

Determination of Lead (Pb), Arsenic (As) and Chromium (Cr) in Urine and Serum of Residents of Gashua, Yobe State, Nigeria

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Abstract: The concentration lead (Pb), Chromium (Cr) and Arsenic (As) in blood serum and urine of male and female donors of different age groups from Gashua were determined. Sample of blood serum and urine of male and female donors of similar age groups were also collected from Damaturu to serve as control. All samples were collected under routine clinical laboratory conditions, pre-treated with acids, digested and heavy metals were analyzed using Atomic Absorption Spectroscopic techniques (ASS). The results indicate that urinary lead, chromium and arsenic levels of the donors from Gashua were significantly higher than those of the control. The concentrations of the metals in blood and urine samples of the male donors were higher than the levels found in samples from the female donors. The high level of these heavy metals in the blood and urine samples might be connected to exposure to the metals through food, air or water, but these need further investigation. The study suggests an immediate assessment of environmental factors responsible for high levels of these metals as they might be connected with the high rates of kidney disease in Gashua.

Keywords: Lead; Arsenic; Chromium; ASS; Urine; Blood; kidney problems; Gashua

1. Introduction

The term “heavy metals” refers to the group of metals and metalloids with atomic density greater than 4 g/cm³, or 5 times or more, greater than water and is toxic or poisonous at high concentration [1].

Exposure to heavy metals may be through food, water, air or soil. Workplace can also bring about heavy metal exposure, as several industries produce or use those metals. In the body each metal is different in how it behaves and where it is found. Exposure alone does not cause any harm or disease. Heavy metals may enter the human body, plants, and animals via ingestion, inhalation and absorption through the mucous membranes or skin, and manual handling of vehicle emissions. The major source of airborne contaminants includes arsenic (As), cadmium (Cd), nickel (Ni), cobalt (Co), palladium (Pd), lead (Pb), platinum (Pt), vanadium (V), zinc (Zn), antimony (Sb), and rhodium (Rh) [2]. Water can also be polluted by leaching from industries and consumer wastes, acid rains can exacerbate this process by releasing heavy metals trapped in soils. Any metal or metalloid species may be considered a “contaminant” if it occurs where it is unwanted, or in a form or concentration that causes a detrimental human or environmental effect. Human exposure to heavy metals through air, water, soil and food is demonstrated in Figure 1 [3].

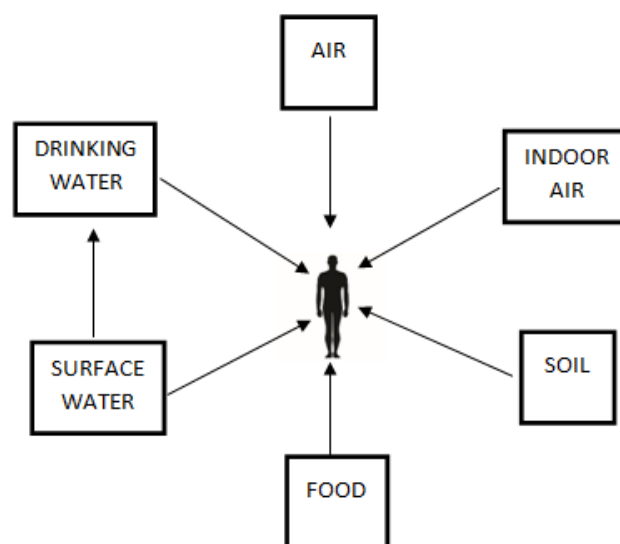


Figure 1 [3]: Pathways of human exposure to heavy metals

1.1 Heavy Metals Toxicity

Other heavy metal toxins also exist but the most important heavy metals with respect to human health is lead, arsenic, cadmium and mercury. These metals exposure may be via ingestion, inhalation and absorption through food, water, soil or air. Exposure these metals may cause symptoms such as persistent fatigue, irritability, loss of appetite, stomach discomfort and constipation, insomnia, headache, anemia, nausea, vomiting, diarrhea, tiredness, muscle and joint pains and sometimes it can be asymptotic. The effects of these metals are potential concern for all humans, which include disruption of metabolic function in different ways, poor muscle coordination, nerve damage, increase blood pressure, reproductive problems, anemia, brain damage, lung cancer, bone defects, mental retardation in children,

damage to nervous system, fatal infant encephalopathy, gastrointestinal problems, sensor neural deafness, spontaneous abortion, congenital paralysis, development delay, metal poisoning, kidney and liver damage and in extreme case, death. These metals are highly toxic and must be monitored on a regular basis. Due to their extreme toxicity, heavy metals must be determined in biological fluids such as blood and urine [4, 5].

1.2 Heavy Metals Diagnosis and Exposures

The determination of heavy metals in human blood serum can be utilized as indicators for several pathological conditions, the simultaneous detection of certain metals in the serum offers a very interesting approach in the diagnosis and treatment of various diseases [6, 7]. As the blood transport metals to the body tissues, the blood test is best for detecting recent heavy metals poisoning and for measuring levels of minerals in the body [5]. Population based reference intervals for trace elements in blood are useful to explore geographical and temporal variation [8].

Like blood analysis, urine analysis also provides a useful indicator of exposure to toxic heavy metals for several diseases. Measuring urine heavy metals is an accepted method for assessing the presence of heavy metals toxic in an individual [9]. The main determinants of heavy metal between individuals in urinary metals were aged, sex, area of residence, and the frequency of certain food intake mainly fish and shellfish [10]. The availability of reference value in human tissue represents an important indicator to the health status of the general population and occupational groups exposed to trace elements in human tissue [11]. Because of differences in the rates of excretion for toxic metals, urine tests are indicative of cumulative exposures or total body burden for some heavy metals (e.g lead) and recent exposure for others (e, g mercury) [12].

Heavy metals can also bind with vital cellular components like enzymes, structural proteins, and nuclear acid, and interfere with their functions. Symptoms and effects of heavy metals may vary from metal to metal compound, and the amount involved. Broadly long-term exposure can cause carcinogenic, central and peripheral nervous system, and circulatory effects. In the entire body heavy metals results in many damage to many organs. Heavy metal poisoning may be instantaneous (acute) or long term (chronic)[5, 13].

Acute exposures to heavy metal occur when people are exposed to high content of the metal at one time. For example, leaded toy swallowing by kids can cause sudden exposure to lead. These types of exposure generally occur from things you are aware of and may cause serious health effects or even death [2]. Chronic exposures on the other hand are long-term exposure to low levels of heavy metals which also causes health problems. These poisoning symptoms can be severe, but obviously often and develop much more slowly over time than symptoms cause by acute exposure. Symptoms of chronic toxicity include headaches, constipation, weakness, muscle and joint pains among others [2].

1.3 Statement of problem

Studies have shown that there is high prevalence of kidney problems in Gashua community, hundreds of people have died and hundreds are still suffering from kidney related diseases. The disease may be associated with exposure to high levels of heavy metals through food, air or water. Therefore, in an attempt to investigate the possible causes of these killer disease in the area, this study will investigate the levels of some heavy metal toxins in blood and urine of the inhabitants of the area, which might be associated with the major health hazard in the area of study.

1.4 Aims and Objective of the Study

The aim of this research is to determine the concentrations of heavy metals in human blood serum and urine samples of people living in Gashua and the objectives are;

- (i) To determine the levels of lead, arsenic and chromium in male and female blood samples of different age groups
- (ii) To determine the levels of lead, arsenic and chromium in male and female urine samples of different age groups
- (iii) To compare the results obtained with cited literature values of heavy metals in blood and urine in order to assess possible risks of heavy metal exposure.

1.5 Significance of the study

The results of this study are expected to provide information which will serve as possible indicators of exposure to lead, arsenic and chromium and the need to explore the possible sources of these metal toxins which are associated to kidney problems and other health problems.

1.6 Scope of the study

The study is limited to assessment of the levels of heavy metals; arsenic (As), lead (Pb), and chromium (Cr) in urine, and blood serum of people (volunteers) living in Gashua.

2. Materials and Methods

2.1 Ethical Approval

The study involved human subjects; therefore I applied for an ethical clearance which was approved by the Hospital Management Board Damaturu, Yobe State, Nigeria.

2.2 Procurement of Relevant Details of Volunteers

Relevant details of all volunteers were obtained through a personal interview and hospital card details with the assistance of the General Hospital Gashua laboratory staff. All subjects were patients who visited the hospital during the period of study. The detail information includes gender, age, and history of any illness or disease. Similar

details were also obtained from volunteers from Damaturu as control samples.

2.2 Sampling

2.2.1 Collection of Urine Samples and Pretreatment

Twenty-five milliliter (25ml) of a spot morning sample was collected from each participant in a sterile polyethylene container. The urine sample was filtered and concentrated nitric acid (0.2ml of acid 20ml of urine) was added to the aliquots and stored at 4°C. All samples were analyzed within 48 hours of collection. Test and control samples were collected at random from male and female subjects across all age groups.

2.2.2 Digestion of Urine Samples

One milliliter (1ml) of sample was transferred into centrifuging bottle and 2ml of a 1:1 mixture of concentrated hydrochloric and nitric acids (1ml conc. HCl + 1ml conc. HNO₃) was added. The sample was digested at 300 W for 4 minutes, which was sufficient to remove any interfering matrix within the samples. The resulting solution was evaporated almost to dryness to remove excess acid, and then diluted with 5ml de-ionized water. Blank sample was prepared using the sample procedure as for the samples but without urine.

2.2.3 Collection of Blood Samples

Five milliliter (5ml) of blood sample (test and control) was collected from each participant and put into a sterile plain plastic bottle. This was done by vein puncture by a qualified nurse using pyrogen free sterile disposable syringes under contamination control conditions.

The blood was allowed to clot for about 30 minutes and was centrifuged to separate serum from the cellular fraction. The stopper was removed and the serum was carefully drawn using micro-pipette and poured into a 4ml plastic vial, avoiding the transfer of the cellular components of the blood. All specimens that were not analyzed within 48 hours were kept frozen. Blank sample was prepared using the sample procedure as for the samples but without blood.

2.3 Samples analysis

2.3.1 Analysis of Metals in Urine

The digested urine sample solutions and blank were analyzed for Pb, As and Cr using Atomic Absorption Spectrophotometer (Buck scientific model 210 VGP)

2.3.2 Analysis of metals in Blood

The blood serum (0.5ml) was diluted with 5ml de-ionized water and the concentrations of Pb, As and Cr were determined using Atomic Absorption Spectrophotometer (Buck scientific model 210 VGP).

3. Results

3.1 Results

The mean concentrations of the studied heavy metals lead(Pb), arsenic (As), and chromium(Cr) in the blood and urine samples of donors are presented in Figures 2 to 7, while Figure 8 presents comparison of the mean concentrations of Pb, As and Cr (mg/l) in blood of donors of different studied age groups.

Figure 2 presents the mean concentrations of the studied heavy metals (Pb, As, and Cr) in the blood of donors based on age group but regardless of sex. Highest levels of all the studied heavy metals were found in donors of ages 21-30 and lowest levels were found in donors of ages 51-60 years.

Figure 3 presents the mean concentrations of the studied heavy metals (Pb, As, and Cr) in the blood of female donors based on age group. The levels of the metals ranged from 0.0 mg/l Cr to 0.065 mg/l Pb.

Figure 4 presents the mean concentrations of the studied heavy metals (Pb, As, and Cr) in blood of male donors based on age group. The levels of the metals ranged from 0.0 mg/l to 0.12 mg/l.

Figure 5 presents comparison of the mean concentrations of Pb, As and Cr (mg/l) in test blood samples (T) and control blood samples (C) of different age groups. The metals levels ranged from 0.0 mg/l to 0.12mg/l

Figure 6 presents mean concentrations of the studied heavy metals (Pb, As, and Cr) in urine of donors of different age groups but regardless of sex. The levels of the heavy metals ranged from 0.0 mg/l to 0.2 mg/l.

Figure 7 presents mean concentrations of Pb, As, Cr in urine of female donors of different age groups. The levels of metals concentration ranged from 0.00 mg/l to 0.06 mg/l.

Figure 8 presents mean concentrations of the studied heavy metals (Pb, As, and Cr) in urine of male donors of different age groups. The levels of metals concentration ranged from 0.0 mg/l to 0.2 mg/l.

Figure 9 presents a comparison of mean concentrations of the studied heavy metals (Pb, As, and Cr) in blood and urine of donors of different age groups. The levels of metals concentration ranged from 0.00 mg/l to 0.25 mg/l.

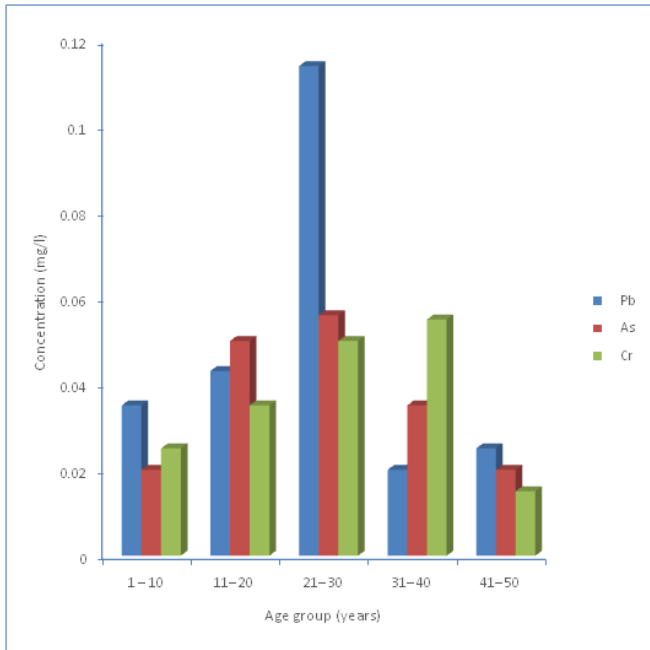


Figure 2: Mean concentrations of Pb, As and Cr (mg/l) in blood of donors of different age groups

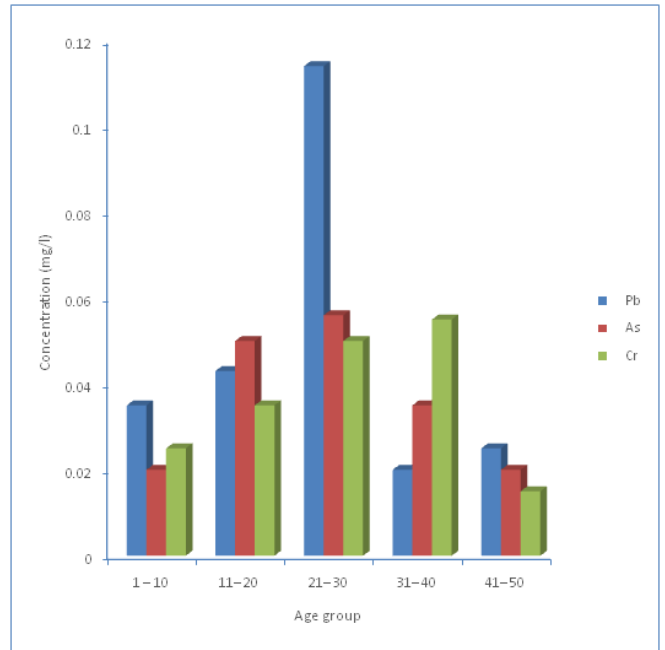


Figure 4: Mean concentrations of Pb, As and Cr (mg/l) in blood of male donors based on age

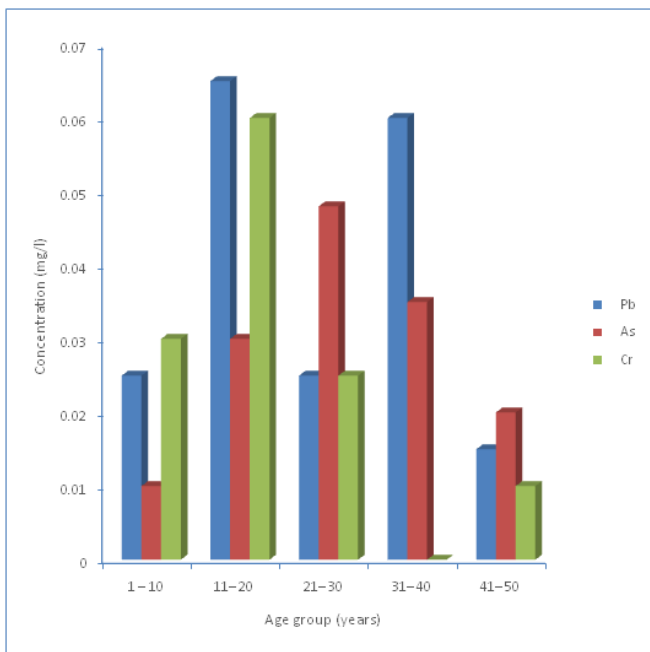


Figure 3: Mean concentrations of Pb, As and Cr (mg/l) in blood of female donors based on age

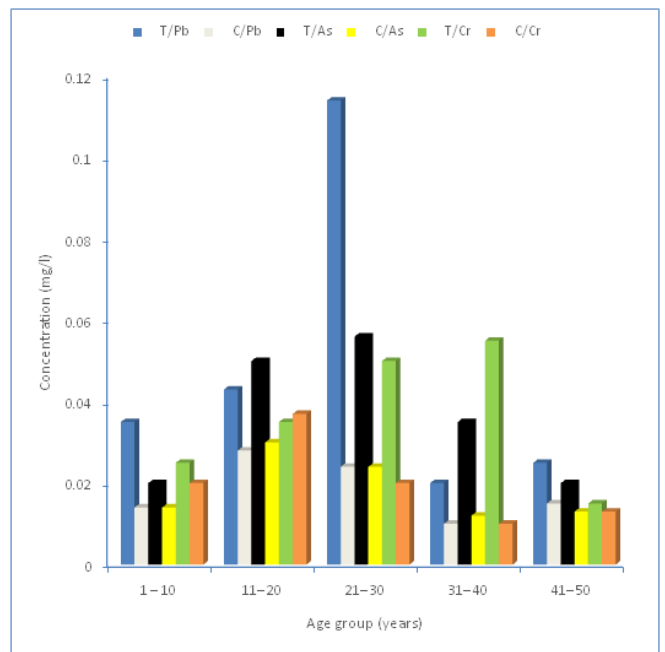


Figure 5: Comparison of the mean concentrations of Pb, As and Cr (mg/l) in test blood samples and control blood samples of different age groups

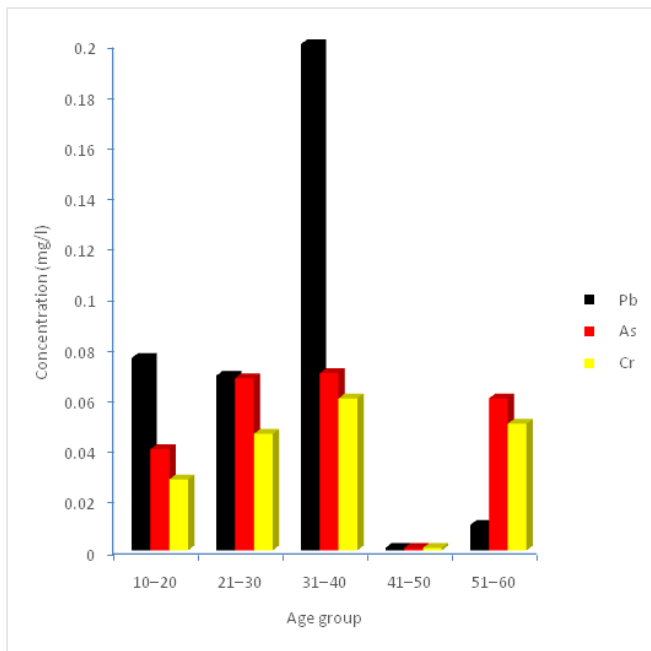


Figure 6: Mean concentrations of Pb, As and Cr (mg/l) in urine of donors of different age groups

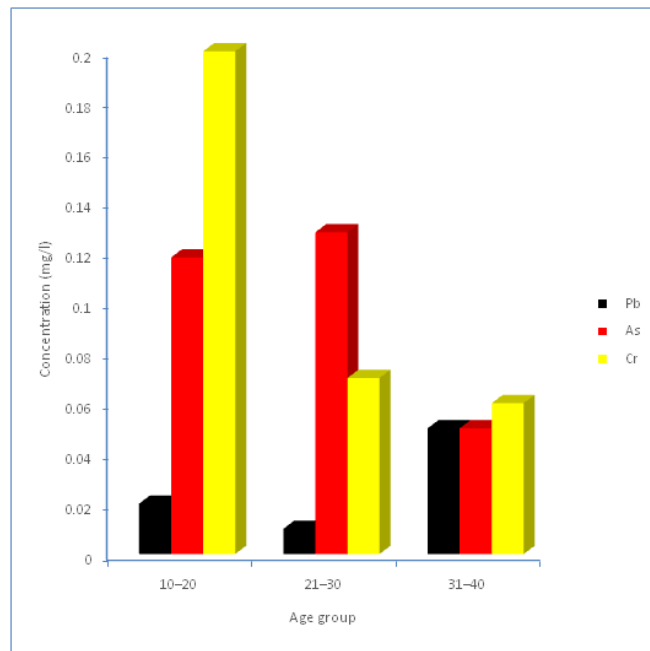


Figure 8: Mean concentrations of Pb, As and Cr (mg/l) in urine of male donors based on age

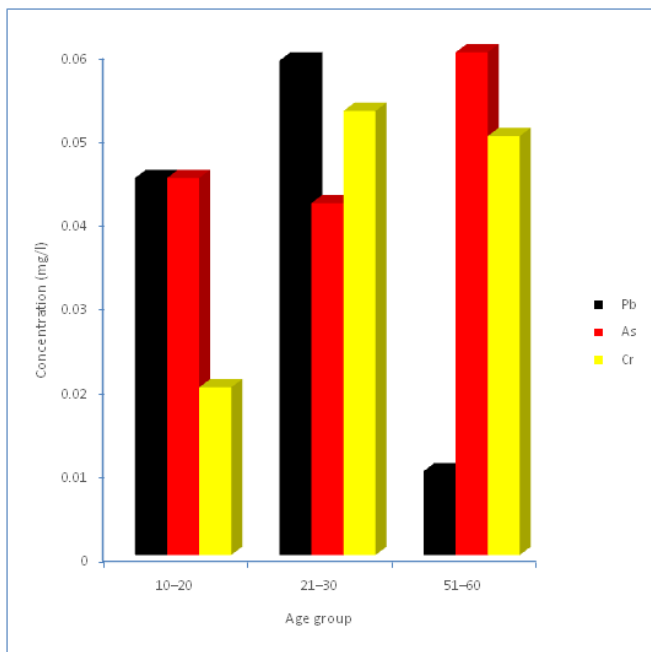


Figure 7: Mean concentrations of Pb, As and Cr (mg/l) in urine of female donors based on age

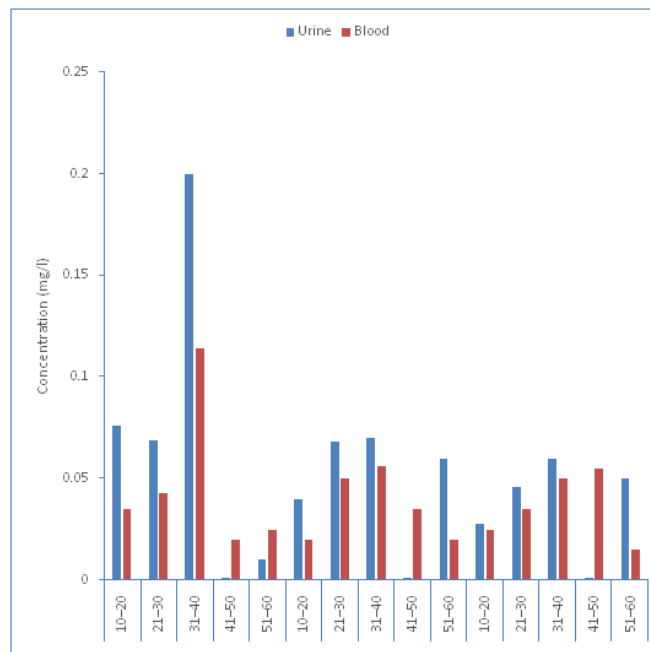


Figure 9: Comparison of the mean concentrations of Pb, As and Cr (mg/l) in blood and urine of donors of different age groups

3.2 Discussion

3.2.1 Lead (Pb) in Blood and Urine

The mean concentration of Pb in the blood of donors regardless of sex (figure 2) shows the following decrease in order of concentration with respect to age group; 21-30 (0.114±0.003 mg/l) years > 11-20 years (0.043±0.002 mg/l) > 1-10 years (0.035±0.003 mg/l) > 41-50 years (0.025±0.002 mg/l) > 31-40 years (0.020±0.003 mg/l). The Pb levels were all lower than reference value of 0.1 mg/l of value lead in the body [14], with exception of Pb in blood of donors of age group 21-30 years. This level of Pb

has reached the level at which source of exposure of lead should be investigated [15]. Age group 1-10 with 0.035 ± 0.003 mg/l is less than $5 \mu\text{g/l}$ level [16], which may impair development in children as indicated [12], and is in line with lower reports [17]. 31-40 years result of 0.020 ± 0.002 mg/l provides a bench mark of successful prevention of health effects [18].

The mean concentration of blood Pb of female samples (figure 3) based on age groups (years) is in order of 11-20 (0.065 ± 0.003 mg/l) > 31-40 (0.060 ± 0.002 mg/l) > 21-30 (0.25 ± 0.002 mg/l) = 1-10 (0.025 ± 0.003 mg/l) > 41-50 (0.015 ± 0.001 mg/l). All the Pb concentrations of the age groups are less than $10 \mu\text{g/dl}$ as recommended [16], but level less than $10 \mu\text{g/l}$ of lead may cause health effect as there is no safer level of lead [12, 15, 18]. The level of lead does not increase with age but variation may be based on physiological and environmental factors [8].

The mean concentration of blood Pb of male samples (figure 4) based on age groups (years) is in order of 21-30 (0.070 ± 0.005 mg/l) > 11-20 (0.055 ± 0.003 mg/l) > 1-10 (0.037 ± 0.002 mg/l) > 41-50 (0.025 ± 0.001 mg/l) > 31-40 (0.002 ± 0.001 mg/l). Pb concentration of 0.002±0.001 of age group 31-40 years is less than the normal group age 20-40 years found in both normal and firefighter groups of Jeddah and Yanbu cities while 0.070 ± 0.005 mg/l of age 21-30 years is higher than the age range of the two group concentrations [19]. All the above Pb concentrations of the age groups less is than CDC recommended level [12], and are greater than the range of males 40-70 years found in Danish subpopulation [20], but only 31-40 years provide a bench mark for prevention [18].

Comparison of the mean concentrations of blood Pb, As, and Cr (mg/l) in blood test samples and blood control samples of different age groups (figure 5) shows that the levels of Pb in the test samples were higher than the control samples. Significant differences were observed between the test and the control samples. The blood lead in the test samples indicates levels of concern as the investigated levels were higher than the safe level exposure [15], but both the result found in children is lower than CDC level which may impair development [12, 21]. The level of blood Pb in this study is similar to other values reported [19, 20].

The mean concentration of urine Pb (figure 6) based on different age group (years) ranged from 0.001 ± 0.001 mg/l for 41-50 years to $0.20 \pm$ mg/l for ages 31-40 years. These Pb concentrations are in line with normal urinary lead level with exception of age group 31-40 (0.2 ± 0.02 mg/l) [22]. The concentration of age group 41-50 is in within range [23].

The mean concentration of urine Pb of female donors based on age groups (years) is in order of 51-60 (0.01 ± 0.005 mg/l) < 10-20 (0.045 ± 0.003 mg/l) < 21-30 (0.059 ± 0.003 mg/l) (figure 7). The female Pb concentrations in the urine are within the normal range [22], and similar results with other studies [10, 12, 16]. They attributed the variations observed to differences on

tribes, age, sex, frequency of food intake (mainly fish) and rate of excretions.

The mean concentration of urine Pb of male donors ranged between 0.01 mg/l for 21-30 years age group to 0.05 ± 0.01 mg/l for age 31-40 years (figure 8). The levels of Pb in the blood of the males were lower than the levels found in the females' blood, but all the male urinary lead concentrations were within the normal levels in human [22]. The result of the male urinary lead levels is similar to urinary lead levels reported in other studies [16, 24].

Comparison of mean concentration of urine Pb to blood Pb based on age groups (years) shows urine lead was higher than blood lead with exception in age group 41-50 and 51-60 years (figure 9). The high range of urine Pb to blood Pb indicates that urine is excreted at fast rate within the age of 10-40 years.

3.2.2 Arsenic (As) in Blood and Urine

The mean concentration of blood As (figure 2) is in order of 21-30 (0.056 ± 0.003 mg/l) years > 11-20 years (0.05 ± 0.01 mg/l) > 31-40 years (0.035 ± 0.002 mg/l) > 41-50 years (0.02 ± 0.01 mg/l) = 1-10 years (0.02 ± 0.01 mg/l). The As concentrations are higher than the level of arsenic of HD patient [25]. The As concentrations of the different age groups are above the range of serum arsenic of higher and lower concentration in urine, urinary protein, and urea of renal insufficiency patients, with age group 41-50 and 1-10 years within the range of arsenic in packed cells of the same patients [26]. The concentration of As in age group 41-50 and 1-10 years are within the normal range, 31-40 years is slightly higher [27]. The difference in results may be based on diet particularly fish intake [27].

The mean concentration of blood As of female donors (figure 3) based on age groups (years) is in order of 21-30 (0.048 ± 0.004 mg/l) > 31-40 (0.035 ± 0.002 mg/l) > 11-20 (0.03 ± 0.01 mg/l) > 41-50 (0.02 ± 0.01 mg/l) > 1-10 (0.01 ± 0.005 mg/l). The difference in concentration agrees with results other reports [27, 28]. Age groups 41-50 and 1-10 are within the normal range and permissible limit of healthy subjects with 31-40 age groups slightly higher [14, 27].

The mean concentration of blood As of male donors (figure 4) based on age groups (years) is in order of 21-30 (0.056 ± 0.002 mg/l) > 11-20 (0.05 ± 0.001 mg/l) > 31-40 (0.035 ± 0.003 mg/l) > 41-50 (0.02 ± 0.01 mg/l) = 1-10 (0.02 ± 0.01 mg/l). The As concentrations are lesser than the normal content found in healthy male subjects [29], but age group 41-50 and 1-10 years are within the normal range of arsenic content with 31-40 slightly higher [14, 27], this indicate that arsenic may play essential role in human health [25]. The concentrations of arsenic of the different age group are within the normal range and firefighters found in Yanbu city [19].

The mean concentrations of blood as of test samples were higher than values found in the blood of control samples (figure 5). Differences in the types of environmental

exposure to pollutants may be responsible for the observed variations and differences in the test and control samples.

The mean concentration of urine As (figure 6) based on different age group (years) is in order of 31-40 (0.07 ± 0.01 mg/l) >21-30 (0.068 ± 0.003 mg/l) slightly >51-60 (0.06 ± 0.01 mg/l) >10-20 (0.04 ± 0.01 mg/l) > 41-50 (0.001 ± 0.001 mg/l). The order of concentration are higher values [16, 29], but within the range of acceptable risk level and normal spot urinary arsenic level [30, 31]. The differences in As concentration is due to rate of excretion, time taken of sample, and nature of diet [24, 27].

The mean concentration of urine As of female donors indicates that As was high in female of age groups 51-60 years as compared to other age groups (figure 7). This variation was contrary to the levels found in male subjects where As levels in urine was high in age group 21-30 compared to the older donors (figure 8). A possible variation may be health status, nutrition and various lifestyles.

Comparative analysis of the levels of As in blood and urine (figure 9) revealed that urinary lead concentrations were higher than blood lead concentrations with exception of age group 41-50 which were lower [27]. The observed levels of As in the blood and urine samples is the cause for concern because long term exposure to As even at low concentration is a great risk to health. Higher fetal and infant mortality, developmental delay, diminished intellectual ability and attention deficit disorders have been associated with high arsenic levels in water, grains, meat, rice etc [32]

4.2.2 Chromium (Cr) in Blood and Urine

The result of variations in mean Cr concentration in blood did not show a regular variation based on age or gender Cr (Figure 2-6) but all the levels were higher than normal range of serum chromium level [17, 33]. High levels of Cr in human system have been shown to lead to kidney, liver, and blood cells disorders [34].

4. Conclusion

4.1 Conclusion

The results show that there is higher level of heavy metals (As, Cr, and Pb) in blood and urine samples of donors from Gashua community when compared with the control sample and other studies. The high concentrations indicate that the tested subjects were exposed to heavy metals probably through food, air, or water. As and Pb have been shown to cause serious health problems such as cardiac and kidney disorders. Though, analysis of the food consumed by the people in the area of the research was not conducted, but food and other routes of exposure may be possible for the high levels of heavy metals in the blood and urine samples. The heavy metals exposure through these routes may be a good transfer pathway which the metal can reach the consumers. Therefore, presence of these metals in blood and urine of Gashua inhabitants may

be a likely cause for the kidney problems experienced in the area of research.

4.2 Recommendation

- There is need to analyse other toxic metals like cadmium and mercury in blood and urine samples of the inhabitants.
- Heavy metal contents of the staple foods in the area of research should be investigated.
- In order to get rid of accumulated heavy metals, the source must be investigated and be eliminated.
- The sample size should be increased and monitoring should be conducted for a period of between six months to one year for proper monitoring and assessment of environmental and physiological factors which changes with time.
- The Government of Yobe State should focus attention on the area of research by involving in all relevant bodies to tackle the problem of kidney in the area of research in order to safeguard human health.

4.3 Acknowledgement

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References

- [1] Hawkes, S.J. "What is a "Heavy Metal?" Journal of Chemical Education, 74(11), pp.1374, 1997.
- [2] Oregon Health Authority. (2001). "Heavy Metals and Your Health: Frequently asked questions about testing, treatment and prevention". Oregon Public Health Division Office of Environmental Public Health.
- [3] Kozłowska, K. Polkowska, Ż. Przyjazny, A. and Namieśnik, J. "Analytical Procedures Used in Examining Human Urine Samples." Polish Journal of Environmental Studies, 12(5):503-521, 2003.
- [4] Nielan, M.W.F. and Marvin H.J.P. (2008). "Challenges in Chemical Food contaminants and Residue Analysis, in Y Pico (ed.), Food Contaminants and Residue Analysis." Comprehensive Analytical Chemistry, vol.51:1-28.
- [5] Great Plain Laboratory, GPL. (2014). "Heavy Metal and Your Health: Heavy Metal Testing." Fax 9130: 841-6207.
- [6] Bárány, E. Bergdahl, I.A. Bratteby, L.E. Lundh, T. Samuelson, G. Schutz, A. Skerfving, S. and Oskarsson, A. (2002). "Trace element levels in Whole blood and serum from Swedish adolescents". Environ. Res, vol.286:129-141.
- [7] Canellas, C.G.L. Carvalho, S.M.F. De Jesus, E.F.O. Anjos, M. Jand Lopes, R.T. (2006). "Trace and major elements in serum of patients with chronic

- myelogenous leukemia". *J. of Radioanal. Nucl. Chem.*, 269(3):631-634.
- [8] Grandjean, P. Nielsen, G.D. Jergensen, P.J. and Harder, M.(1992). "Reference intervals for trace elements in blood: Significant of risk factors." *Scand J Clin Lab Invest*, 52(4):321-37.
- [9] Walter, C.J. (2009). "The Benefits of Pre- and Post-Challenge Urine Heavy Metal Testing: Part 1." *Alternative Medicine Review*, 14(1):3-13.
- [10] Aguilera, I. Daponte, A. Gil, F. Hernandez, A.F. Godoy, P. Pla, A. and Ramos J.L. (2010). "Urinary levels of arsenic and heavy metals in children and adolescents living in the industrialized area of Ria Huelva (SW Spain)." *Environmental Interaction*, 36(6):563-9.
- [11] Kucera, J. Bencko, V. Sabbioni, E. and Vander Vanne. M.T. (1995). "Review of trace elements in blood, serum, and urine for the Czech and Slovakia populations and critical evaluation of their possible use reference value." *Sci Total Environ*, vol. 166:21-34.
- [12] Agency for Toxic Substances and Disease Registry (ATSDR). (2014). "Toxicological profile." *Us department of Health and Human Services, Atlanta GA: Public Health Service, August.*
- [13] Landis, W.G. Sofield, R.M. and Yu, M-H. (2000). "Introduction to Environmental Toxicology: Molecular Substructures to Ecological Landscapes, 4th ed.", Florida: CRC Press, Boca Raton, pp.269.
- [14] Singh, R. Gautam, N. Mishra, A. and Gupta, R. (2011) "Heavy metals and living systems: An overview". *Indian J Pharmacol*, 43(3):246-253.
- [15] NHMRC. (2009). "Information Paper-Blood Lead Levels for Australians." Australia: Australian Government.
- [16] Centers for Disease Control and Prevention (CDC). (2013). "Third National Report on Human Exposure to Environmental Chemicals." Atlanta, GA: CDC.
- [17] Bazz, A.Nriagu, J.O. and A.M Linder, A.M. (2008). "Determination of toxic and essential elements in children's blood with inductively couple plasma-mass spectrometry." *J Environ Monit*, 10(10): 1226-32.
- [18] Steven, G.G. and B. Weiss, B. (2006). "Neurotoxicology." *PMCID*, 27(5):693-701.
- [19] Al-Malki, A.L. (2009). "Serum heavy metals and hemoglobin related compounds in Saudi Arabia Firefighters." *Journal of Occupational Medicine and Toxicology*. vol.4:18.
- [20] Kristiansen, J. Christiansen, J. M. Iversen, B. and Sabbioni, E. (1997). "Toxic trace element reference levels in blood and urine: influence of gender and lifestyle factors." *Sci Total Environ*, 204(2):147-60.
- [21] David, C.D. (2013). "Lead levels-blood." *Medical Encyclopedia: Medline Plus.*
- [22] Tiez, N.W.(1995). "Clinical Guide to laboratory Test 3rd ed." Philadelphia, PA: W.B, Saunders.
- [23] Saracoglu, S. Soylak, M. and Elci, L. (2003). "Separation/Preconcentration of trace heavy metals in urine, sediment and dialysis concentration by coprecipitation with samarium hydroxide for atomic absorption spectrometry." *Elsevier. Talanta* 59:287-293.
- [24] Bjermo, H. Sand, S. Nalsen, C. Lunth, T. Enghardt, Barbieri, H. Pearson, M. Lindroos, A.K. Johnson, B. A. Barregard, L. and Darnerud, P.O.(2013). "Lead, mercury, and cadmium in blood and their relation to diet among Swedish adults. *Food Chem Toxicol*, vol. 57:161-9.
- [25] Mayer, D.R. Kosmus, W. Pogglichtsch, H. Mayer, D.and Bayer, W. (1993). "Chemistry and Analysis of Arsenic Species in Water, Food, Urine, Blood, Hair, and Nails." *Biol Trace Elem Res*, 37(1):27-28.
- [26] Zhang, X. Cornelis, R. De Kimpe, J. Mees, L. Vanderbiesen, V.and Vanholder, R. (1995). "Total arsenic determination in serum and packed cells of patients with chronic renal insufficiency." *Fresenius J Anal Chem*, vol. 353:143-147.
- [27] Zhang, X Cornelis, R. De Kimpe, J. Mees, L. and Lamiere, N. (1997). "Specification of Arsenic in Serum, Urine, and Dialysate of Patients on Continuous Ambulatory Peritoneal Dialysis." *Clinical Chemistry*, 43(2):406-408.
- [28] Rusyniak, D.E. Arroyo, A. Acciani, Froberg, B. Kao, L. and Furbee, B.(2010). "Heavy metal poisoning: management of intoxication and antidotes." *EXS*, vol. 100:96-365.
- [29] Zhang, X. Cornelis, R. De Kimpe, J. Mees, L. Vanderbiesen, V. De Cubber, A. and Vanholder, R. (1996) "Accumulation of arsenic species in serum patients with chronic renal disease." *Clin Chem.*, vol. 353:1231-1237.
- [30] Murray, L. Daly, F. Little, M. and Cadogan. M. (2011). "Toxicology Handbook 2nd Ed." Australia: Churchill living stone.
- [31] WHO. (2000). "Towards an assessment of the socioeconomic impact of arsenic poisoning in Banglades." World Health organization, Geneva.
- [32] Lewis, M.Worobey, J. and Ramsay, D.S. (1992). "Prenatal exposure to heavy metals: effect on childhood cognitive skills and health status." *Pediatrics*, vol. 89:1010-5.
- [33] David, C.D. (2013). "Chromium-blood test." *Medical Encyclopedia: Medline Plus.*
- [34] Dayan, A.D. and Paine, A.J. (2001) "Mechanisms of Chromium toxicity, carcinogenicity and allergenicity: Review of literature from 1985 to 2000". *Human and Experimental Toxicity*, 20(9):439-451