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Evaluation of possible human exposure to metals in chicken-livers obtained from market in Mafikeng, South Africa

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ABSTRACT

This study evaluates the health risk associated with consumption of chicken livers due to heavy metals. One hundred and thirty five bags 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. Three sets of forty five packaging bags each filled with chicken-livers were obtained from shops; SRL3, SPL1 and FVL2 respectively at different periods of the year in 2016. They were analyzed for macro-metals, trace metals, possibly required trace metals and human carcinogens using 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 Cr for all the age groups and the three shops was found to be 9.99×10^{-4} implying that 1 person in every 1000 would be affected.

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1. Introduction

The South Africa legislation entails that the owners of slaughterhouses and the processors of poultry meat should be of high quality as the consumer's demand is increasing. This means that a careful monitoring must be imposed on all the slaughterhouses and the processors of poultry meat. This affects the finished product from farm to consumers (1). Edible offal inspection including the liver is one of the basic steps of health impact monitoring. The liver being one of the major organ involved in metabolic processes, is considered to be one of the most eloquent witness of different types of etiologic attacks: infectious, toxic, metabolic, nutritional and traumatic (3). Food from the animal origin is one of the possible causes of exclusion from its consumption. It is certain that some heavy metals tend to accumulate in livers during their lifespan, according to the European Food Safety Authority (4) and the U.S. Environmental Protection Agency (5). These accrued toxins are then passed on to humans who eat the livers. Indeed, poultry liver consumption needs a special attention. The liver is considered to be an important source of nutrients, such as vitamins, macro elements and microelements (6). It is eaten as a diet by some pregnant women for nutritional disorders in some countries (7). Although, studies in different countries were conducted

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regarding dioxins contents of different foods, very few reports have been published on the contaminants levels in poultry liver (8, 9). According to the U.S. Department of Agriculture, the chicken livers can also contain contaminants metals which may be harmful for human consumption (10). Since the liver filters toxins and other potentially harmful substances out of the blood, it is assumed that eating chicken liver may be harmful or our health (11). 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 (12). 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 (13). 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 (5). The occurrence of heavy metals in the environment is very common and it has resulted in human exposure for an immeasurable amount of time. An elevated amount of these contaminants in the environment has been recorded as a result of anthropogenic activities (14).

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 (15). 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 (smooth maize meal porridge). Research works of this nature are needed to ascertain concentration levels in human exposure due chicken liver consumptions. The sources or places where the chicken livers are obtained, sold (shops) or packaged may contain some high levels of contaminant. The industrial activities and consumer products for chickens have led to the creation of over 70,000 chemicals (16). The rate at which these new chemicals are formulated outpaces the rate at which their safety can be evaluated. So there are a lot of health hazard effects due to exposure to these chemicals (17). Carcinogenic and non-carcinogenic metals always cause a risk no matter how low the dose is (18). This study aimed to analyze the contaminants due to the ingestion pathway for human exposure in consuming chicken livers.

2. Materials and methods

2.1. The study site

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.

2.2. Sample collections and Analysis Using the NexION 300Q ICP-MS Instrument

A group of 135 bags filled of chicken-livers with each bag weighing 0.5 kg was obtained from three shops around Mafikeng local government municipality and were transported to the laboratory for analysis. From each of the shops with coded names; SRL3, SPL1 and FVL2, a group of 15 bags 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. A solution analytical method was used with internal multi-standard calibration method at the Centre for Applied Radiation Science and Technology, North West University.

In the laboratory, 0.5 kg of each of the groups of the chicken-liver sample was air-dried for two weeks. They were grinded using an electric mill and ashed for 18 hr at 600 °C. A MARS 5 microwave digester (CEM GmbH, Kamp-Lintfortstate, Germany) was used to digest each 100 mg and after which, the samples were evaporated to near dryness at 800 °C for another 18 hr. 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) and the water (>18:1 MΩ) was treated by a Milli-Q water system (Millipore Co., Massachusetts, USA). After the dilution of the acid solutions to a suitable concentration the metals in the

Table 1. Ingestion dose evaluation parameters.

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

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

chicken-liver samples were measured using ICP mass spectrometry. For accuracy, the Perkin Elmer Pure Plus NexION Dual Detector Calibration Solution was analysed using the Atomic Spectrometric Standard with the specifications: 200 micro-grams per litre of Al, Ba, Ce, Co, Cu, In, Li, Mg, Mn, Ni, Pb, Tb, U and Zn.

2.3. 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;

- Macro-metals (needed in large quantity),
- Trace metals (required in trace quantity),
- Possibly required trace metals,
- Toxic metals,

The LADD of the above five classified contaminant in the liver were calculated based on the relation;

$$\text{Life time average daily dose (mg/kg/day)} = \frac{\text{CF}\times\text{CR}\times\text{EF}}{\text{BW}}$$

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,
BW= the average body weight for groups,
EF= the exposure factor which 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 data presented in Table 1.

$$\text{EF}= \frac{\text{Eating exposur in a year}\times 50 \text{ week per year}}{\text{Eating exposur in a year per lifetime}\times 365 \text{ days per year}}$$

2.4. Cancer Risks

The cancer risks associated with the exposure to a measured dose of chemical contaminant were evaluated using the incremental lifetime cancer risk of 24 hour/day exposed for 70 years (19). The US EPA

cancer risk for regulatory purposes ranged from 1 $x10^{-6}$ to 1 x 10^{-4} (20). The incremental lifetime cancer risk of Cr was calculated as (21);

$$\text{ILCR of Cr in the Liver} = \text{LADD} \times \text{CFS}$$

CSF = Cancer Slope Factor considered to be 0.5 (mg/kg/day)-1 for Cr.
LADD = Lifetime average daily dose of exposure to Cr measured in mg/kg/day.

2.5. Non-Carcinogenic Risk Assessment

The hazard quotient (HQ) characterized the non-carcinogenic risks and is a unit less. This indicates the probability of an individual suffering an adverse effect and evaluated as shown below; $\text{HQ} = \frac{\text{LADD}}{\text{RfD}}$

RfD= toxicity threshold value i.e. the chronic reference dose measured in mg/kg/day of a specific heavy metal.

The non-carcinogenic effect to the population is obtained from the summation of all the HQs due to each of the nth individual heavy metals. This is referred to as the Hazard Index (HI) as described by the USEPA document (22). The Hazard Index (HI) is obtained using the relation below; $\text{HI} = \sum_{j=1}^{n} \text{HQj} = \sum_{k=0}^{n} \left(\frac{\text{LADD}}{\text{RfD}}\right) \text{j}$

where HQ, EDIL and RfD are values of heavy jth metal.

3. Results

The results of metals analyzed in the liver samples

Table 2. Ingestion dose evaluation parameters.

Metal	SPL1 (Conc. in ppm)		FVL2 (Conc. in ppm)		SRL3 (Conc. in ppm)	
	Mean*	Range*	Mean*	Range*	Mean*	Range*
Na	126.405	98.675-129.561	59.229	40.561-65.291	157.284	124-165.768
Mg	31.937	19.815-39.067	16.995	12.679-25.532	40.18	30.567-52.679
P	206.696	194.671-215.213	105.796	98.452-115.061	263.824	198.578-289.901
S	3.139	1.359-5.254	0	ND	4.882	3.417-6.452
Ca	6.85	2.091-8.765	6.375	3.547-9.746	8.855	4.856-9.976
Fe	1.3	0.167-3.056	0.542	0.352-0.781	1.022	0.985-1.871
Cu	0.022	0.001-0.045	0.01	0.001-0.051	0.023	0.015-0.056
Zn	0.149	0.057-0.356	0.094	0.001-0.158	0.156	0.067-0.276

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

Table 3. Trace metals (required in trace quantity)

Metal	SPL1 (Conc. in ppm)		FVL2 (Conc. in ppm)		SRL3 (Conc. in ppm)	
	Mean*	Range*	Mean*	Range*	Mean*	Range*
Li	0.074	0.054-0.095	0.424	0.257-0.769	0.286	0.057-0.586
Mo	0.004	0.002-0.007	0.003	0.001-0.005	0.005	0.001-0.008
Br	ND	ND	ND	ND	0.002	0.001-0.005
Cr	0.001	0-0.003	0.002	0.001-0.004	0.002	0-0.004
Mn	0.028	0.001-0.058	0.013	0.004-0.024	0.027	0.003-0.056
Si	0.111	0.045-0.252	0.089	0.025-0.135	0.757	0.35-0.972
B	0.041	0.029-0.079	0.029	0.005-0.045	0.073	0.046-0.097
Sc	0.007	0.003-0.009	ND	ND	ND	ND

ND = Not Detected, * = 15 liver samples

from the three shops were classified into five groups as shown in Tables 2-6. 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.

4. Discussion

4.1. Macro elements

Table 2 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 9.976 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.

4.2. Trace elements

Although required in very small amounts (Table 3), 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. These elements act as cofactors for many enzymes and serve as centers for stabilizing structures of enzymes and proteins (23).

4.3. Possibly required trace elements

As seen in Table 4, the possibly required trace elements bind to the molecules on the receptor site of cell membrane or alternate the structure of membrane to prevent entry of specific molecules into the cell (24). The functions of trace elements have a double role; (a) in normal levels, they are important for stabilization of the cellular structures, (b) in deficiency states, they may stimulate alternate pathways and cause diseases.

Table 4. Possibly required trace metals

Metal	SPL1 (Conc. in ppm)		FVL2 (Conc. in ppm)		SRL3 (Conc. in ppm)	
	Mean*	Range*	Mean*	Range*	Mean*	Range*
Ni	ND	ND	0.001	0-0.003	ND	ND
Rb	0.049	0.024-0.065	0.016	0.011-0.025	1.417	1.014-1.945
Sr	0.001	0-0.043	0.002	0.001-0.005	0.003	0.001-0.007

ND = Not Detected, * = 15 liver samples

Table 5. Toxic metals

Metal	SPL1 (Conc. in ppm)		FVL2 (Conc. in ppm)		SRL3 (Conc. in ppm)	
	Mean*	Range*	Mean*	Range*	Mean*	Range*
Ba	0.001	0-0.004	0.001	0-0.004	0.001	0-0.003
Bi	0.001	0-0.003	ND	ND	ND	ND
Ti	0.100	0.025-0.145	0.048	0.023-0.087	0.115	0.079-0.136
Cr	0.001	0-0.002	0.002	0.001-0.005	0.002	0.001-0.004
Al	0.07	0.025-0.095	0.98	0.076-1.241	1.421	0.712-1.658

ND = Not Detected, * = 15 liver samples

Table 6. Human carcinogens

Metal	SPL1 (Conc. in ppm)		FVL2 (Conc. in ppm)		SRL3 (Conc. in ppm)	
	Mean*	Range*	Mean*	Range*	Mean*	Range*
Cr	0.001	0-0.002	0.002	0.001-0.005	0.002	0.001-0.004
Ni	ND	ND	0.001	0-0.003	ND	ND

ND = Not Detected, * = 15 liver samples

Trace elements have clinical significance and in large quantities may be harmful to the body. 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 almost always 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 (25).

4.4. Toxic metals

As revealed in Table 5, the toxic metals such as Ti, Cr, Ba, and Bi are directly toxic to cells and demonstrate hepatotoxicity in vitro (26). At little concentrations in the diet, these elements are safe. Indeed at trace concentration some of these elements are included in homeopathic medications and in dietary supplements advertised as being effective in enhancing vitality or improving immune function (12). 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 (27, 28).

4.5. Human health risk assessment

Table 6 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.

4.6. Lifetime Average Daily Dose (LADD) of macro-metals

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 4.93×10^{2} mg/kg/day, 2.94×10^{2} mg/kg/day and 7.51×10^{1} mg/kg/day respectively for the age group 0.5 – 5.0 years.

4.7. LADD of the trace elements

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), 6.40×10^{-1} mg/kg/day (age 0 – 0.5 years), 7.63×10^{-1} mg/kg/day (age 12 – 20 years) and 5.74×10^{-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; 7.93×10^{-1} and 5.35×10^{-1} mg/kg/day (age 0.5 – 5.0 years), 6.41×10^{-1} and 4.32×10^{-1} mg/kg/day (age 5.0 – 12.0 years), 3.59×10^{-1} and 2.42×10^{-1} mg/kg/day (age 0 – 0.5 years), 4.27×10^{-1} and 2.88×10^{-1} mg/kg/day (age 12 – 20 years) and 3.21×10^{-1} and 2.17×10^{-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.

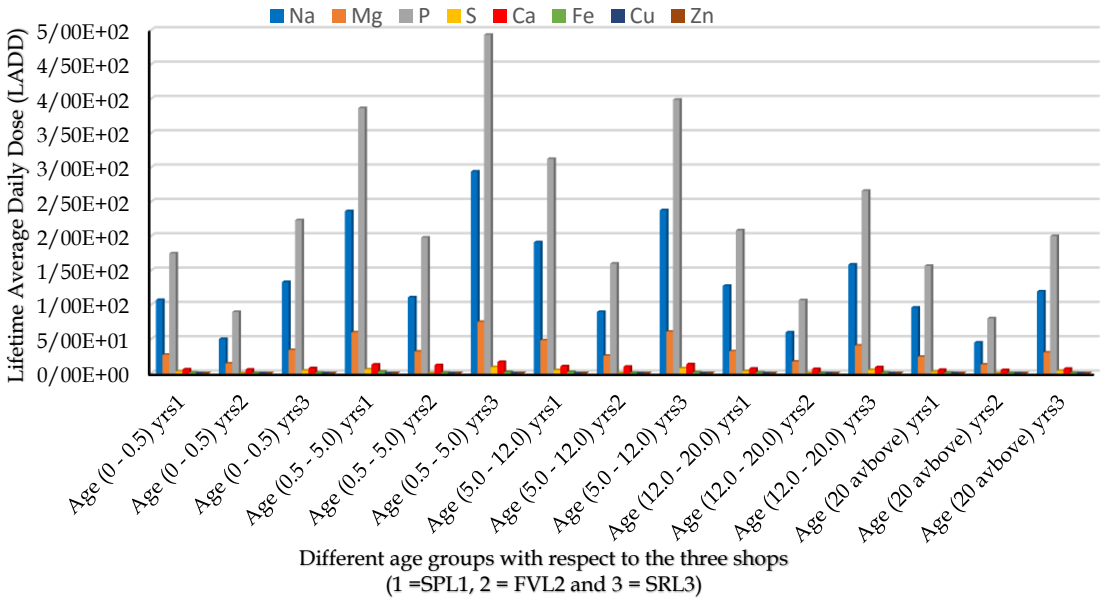

Figure 1. Lifetime Average Daily Dose of macro-metals in the experimented liver samples from three shops for different age groups

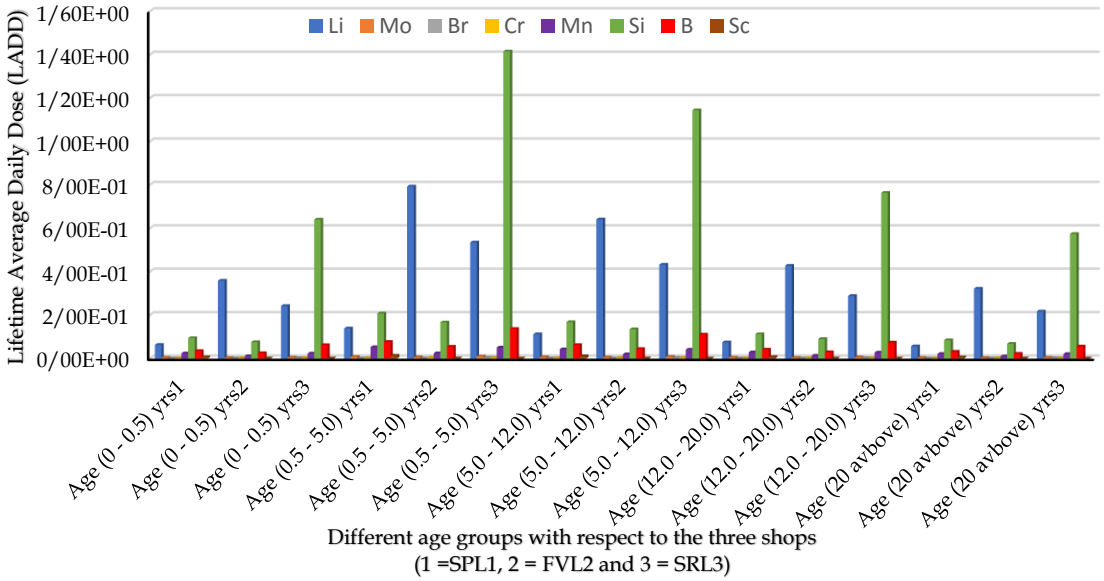

Figure 2. The LADD of trace metals in the liver samples from three shops for different age groups

4.8. LADD of possibly required trace metals

The Lifetime Average Daily Dose (LADD) of possibly required trace metals in the liver samples from the three shops for different age groups are 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.

4.9. LADD of toxic metals

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

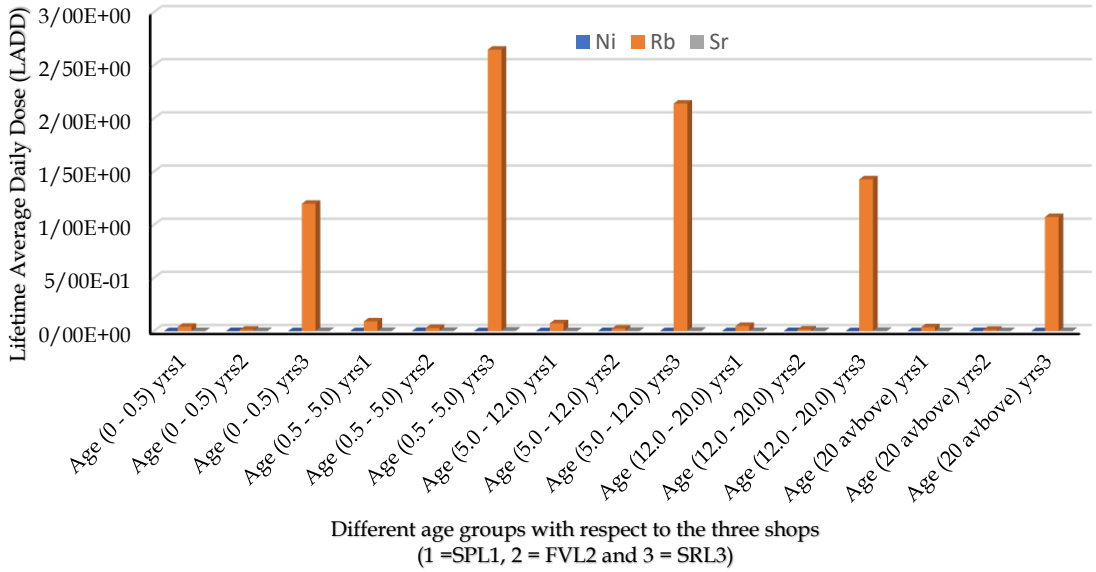

Figure 3. The LADD of possibly required trace metals in the liver samples from three shops for different age groups

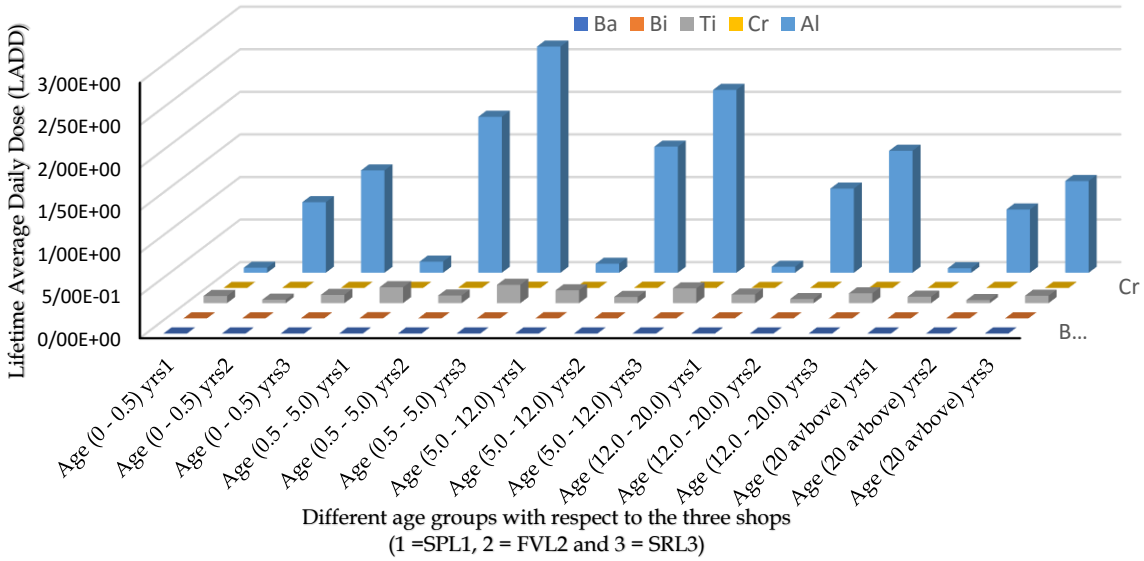

Figure 4. The LADD of toxic metals in the liver samples from three shops for different age group

mg/kg/day (SRL3). A general trend was observed in this study and all the elements/LADD analyzed from liver sample obtained from shop SPL1 were low.

4.10. LADD of human carcinogens

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}.

4.11. Hazard Index and ILCR for 0 to 20 years and above

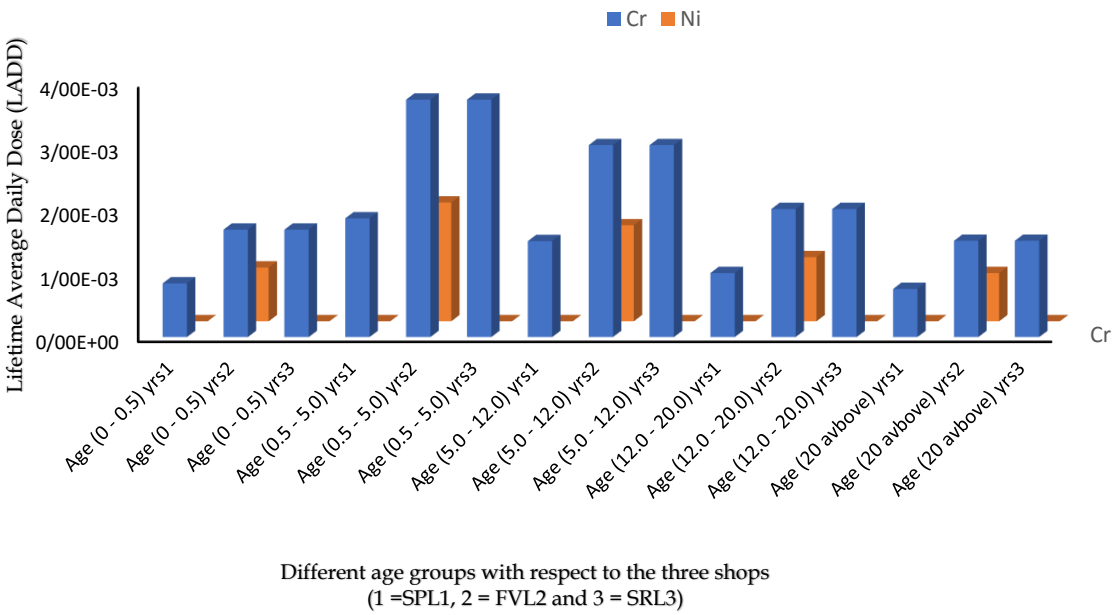

Figure 5. The LADD of human carcinogens in the liver samples from three shops for different age groups

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 posed 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. In shops FVL2 and SRL3 ILCR were high with similar values of 8.46×10^{-4} (0 - 0.5 years), 1.87×10^{-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 7.58×10^{-4} (20 years above), compared to shop SPL1 with values of 4.23×10^{-4} (0 - 0.5 years), 9.34×10^{-4} (0.5 – 5.0 years), 7.56×10^{-4} (5.0 – 12.0 years), 5.04×10^{-4} (12.0 - 20.0 years), and 3.79×10^{-4} (20 years above).

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 shops was found to be 9.99×10^{-4} implying that 1 person in every 1000 is affected. However, the macro elements, trace metals and possibly required trace metals were obtained from the liver samples from the three shops which are healthy for cell, skeletal or bone development. Among the long list of human carcinogens, only Cr and Ni were identified at low amount compared to other studies in the world.

Conflict of interest

The authors have no conflict of interest.

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