following values were obtained in both shop FVL2 and SRL3 respectively; 7.93 x 10⁻¹ and 5.35 x 10⁻¹ mg/kg/day (age 0.5 – 5.0 years), 6.41 x 10⁻¹ and 4.32 x 10⁻¹ mg/kg/day (age 5.0 – 12.0 years), 3.59 x 10⁻¹ and 2.42 x 10⁻¹ mg/kg/day (age 0 – 0.5 years), 4.27 x 10⁻¹ and 2.88 x 10⁻¹ mg/kg/day (age 12 – 20 years) and 3.21 x 10⁻¹ and 2.17 x 10⁻¹ mg/kg/day (age 20 years above). The LADD of Mo, Br, Cr, Mn B and Sc were low especially in the shop SPL1.

Figure 3: The LADD of possibly required trace metals in the liver samples from three shops for different age groups.
The Lifetime Average Daily Dose (LADD) of possibly required trace metals in the liver samples from the three shops for different age groups is shown in Figure 3. It shows that the LADD in mg/kg/day was high for Rb in shop SRL3 with the values of 1.2, 2.65, 2.14, 1.43 and 1.07 among the age groups 0 - 0.5 years, 0.5 – 5.0 years, 5.0 – 12.0 years, 12.0 – 20.0 years and 20.0 years above respectively.

**Figure 4:** The LADD of toxic metals in the liver samples from three shops for different age groups

The LADD of toxic metals in the liver samples from shops SPL1, FVL2 and SRL3 are shown in Figure 4 for the different age groups. The LADD of Al was high in shops FVL2 and SRL3 for all the age groups. A comparative high dose was obtained between age groups 0.5 – 5.0 years and 5.0 – 12.0 year in shops FVL2 and SRL3 with values of 1.83 mg/kg/day (FVL2), 2.66 mg/kg/day (SRL3) and 1.48 mg/kg/day (FVL2), 2.15 mg/kg/day (SRL3). A general trend was observed in this study and all the elements/LADD analysed from liver sample obtained from shop SPL1 were low.
The LADD of human carcinogens in the liver samples is illustrated in Figure 5. The intake of Cr was high for all the different age groups and from the samples obtained from the three shops. Based on this value, cancer risk associated to the intake of Cr was further evaluated. Ni was only observed in shops FVL2 for age groups 0 – 0.5 years, 0.5 – 5.0 years, 5.0 – 12.0 years, 12.0 – 20.0 years and 20.0 year and above with the following values in mg/kg/day respectively; 8.46 x 10^{-4}, 1.87 x 10^{-3}, 1.51 x 10^{-3}, 1.01 x 10^{-3} and 7.58 x 10^{-4}.

4.2 Cancer Risks: The cancer risks associated with the exposure to a measured dose of chemical contaminant were evaluated using the incremental lifetime cancer risk. This is the incremental probability of an individual developing any type of cancer over a lifetime due to a 24 hour/day human carcinogenic exposure to a given daily dose of a chemical for 70 years (Winde and Stoch, 2010). The US EPA cancer risk considered acceptable values for regulatory purposes within the range of 1 x10^{-6} to 1 x 10^{-4} (Cairncross et al, 2013). The incremental lifetime cancer risk was measured using the Cancer Slope Factor (CSF). This is the risk produced by a lifetime average dose of 1 mg/kg/day for a specific contaminant (Liefferink, 2011). This risk was worked out for Cr using the slope factors of 0.5 (mg/kg/day)^{-1} in the relation below;

\[ \text{ILCR of Cr in the liver} = \text{LADD} \times \text{CSF} \]

The LADD of Cr in mg/kg/day represents the lifetime average daily dose of exposure to Cr. The incremental lifetime cancer risk (ILCR) over a lifetime due to a 24 hour/day Cr exposure in the liver to a given daily dose for 70 years was measured. In shops FVL2 and SRL3 ILCR were high with similar values of 8.46 x 10^{-4} (0 - 0.5 years), 1.87 x 10^{-3} (0.5 – 5.0 years), 1.51 x 10^{-3} (5.0 – 12.0 years), 1.01 x 10^{-3} (12.0 - 20.0 years), and 7.58 x 10^{-4} (20 years above), compared to shop SPL1 with values of 4.23 x 10^{-4} (0 - 0.5 years), 9.34 x 10^{-4} (0.5 – 5.0 years), 7.56 x 10^{-4} (5.0 – 12.0 years), 5.04 x 10^{-4} (12.0 - 20.0 years), and 3.79 x 10^{-4} (20 years above).
4.3 Non-Carcinogenic Risk Assessment:
The hazard quotient (HQ) characterized the non-carcinogenic risks and is a unit less number that is expressed as the probability of an individual suffering an adverse effect. It is defined as the

$$HQ = \frac{LADD}{RfD}$$

The non-carcinogenic effect to the population is obtained from the summation of all the HQs due to each of the n\textsuperscript{th} individual heavy metals. This is referred to as the Hazard Index (HI) as described

$$HI = \sum_{j=1}^{n} HQ_j = \sum_{j=1}^{n} \left( \frac{LADD}{RfD} \right)_j$$

Where, $HQ_j$, $EDI_j$, and $RfD$ are values of heavy j\textsuperscript{th} metal. An interpretation of HI is based on two extreme conditions: (i) If HI $< 1$, then the exposed population is unlikely to experience adverse health effects (ii) If HI $> 1$, then there may be concern for potential non-carcinogenic effects (USEPA, 1989). In this study, we measured the non-carcinogenic effect due to the following metals; Cr, Ni, Cu and Zn using the reference doses (RfD) in (mg/kg/day) of 0.003, 0.02, 0.037 and 0.30 respectively (USEPA, 2007, DEA, 2010).

![Figure 6: Hazard index measurement among the five different age groups](image)

This section deals with the human health risk assessment that might result from exposure to non-carcinogenic chemicals identified in this study to be; Cr, Ni, Cu and Zn. The exposure assessment was carried out by measuring the Lifetime Average Daily Dose (LADD) divided by the toxicity threshold value i.e. the chronic reference dose (RfD) in mg/kg/day of a specific heavy metal as shown in the relation below;
between the age groups 12.0 – 20.0 years and 20
shops SPL1, FVL2 and SRL3 respectively. Also between the
obtained from shops SPL1, FVL2 and SRL3.
Hazard Index for 0 to 0.5 years were found to be
categories as shown in Figure 6 because of their
behavioural and physiological differences. The
Hazard Index for 0 to 0.5 years were found to be
1.2, 1.48 and 1.53 respectively from samples
obtained from shops SPL1, FVL2 and SRL3.
This factor was high with the following values;
2.66, 3.27 and 3.38 for the age groups 0.5 – 5.0
years with respect to samples from shops SPL1,
FVL2 and SRL3 respectively. Also between the
age groups 5.0 - 12 years the following values
were obtained; 2.15, 2.64 and 2.73 with respect to
shops SPL1, FVL2 and SRL3 respectively.
Between the age groups 12.0 – 20.0 years and 20
years above, the HI values revolve about the
value of one. However, the HI values obtained
for all the age groups were above the value of one
making the non-carcinogenic effects significant.
Because HI > 1, we may say that the exposure
may pose serious non-carcinogenic effect to the
population living around Mafikeng metropolis.
The average value of the carcinogenic risk due to
Cr for all the age groups and the three shops was found to be 9.99 × 10⁻⁴ implying that 9 persons
in every 10000 may be affected.

5 CONCLUSION
Dose response assessment estimates the toxicity
due to exposure levels of chemicals. The cancer
slope factor (CSF, a carcinogen potency factor)
and the reference dose (RFD, a non-carcinogenic
threshold) were used to validate human health
risks in Mafikeng metropolis. From this study,
the HI values were above the threshold value of
one. The average value of the carcinogenic risk
due to Cr for all the age groups and the three

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