

## ANALYSIS OF HEAVY METALS IN URINE SAMPLES OF PREGNANT WOMEN FROM MAKURDI METROPOLIS

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### Abstract

Analysis of heavy metals in urine samples of pregnant women was carried out across various occupational and geographical location of Makurdi using Atomic Absorption Spectrophotometry AAS. Results of the analysis shows the mean concentration and standard deviation to be  $0.045 \pm 0.10$  for As,  $0.070 \pm 0.07$  for Pb,  $0.130 \pm 0.10$  for Ni  $0.150 \pm 0.10$  for Cd and  $0.610 \pm 0.40$  for Cu. From the analysis, Cu had the highest concentration in all the five hospitals followed by Cd. Fifty urine samples were analyzed in order to assess the body burden of the pregnant women with the metals and health implication of the latter to the pregnant women and their fetuses. The concentration of Cu, As and Ni were within the permissible limit but that of Pb and Cd were above the recommended values by WHO. The study recommends that before conception, women of child bearing age should include levels of heavy metals check and possibly detoxification to avoid transfer of these metals to the growing fetus as these metals are not filtered out by the placenta.

**Keywords:** Urine, AAS, Follicular Fluid, Fetus, anthropogenic, bio-accumulate, ceruloplasmin

**Introduction**

Man exists in an environment which is no longer friendly to him as a result of high anthropogenic pressure, which releases high levels of contaminants to the environment. These contaminants affect man, plant and the environment in general. It can take the form of chemical substances or energy, such as noise, heat or light contaminants or pollutants.

The term heavy metal assumes a variety of different meaning throughout the different branches of science. Although "heavy metal" lacks a consistent definition in medical and scientific literature, the term is commonly used to describe the group of dense metals or their related compounds, associated usually with environmental pollution or toxicity [1]. Elements fitting this description include lead, mercury and cadmium. The presence of heavy metals in the environment constitutes a major global source of concern to environmental scientists and engineers because heavy metals are not only harmful to humans and animals, but tend to bio-accumulate in the food chain [2]. According to WHO [3], in a bid to improve their living conditions, human beings try to exploit nature by manufacturing new products, establishing more industries and improving on old ones. Fossil fuel combustion, agrochemical applications, metallurgical industries activities and industrial wastes generation over the last century [4] have undoubtedly intensified the emission of various heavy metals and other pollutants into the environment thereby stressing the terrestrial, aquatic and atmospheric ecosystem beyond their natural recycling capacity.

Heavy metal exposure happens over a life time depending on where you live, your exposure, lifestyle, culture and man's activities including dumping of waste and agricultural activities (pesticides, herbicides etc) [5]. Unfortunately, many infants today are born toxic with heavy metals as they pass to the baby through the placenta and breast milk. Exposure to high concentrations of trace metals may diminish fecundity; defined as the biologic capacity for reproduction. Several metals including (As, Cd, Mg and Zn) have been detected in human follicular fluid [6].

Pregnancy and lactation increase demand for some essential metals, particularly copper, zinc, and iron [7]. References to women as being highly susceptible to metal toxicity usually refer to effects on the fetus during pregnancy.

Urine is a liquid that is secreted by the kidneys through a process called urination. The kidneys extract the soluble wastes from the bloodstream, it filters in the kidneys. The density varies between 1.003-1.03g/cm<sup>3</sup> [8].

The tolerable limits of some metals in drinking water given in mg/L are 0.05 for As, 0.001 for Cd, 0.05 for Cr, 1.2 for Cu, and the tolerable limit for heavy metals in sea food is as reported by WHO given in mg/Kg are 0.015 for cobalt (Co), 0.030 for cadmium (Cd), 0.050 for lead (Pb), 0.3 for iron (Fe) and 1.000 for copper (Cu) [3].

## **MATERIALS AND METHODS**

### **The Study Area**

The study area was Makurdi town; the administrative headquarter of Benue State. It lies between Lat. 7° 44'N and Long. 8° 54'E and is located within the floodplain of the lower River Benue valley the physiographic span between 73-16m above sea level. Due to the general low relief, sizeable portions of Makurdi is waterlogged and flooded during heavy rainstorms. Makurdi town is drained principally by river Benue which divides it into Makurdi North and South with the banks connected by two bridges [9]. Other minor rivers that drain Makurdi town and empty their waters into the River Benue includes Idye, Genebe, Urudu, Kpege and Kereke Mu, Anmbira Rivers. The climatic condition in Makurdi town is influenced by two air masses: the warm moist southwesterly air mass and the warm dry northeasterly air mass.

### **Sample Collection**

From five different hospitals, urine samples were gathered from ten pregnant women representing various age groups. Standard methods of collection were used as described by Radojevic and

Bashkin [10]. This was done after approval had been provided by the Research Ethics Committee of the hospitals and with the assistance of health personnel at the hospitals. The various hospitals include: Hospital 1 = A = Nigeria Air Force Base hospital.

Hospital 2 = B = Bishop Murray Teaching Hospital, Makurdi.

Hospital 3 = C = Benue State University Teaching Hospital.

Hospital 4 = D = Federal Medical Centre, Makurdi.

Hospital 5 = E = Family Support Clinic, Makurdi. The urine samples were collected in Sandoz sterile vials. Urine samples from 50 patients whose ages ranged from 15 to 45 were used for the heavy metals analysis. Variation of the heavy metals including lead, cadmium, copper, arsenic and nickel in urine samples was correlated with respect to age, professions, and personal habits. A thorough history with particular attention to environmental exposure to pollutants was taken from each woman. Demographic data and common risk factors for developing toxicity were gathered from each participant; they included age, residence place, living near a busy street, occupation, their husband occupation, passive smoking, use of traditional cosmetics and kohl, receiving vitamins, materials used for building their house, history of house renovation, use of canned food, use of lead-glazed ceramic and use of herbal medicinal products.

### **Preparation of Urine Samples for Heavy Metal Determination**

#### **Microwave Assisted Analysis**

The samples of urine collected from the women were analyzed using AAS. After collection, the urine samples were placed in a box and transferred to the laboratory. About 1mL nitric acid ( $\text{HNO}_3$ ) was added for preservation until analysis. Samples were prepared following the method suggested by Teresa [11]. Urine 2mL with addition of 4mL nitric acid ( $\text{HNO}_3$ ) and 1mL hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) which was placed in a microwave. The main advantages of microwave digestion are that it requires

smaller amounts of sample and oxidising materials, shorter digestion time and easiness of sample handling [12].

### Statistical Analysis

Results are presented as mean  $\pm$  standard deviation. The data were carefully, entered, checked and properly coded. The significant difference between heavy metal concentration in the samples of various women were determined using One-Way analysis of variance (ANOVA). Differences in metal concentration were examined using t-test. The level of significance was set at  $p=0.05$ .

### Results and Discussion

The mean concentration of heavy metals in urine samples of pregnant women is shown in Table 2 where Cu has the highest concentration followed by Cd, Ni, Pb and As

**Table 2: Mean Concentration of Heavy Metals in Urine Samples of Pregnant Women**

	Number of samples	Samples code					Mean
		A	B	C	D	E	
As	10	0.033 $\pm$ 0.01	0.039 $\pm$ 0.01	0.049 $\pm$ 0.02	0.056 $\pm$ 0.02	0.048 $\pm$ 0.02	0.045 $\pm$ 0.02
Pb	10	0.032 $\pm$ 0.03	0.041 $\pm$ 0.01	0.086 $\pm$ 0.07	0.110 $\pm$ 0.09	0.085 $\pm$ 0.09	0.070 $\pm$ 0.07
Ni	10	0.027 $\pm$ 0.01	0.082 $\pm$ 0.07	0.130 $\pm$ 0.10	0.220 $\pm$ 0.20	0.210 $\pm$ 0.20	0.133 $\pm$ 0.10
Cd	10	0.059 $\pm$ 0.09	0.096 $\pm$ 0.10	0.130 $\pm$ 0.10	0.240 $\pm$ 0.20	0.230 $\pm$ 0.10	0.150 $\pm$ 0.10
Cu	10	0.380 $\pm$ 0.08	0.520 $\pm$ 0.10	0.810 $\pm$ 0.60	0.550 $\pm$ 0.40	0.800 $\pm$ 0.50	0.610 $\pm$ 0.40

The mean values are as follows:

Metals	Mean values
As	0.045±0.02
Pb	0.070±0.07
Ni	0.133±0.10
Cd	0.150±0.10
Cu	0.610±0.40

### Concentration of Heavy Metals

#### Copper (mg/L)

From the result, it was observed that the mean concentration of copper (Cu) was highest in the analyzed samples with a value 0.61 mg/L followed by that of cadmium. The concentration of copper may be high because it is said that copper is required to hold onto a pregnancy and studies indicates that women with low estrogen and often low copper have more miscarriages [13]. Estrogen, birth control pills and copper intrauterine devices are known to increase the amount of copper in humans. Estrogen increases the absorption of copper making women more likely to carry excess copper even when no birth control is used

#### Cadmium (mg/L)

The mean concentration of Cd was 0.15mg/L which is above WHO standard of 0.06mg/L. The largest source of cadmium exposure is through tobacco products. An average cigarette contains 1 - 2µg of Cd, of which smokers absorb a portion via inhalation. One Cigarette has been shown to increase the blood level of Cd level by approximately 0.1-0.2µg [21]. Diet is the

most common exposure source. Cd can be found at elevated levels in food such as shellfish, organ meats, cereals, root vegetables and green leafy vegetables. It is estimated that the average Cd intake from various food varies between 8 $\mu$ g and 25 $\mu$ g per day [22]. Cd exposure may also occur via house dust and consumer products as well as due to proximity to industrial sources [23]. The half- life of Cd in blood can range up to 10 years and thus may also serve as a good reflection historical exposure [22]. About 32% of the women had high Cd level as a result of smoking as attested by the women on the questionnaire issued but it is noticed generally that the concentration of Cd was higher than WHO Standards of 0.06 [3]; these levels are likely as a result of a combination of exposure sources. Some have estimated that 98% of the ingested cadmium comes from terrestrial food such as rice, vegetables while only 1% comes from aquatic food such as fish and shellfish and 1% arises from cadmium in drinking water.

#### **Nickel (mg/L)**

From this study, nickel had a mean concentration of about 0.133mg/L which is above the permissible limit and the possible sources leading to such a concentration are nickel and nickel compounds widely used in modern industries such as electroplating, battery alloy production and dye industries due to its unique chemical and physical properties. Most people are familiar with attractive mirror finish that can be achieved by nickel plating. Jewelry, stainless steel, coins, magnets and components of industrial machine are all made of the metal nickel. Despite the beautiful appearance, nickel exposure, especially in industrial and occupational settings can present significant health hazards. Nickel is believed to bind to estrogen receptors and mimic the action of estrogen. It is well established that lifetime estrogen exposure is a breast cancer risk factor and unfortunately even this "imposter estrogen" contributes to the risk [27]. Urine reflects recent exposure to nickel and may vary widely in nickel content from day to day. Sources of nickel are numerous and include cigarettes (2 $\mu$ g to 6 $\mu$ g Ni per

average cigarette), diesel exhausts dental or orthopedic implants, stainless steel kitchen utensils, inexpensive jewelry, foods especially cocoa, chocolate, soya products, nuts and hydrogenated oil [28].

Nickel finds its way into the ambient air as a result of the combustion of coal, diesel oil and fuel oil, the incineration of waste and sewage miscellaneous sources [29].

The effect of living near a refuse dump tend to increase the concentration of the heavy metals in the women but in the case of Ni, the concentration was higher in respondents that said they were not living close to refuse dumps probably due to the fact that nickel occur at a very low levels in the environment and the possible sources of Nickel are from Jewelry and food source.

Food processing methods apparently add to the Nickel levels already present in foodstuff via: leaching from nickel-containing alloys in food processing equipment made from stainless steel; the milling of flour; catalytic hydrogenation of fats and oils by use of Nickel as catalyst [29].

### **Lead (mg/L)**

Lead is a common element associated with mining, waste incineration, pesticide etc. The element occurs in the earth crust primarily as galena (Pbs). The solubility of Pb in most surface and ground water is low with the highest values being recorded in soft acidic water. Also, exposure from road which is a source of lead from vehicular exhaust as a result of leaded petrol that is still in use in the country despite its ban since 2004 [30].

From this study, the concentration of Pb was high (0.07mg/L) but not above the permissible limit of 0.1 mg/L. the high concentration is basically from anthropogenic sources which includes the use of pesticides mostly by the farmers, burning of coals in most homes, use of traditional cosmetics like Kohl attested by the participants and living near busy streets where exhaust from automobile tend to increase the Pb concentration. Despite the fact that the population for those that said they renovated their homes recently is small (32%) as compared to

those who didn't, (68%) the level of heavy metal in correspondent that renovated their house recently is higher because paints contain heavy metals like lead. Lead on paints and automobile exhaust are still recognised for its toxicity [31].

Spider webs were shown to contain high lead concentration of about 503.34  $\mu\text{g/g}$  and 662.50 $\mu\text{g/g}$  in both indoors and outdoors areas in Nigeria. Also, participants that live near refuse dump had high level of Pb due to the fact that Pb can be found in such environment and incinerating of those refuse sites leads to an increase in the concentration of Pb. Indiscriminate disposal of toxic waste therefore possesses a great threat to human health [32].

#### **Arsenic (mg/L)**

The dominant basis of arsenic poisoning is from ground water that naturally contains high concentration of arsenic. A study found that over 137 million people in more than 70 countries are probably affected by arsenic poisoning from drinking water [33]. Chronic arsenic poisoning result from drinking contaminated well water over a long period of time. It has also been found that rice is particularly susceptible to accumulation of arsenic from soil [34]. Also, arsenic poisoning can also come from wood dust because arsenic is used in preserving wood. From this study, it can be shown that most arsenic poisoning came from drinking sources (boreholes and well) while rice eaters also have high arsenic concentration. The concentration of arsenic from this work was not above the permissible limit given by WHO [3].

#### **Recommendation**

Just like AIDS awareness and campaign was introduced in the society such that the old and young in rural areas got to know about it and prevent it, heavy metals, its toxic effect and possible sources should be made known to individuals so that they can watch their lifestyle, mind the things they eat and sources from which it is gotten. All women planning to have children need to detoxify before conception. In detoxification of heavy metals

first, the source must be identified and removed. Also, research should be carried out on pregnant women till the time of delivery and also their babies so as to know the effects of these metals on the baby, how it affects its birth size and weight and any other negative effect. Research should also be carried out on the soil to know the level of heavy metal contamination in a particular land before it is used for agricultural purposes. Also indiscriminate dumping of refuse should be checked and food should be consumed from the right sources. Roadside trading should be discouraged. Further test is needed to identify the direct source of cadmium exposure among non-smokers in order to improve public health. Finally, concentration of heavy metals should be carried out on other body fluid like blood.

#### References

1. Duffus, J.H. (2002). Heavy metals- A meaningless term? IUPAC Technical Report pure *Applied Chemistry*, 74(5), 793-807.
2. Yoon, J. Cao, X. Zhou, Q. and Ma, L. (2006). Accumulation of Pb, Cu and Zn in Native Plant Growing on a Contaminated Florida Site, *Science of the Total Environment*, 368 (2-3): 456-464
3. WHO- World Health Organization (1996) "Health Criteria other Supporting Information in Guideline for Drinking Quality Water, Geneva 2(1): 31-388
4. Mason, R. Fitzgerald, W. and Morel, M. (1994). The Biogeochemical Cycling of Elemental Mercury: Anthropogenic Influences. *Geochimica et Cosmochimica Acta*, 58, 3191-3198
5. Ademoroti, C. M. A. (1994). Environmental Chemistry and Toxicology. 1<sup>st</sup> Edn. Fodulex Press Ltd Ibadan. Pg 61-67.
6. Bloom, M.S Louis, G.M Sundaram, R. Kostyniak, P.J and Jain, J. (2011). Association between Blood Metals and Fecundity among Women Residing in New York State. *Reproductive Toxicology*, 31(2):158-63. doi:10.1016/j.reprotox.2010.09.013.Epub2010 oct 8.

7. Piccano, M.F. (1996). Pregnancy and Lactation. In: Ziegler, E.E and L.J. Filter, Jr., eds. Present Knowledge in Nutrition, 7th ed. Washington, DC: ILSI Press, pp. 384-395.
8. Wikipedia, (2009). The free encyclopedianoco/acba/cong/tumr/, sysi/epon.utre
9. Ochieri, M.I (2010) Assessment of Ground Water Quality for Rural Water Supply in Benue State. Unpublished MSc Thesis, Department of Geography, University of Nigeria, Nsuka.
10. Radojevic, M. and Bashkin, V.N. (1999) Practical Environmental Analysis. The Royal Society of Chemistry, Cambridge 466
11. Teresa, L. (2009). Application of ICP.OES to Multiological Analysis of Biological Material in Forensic inorganic toxicology problems of Forensic Science, *System Pharmacology*, 77:64-78
12. Ingrid, G. (1998). Heavy Metals and Fertility. *Journal of Toxicology and Environmental Health*, 54:593-611
13. Ren,/ M., Wang,/ L., Wen,/ L., Chen,/ J., Quan,/ S., & Shi,/ X. (2023). *Association between female circulating heavy metal concentration and abortion: A systematic review and meta analysis. Frontiers in Endocrinology*, 14 : 1216507. <https://doi.org/10.3389/fendo.2023.1216507>
14. Miline, D.B. Klevvay, L.M, Hunt J.R. (1988). Effects of Ascorbic Acid Supplements and diet Marginal in Copper on Indices of Copper Nutrients in Women. *Nutritional Research*, 8:865-873
15. Rama, L and Leela, (1992). Role of Material Nutrition. Annual Report. National Institute on Nutritional. 93:47-50
16. Ebbs, J.H Tisdal F.F and Scott W.A (1984). The Influence and Prenatal Nutrition on Mother and child. *Journal on Nutrition*, 22:515-526
17. Ajose, A.B Fasubi, J. Ametar, D.A, Adelekan and Maki

- N.O. (2001). Serum Zinc and Copper Concentration in Nigerian women with Normal Pregnancy. *Nigeria Post graduate Medical Journal*, 8:161-164.
18. Izquierdo, A.S. Castanon, S.G Ruata, M.L Arigues, E.F and Terraz P.B (2007). Updating of Normal Levels of Copper, Zinc and Selenium in Serum of Pregnant Women. *Journal on Trace Elements. Medical Biology*, 22(1)49-52
  19. Black, M.M (2001). Zinc Deficiency and child Development. *American Journal on Clinical Nutrition*, 68(2):4645-4695.
  20. Pathak, P. and Kapil, U. (2004). Role of Trace Elements Zinc Copper and Magnesium during Pregnancy and its outcome in Paediatric 71:1003-1005
  21. Jarup, L. Berglund, M. Elinder, C.G. Nordberg, G.I and Vahter, M. (1998). Health Effects of Cadmium Exposure. *Journal on Environmental Health*, 24:1-51
  22. Jarup, L. and Akesson, A. (2009). Current Status of Cadmium as an Environmental Health Problem. *Applied Pharmacology*, 228:201-206
  23. Satarug, S. Baker, J.R. and Urbenjapol, S. (2003) A Global Perspective on Cadmium Pollution and Toxicity in Non-Occupationally Exposed Population. *Toxicology letters*, 137: 65-83
  24. Alam, D.S (2013) Smoking Attributable Mortality in Bangladesh: Proportional Mortality Study. *Environmental Health Perspectives*, 120: 758-763.
  25. Khan, A.R. (2013). Cadmium Found in Bangladeshi Rice. *Environmental Health perspectives*, 47:417-422
  26. Ahmaruzzaman, M (2011) Industrial Waste as Low cost Potential Sources for Treatment of Waste Water Laden with Heavy Metals. *Advances in Colloid and Interface Science*, 166 (1-2), 36-59.
  27. Aquino, N.B Sevingy, M.B Sabanga, J and Louie, M.C. (2012) The Role of Cadmium and Nickel in Estrogen Receptor Signaling and Breast Cancer. *Journal on Environmental Science Health*, 30(3):189-224.

28. Nielson, F. H. (1994). In M. E. Shils, J.A. Olson & M. Shike (Eds), *Modern Nutrition in Health and Disease*. Philadelphia *Lea and Febige* pp 273-285
29. Von-Burg R. (1993). Toxicology Update: toluene. *Journal of applied Toxicology* 13(6), 441-446
30. Ogunfowokan, A.O. Kaisam, J.P and Balogun, (2009). Study of Trace Metals in Urine of some Nigerian Medical Patients. *Toxicology Environmental Chemistry* 91:435-447
31. Huges, W.W (1996). *Essentials of Environmental toxicity. The Effects of Environmental Hazardous Substances on Human Health*. Conas ltd California. Taylor and Francis Publishers pp3, 87-95
32. Ibeto, I.C Okoye, C. Ofoefule, A and Uzodinma, U. (2012). Analysis of Environment pollutants by Atomic Absorption Spectrophotometry. *Macro to Nano Spectroscopy, Pp* 25-50
33. Smith, D.R. and Flegal A.R (1995). Lead in the biosphere, Recent trends *Ambio*,24(1):21-23
34. Deborah, K. (2011). Do you worry about Cancer? *Boston Globe*, 82(2);243-249.