AN ASSESSMENT OF THE METALS ACCUMULATED IN NEWLY WEANED MICE AFTER INGESTING 'NZU CLAY' USING NEUTRON ACTIVATION ANALYSIS AND ATOMIC ABSORPTION SPECTROSCOPY

ISSN 214-453-2

OMONIYI K. I.*[1] AND OKUNOLA J.O.[2]

[1]*Department of Chemistry, Ahmadu Bello University, Zaria, Kaduna, Nigeria.*

[2]*Department of Applied Chemistry, Federal University, Dutsin-Ma, Katsina State, Nigeria.*

[*israelflourish@yahoo.com*]

ABSTRACT

'Nzu clay' is a geophagious clay consumed in Africa for some medicinal purposes. This study reports the level of accumulated metals in mice that ingested 'Nzu clay' for 14 days. Eight female mice (10 ± 2 g) were each fed 0.02 g of 'Nzu' mixed with Bendel Plc Feed per day. The 'Nzu clay' contained 2.900 mg/kg Pb and 2.143 mg/kg Cd, while the experimental mice accumulated 0.893 mg/kg of Pb and 2.750 mg/kg Cd, but the control accumulated 0.714 mg/kg Pb and 2.250 mg/kg Cd. By extrapolation for 65 kg human weight, 0.014 mg/kg of Cd is anticipated to be accumulated at the end of two weeks. This is 13-fold the WHO tolerable weekly intake of 0.007 mg/kg body weight of human; and 10-fold increase in Pb compared to the WHO tolerable weekly intake. Neutron activation analysis indicated that the clay contained As, Cd and Pb at levels above WHO limits in foods. The accumulated concentration of Al in the mice was four-fold the 120.24 mg/kg in the control, while the accumulated Al concentration was 230-fold the tolerable intake. The accumulated concentration of manganese was 2.05 mg/kg compared to 0.07 mg/kg in the control. 'Nzu clay' is rich in K (96.0 mg/kg) and the mice also accumulated 11.5 mg/kg K; the National Academy of Sciences maximum level of daily dietary intake of K is approximately 72 mg/kg. The concentration of As in the clay was 51 mg/kg, which is of health concern. The level of Cr in the experimental mice was significantly elevated (Students' t-test, $P < 0.05$) compared to the control. The accumulated level of Cr in the experimental mice was about 36-fold higher than the tolerable intake. The accumulated Fe and Mg in the mice was about 33-fold and 2-fold respectively. This implies that consumption of 'Nzu clay' result to bio-accumulation of essential and non-essential metals. Accumulation of heavy metals of health concern at long-term ingestion of the clay call for continuous ban on its consumption.

Keywords: *geophagious, Nzu clay, tolerable intake, bio-accumulation, heavy metals, mice, hair*

1. Introduction

The Lord God formed man of dust from the ground, and breathed into his nostrils the breath of life (HB, Gen 2: 7). This could possibly account for the reason why clay has been ingested by man for thousands of years. Long before recorded history, humans have used healing clays externally and internally to cure illnesses, sustain life and promote general health. Perhaps, the earliest recorded mention of clay consumption for healing remedy was the healing tradition of India that originated approximately 5,000 years ago (Alexander *et al*., 2002). Ancient tribes of the high Andes, Central Africa and the Aborigines of Australia used clay as a dietary staple, a supplement and for healing purposes (www.aboutclay.com). Geophagia is not limited to any particular age group, race, sex, geographic region or time period (Abraham *et al*., 2013); and is practised so as to born beautiful children (Mcloughlin, 2004), have lighter and softer skin (Alexander *et al*., 2002), medicinal purposes (Bisi-Johnson *et al*., 2010) and a plethora of others.

'Nzu clay' also known as 'Calabash chalk', 'La caire' or 'Agrile' in French, 'Nzu' and 'Ndom' by the Igbos and Efiks/Ibibios of Nigeria respectively is one among series of clay being consumed in Africa, for remedy from morning sickness during pregnancy and pica by children (Campbell, 2002). 'Nzu clay' is a type of clay that is especially high in calcium and such is consumed mostly by pregnant and breast feeding women (Figure 1). In parts of Africa, rural areas of the United States and villages in India, clay consumption coats the gastrointestinal tract and may absorb dangerous toxins (Padilla and de la Torre, 2006). The clay may also provide critical calcium for foetal development (Corwin, 1999; Natural Nigerians, 2011). The clay is an aluminium silicate hydroxide from the kaolin clay group with the possible formula: $Al_2Si_2O_5OH_4$. It is obtained from hills or near rivers mostly in the south-eastern part of the country, and has been in circulation for decades. It is available in markets in many parts of the country, Nigeria. It is cheap, with 3 balls sold for just 5 naira (Food Standard Agency, 2002; Northumberland Country Council, 2008). The clay may then be mixed with other ingredients including sand, wood ash and sometimes salt.

The chalk contains lead (40 mg/kg) and arsenic (Health Canada, 2007) with some other organic pollutants. The presence of these pollutants have raised health concern for pregnant and breast feeding women, as well as developing unborn babies and breast feeding infants (Campbell, 2002; Health Canada, 2007). Speculated long term effects on the consumers of 'Nzu' include cancers of the urinary bladder, lungs and skin (Dean *et al*., 2004).

'Nzu' like some other prized therapeutic grade clays can be used for skincare, detoxification/ cleansing protocols – since clay is an highly efficient adsorbent - , it can eliminate food allergies, food poisoning, mucus colitis, spastic colitis, viral infections, stomach flu, and parasites (parasites are unable to reproduce in the presence of clay) (Ekong *et al*., 2009; Vermeer, 1971; www.clayforall; Dominy *et al*., 2004; Aghamirian *et al*., 2009). The work by Ekong *et al.* (2009) showed that the liver sections of Wistar rats that ingested non-salted 'Nzu' showed sinusoids enlargement and fragmented parenchyma, while ingestion of salted 'Nzu' presented no obvious pathology compared to the control rats.

Cadmium accumulation in the organ of *Cavia porcellus* after being injected intraperitoneally with cadmium chloride solution in three different doses, TDI (tolerable daily intake), intermediate doses and lethal doses (LD_{50})-5% for 60 days. This indicated that the liver accumulated the greatest burden of cadmium followed by the kidneys using an atomic absorption spectrophotometer (Shakoor *et al*., 2000).

This study is aimed at determining the concentrations of accumulated metals in mice ingested with 'Nzu clay' using neutron activation analysis (NAA) and atomic absorption spectroscopy (AAS) and to assess the levels of metals in 'Nzu clay' using NAA and AAS. This is undertaken towards assessing the short-term systemic bio-accumulation of metals in consumers of 'Nzu' clay'.

Figure 1. Processed 'Nzu clay' and raw unprocessed edible clay

2. Materials and Methods

2.1 Ingestion of 'Nzu' by the Animal

'Nzu clay' was purchased from three different markets in Zaria (latitude: 11° 07' 51'') in May 2012.

Sixteen female mice (10 ± 2 g) that were just weaned were used for the study. The experimental group (n = 8) were each fed 0.02 g of 'Nzu' mixed with Bendel Plc Feed per day for 14 days and deionised water *ad libitum*. The questionnaire administered to some consumers of 'Nzu' in Zaria, Nigeria indicated that the average amount of 'Nzu clay' consumed per day is 11 balls of the clay (average weight 10.40 g). By considering an average weight of 65 kg for human and 12.0 g for mice, the average intake of 'Nzu' for each mice per day was obtained to be 0.02 g. This amount was mixed with the feed to obtain the food intake in mice, 1.50 g per 10.0 g body weight.

The control group was fed only Bendel Feed of the same amount as the experimental animal and deionized water *ad libitum* for 14 days. The weight, the level of water intake and physical activity of the mice were taken daily. At the end of 14 days, the mice were sacrificed by lethal dose of chloroform, then each mouse was oven dried at 60ºC for 72 hours.

2.2 Analyses of Mice and 'Nzu' Clay

Quality assurance of the atomic absorption machine was carried out by using spiked digested samples of 'Nzu' clay. Each of the dried mice was pulverized using an agate mortar and pestle, and wet digestion was carried out by using concentrated HNO_3 and HCl (1: 3) on a hotplate at 90°C for 10 minutes. The levels of Pb and Cd in the two animal groups were determined using atomic absorption spectrophotometry at the Multi-user Laboratory of Ahmadu Bello University, Zaria, Nigeria.

Fast neutron activation analysis (FNAA) technique was utilized to determine the elemental composition of the clay and mice using the D-T mode (mono energetic fast neutrons 14 Mev). Standard gamma sources of 22Na, 57Co, 133Ba, 137Cs and 152Eu were used for both energies according to Das *et al*. (1989); Al-Mughrabi and Spirou (1987).

2.3 Statistical Analysis

The levels of the metals accumulated in the two groups of mice were compared using Students' t-test at $P < 0.05$.

3. Results and Discussion

3.1 Physical Behaviour of the Animal

The physical activities of the experimental mice indicated that they were active from day 1 to 14; though the body weights of the experimental mice increased only slightly with time compared to the control group, from day 3 to day 5, there was steady increase in body weight of the experimental and control group (2.6 - 3.4 g). The experimental group consumed more water (3.0 cm^3) compared to mean volume of 2.6 cm^3 by the control group.

3.2 Elemental Composition of the Mice and 'Nzu Clay'

'Nzu clay' has higher concentration of both lead and cadmium than in the experimental mice. The average concentration of lead and cadmium in 'Nzu clay' was 2.900 ± 0.141 mg/kg and 2.143 ± 0.505 mg/kg respectively; while the mean levels of lead and cadmium in the experimental was 2.750 ± 0.354 mg/kg and 0.893 ± 0.253 mg/kg respectively, and the control group had the lead and cadmium levels being 2.250 ± 0.200 mg/kg and 0.714 mg/kg respectively. It can be extrapolated that the concentration of cadmium accumulated in the mice after 14 days of experiment was 0.179 mg/kg body weight and the concentration of lead accumulated in the mice was 0.500 mg/kg body weight for mice of weight of 12 g.

By considering human of average body weight 65 kg that consumes 114.51 g of 'Nzu' per day, the body would accumulate approximately 0.1790 mg/kg of cadmium and 0.500 mg/kg of lead after 14 days of consuming 'Nzu' at this rate; since human and mice share the same homology. The current cadmium provisional tolerable weekly intake established by the Joint Expert Committee on Food Additives (JECFA) of WHO is 0.007 mg/kg body weight of human (WHO, 2010), which implies that about 0.014 mg 'Nzu'/kg body weight is anticipated to be accumulated after two weeks of ingestion. This indicates a 13 fold accumulated cadmium above the tolerable intake. The World Health organization (WHO) provisional tolerable weekly intake of lead is 0.025 mg/kg body weight of human ((WHO, 2010). This implies that consumption of 'Nzu' for two weeks would result to accumulation of 0.50 mg 'Nzu'/kg body weight of human. The systemic accumulated lead and cadmium after the two weeks ingestion of 'Nzu clay' was higher than the WHO specification.

The results for the level of Pb in 'Nzu' obtained from this study (2.900 ± 0.141 mg/kg) is greater than the below detection limit reported by Kelle *et al*., (2004). Likewise, the concentration of Cd in the 'Nzu' studied (2.143 ± 0.505 mg/kg) is greater than 0.327 ± 0.130 mg/kg reported by Kelle *et al*., (2004).

3.3 Levels of Accumulated Metals from Neutron Activation Analysis

The concentrations of the elements in various samples analyzed using Neutron Activation Analysis is represented in Table 1. 'Nzu clay' contained 30 elements out of which 23 are within the detection limit of the machine. 'Nzu clay' contains 28 metals and 2 non-metals (chloride and bromide). Though, not all the elements detected in the clay were accumulated in the mice after consuming the clay. It is common to detect as many as 75 different trace minerals in Montmorillonite clays (Mcloughlin, 2004).

Table 1: Mean concentration of the elements in 'Nzu clay', the control and experimental mice using NAA

Elements	**Level in Nzu clay (mg/kg)**	**Level in the Experimental mice (mg/kg)**	**level in the control mice (mg/kg)**	**Accumulated Concentration (mg/kg)**
Aluminium	133800±304	579.50	114.25	465.25
Titanium	7721±486	NA	NA	NA
Vanadium	221±8	BDL	BDL	BDL
Manganese	27±1	9.20	7.15	2.05
Dysprosium	7.4±0.3	NA	NA	NA
Sodium	629±1	3690.00	3449.50	240.50
Potassium	96±16	8965.00	8953.50	11.50
Arsenic	51±3	BDL	BDL	BDL
Lanthanum	65±0.2	68.00	37.00	31.00
Samarium	12.36±0.05	BDL	BDL	BDL
Uranium	1.36±0.15	BDL	BDL	BDL
Scandium	85±1	645.00	569.00	76.00
Chromium	382±10	2.25	BDL	2.25
Iron	17480±489	247.50	150.50	97.00
Elements	**Concentration in Nzu clay (mg/kg)**	**Concentration In the experimental group (mg/kg)**	**Concentration control group (mg/kg)**	**Accumulated Concentration (mg/kg)**
Cobalt	10.00±2.0	36.80	BDL	36.80
Antimony	17.5±1.2	BDL	BDL	BDL
Caesium	4.1±0.8	NA	NA	NA
Barium	213.0±68.0	BDL	BDL	BDL
Europium	4.2±0.5	52.00	49.00	3.00
Ytterbium	7.7±0.4	BDL	BDL	BDL
Hafnium	2.0±0.1	NA	NA	NA
Tantalum	1.5±0.2	NA	NA	NA
Thorium	16.2±0.2	BDL	BDL	BDL
Magnesium	11.90	1312.50	1177.50	135.00
Chloride	BDL	4734.00	4322.00	412.00
Calcium	1122.0±48.0	21175.00	21000.00	175.00
Zinc	BDL	116.00	100.50	15.50
Bromide	BDL	40.50	38.00	2.50
Rubidium	BDL	32.50	28.50	4.00
Lutetium	BDL	BDL	BDL	BDL

BDL = Below detection limit

The element with the highest concentration in the clay was aluminium, this was 133800 ± 304 mg/kg, 579.50 mg/kg in the experimental and 114.25 mg/kg in the control mice. The accumulated amount of Al in the mice due to 'Nzu' consumption was 465.25 mg/kg. This was followed by iron in the clay being 17480 ±

489 mg/kg, 247.50 mg/kg in the experimental and 150.50 mg/kg in the control mice. The accumulated amount of Fe in the mice due to 'Nzu' consumption was 97.00 mg/kg.

The accumulated concentration of aluminum was 465.25 mg/kg and WHO provisional tolerable weekly intake is 1.0 mg/kg Al which is equivalent to 2 mg/kg for two weeks. The accumulated concentration of aluminum is about 233 fold greater than the tolerable intake. The presence of essential elements like Na, K, Mg, Mn in the clay and in the experimental mice is a benefit, the clay has enhanced these minerals in the body.

The concentration of arsenic in 'Nzu clay' was 51 mg/kg and the standard concentration of arsenic in the earth crust is 1.8 mg/kg which implies 'Nzu clay' contains very high concentration of arsenic. Though, the result indicated that the mice did not accumulate any measurable level of As (Table 1). The amount of Na in the clay was 629.00 ± 1.00 mg/kg, while the amount accumulated in the mice by the consumption of the clay was 240.50 mg/kg. The level of Ca in the clay was 1122.00 ± 1.00 mg/kg, while the amount accumulated in the mice by the consumption of the clay was 175.00 mg/kg. The clay had 96.00 mg/kg of K, however the amount of K that was accumulated in the experimental mice was 11.50 mg/kg. This results corroborate with the assertion that edible clay are supplements for some essential elements (Mohapatra *et al*., 2007).

In addition, the level of radioactive elements such as uranium and thorium in the clay were 1.36 ± 0.15 mg/kg and 16.2 ± mg/kg respectively; however, the mice did not accumulate these elements (Table 1). The consumption of 'Nzu' significantly ($P < 0.05$) elevated the concentrations of aluminium, lanthanum, iron, cobalt and magnesium in the experimental mice compared to the control.

4. Conclusion

'Nzu clay' contains both metals and non metals that are beneficial and toxic. Most of the beneficial metals in 'Nzu clay' are toxic because their accumulated concentrations are more than the tolerable intake, for metals such as iron, magnesium, zinc and manganese. Heavy metals such as As and Cr are present in 'Nzu clay' and the accumulated levels in the mice were more than the tolerable intake, these include cadmium, lead, aluminium, chromium and iron**.** Therefore, regulatory agencies for food and drugs should continually ban the consumption of 'Nzu clay' because of the toxicity tendency of 'Nzu clay' especially with long-term consumption. Further studies on the physico-chemical, radioactivity, microbial properties of the clay, and the elemental composition of the biological samples collected short- and long-term consumers of 'Nzu clay' is on-going.

References

Abrahams, P. W., Davies, T. C., Solomon, A. O., Trow, A. I. and Wragg, J. 2013. Human geophagia calabar chalk and undongo: mineral element nutritional implication. Accessed 4 July 2013.

Aghamirian, M. R. and Ghiasian, S. A. 2009. Isolation and characterization of edically important aerobic *Actinomycetes* in soil of Iran (2006 - 2007). *Open Microbiol. J.,* 3: 53-57.

Alexander, M. D., Wodwodt, A. and Akos- Kiss, F. C.S. 2002. Geophagia: the history of earth – eating. *J. Royal Soc. Med*. 95(3), 143–146.

Al-Mughrabi, M. and Spirou, N. 1987. Applications of neutron activation analysis. *J. Radioanalysis and Nuclear Chem.*, 110: 67.

Bisi-Johnson, M. A., Obi, C. L., Ekosse, G. E. 2010. Microbiological and health related perspectives of geophagia: an overview. *Afric. J. Biotech.*, 9(19), 5784–5791.

Campbell, H. 2002. Calabash Chalk (Calabar stone, La Craie, Agraile, Nzu, Mabele). DOI= www.lef.org accessed on 12/06/2012.

Corwin, T. 1999. The clay eaters. DOI= http://chronicle.augusta.com/stories/031899/fea clay. shtml. Accessed on 12/06/12.

Das, H., Faanhof, A. and Van-Der, S. H. 1989. *Radioanalysis in geochemistry*. Occupational Exposure to Hazardous Agents, Elsevier, Amsterdam, 435 - 439.

Dean, J. R., Deary, M. E., Gbefa, B. K. and Scott, W. C. 2004. Characterization and analysis of persistent organic pollutants and major, minor and trace element in Calabash Chalk. *Chemosphere*, 57 (1), 21-25.

Dominy, N., Davoust, E. and Minetus, M. 2004. Adaptive function of soil consumption an *in vitro* study mideling the human stomach and small intestine. *J. Experiment. Biol.,* 2004 (2), 319-334.

Ekong, M. D., Akpantah, A., Ibok, S. O., Eluwa, A. M. and Ekanem, B. T. 2009. Differential effect of Calabash Chalk on the histology of the liver of adult Wistar rats. *The Internet J. of Health,* 8, (2).

Food Standard Agency, 2002. Lead contamination of Calabash Chalk category. DOI= www.food.gov.uk accessed on 25/05/2012.

French clay for all. Natural healing for all living creature. DOI= www.clayforall accessed on 15/06/2012.

Health Canada 2007. Calabash chalk may pose health risk for pregnant and breastfeeding women. DOI= www.ctvnews.ca accessed on 12/06/2012).

History of healing clays. DOI= www.aboutclay.com Holy Bible, RSV Gen 2: 7.

Kelle, H. I., Otokpa, E. O., Oguezi, V. U. and Ibekwe, F. C. 2004. Assessment of Heavy Metals in Edible Clays Sold in Onitsha Metropolis of Anambra State,Nigeria. *British J. Appl. Sc. & Tech.,* 4(14), 2114-2124.

Mohapatra D., Mishra D., ChaudhuryG.R. and Das R.P. (2007), Arsenic adsorption mechanism on clay minerals and its dependence on temperature, Korean J. Chemical Eng., 24, 426–430.

Mcloughlin, I. J. 2004 The picas. *Br. J. Hosp. Med*. 37, 286–290.

Natural Nigerians, 2011. DOI= www.naturalnigerian.com/2011 accessed on 15/06/2012.

Northumberland Country Council 2008. Press Release Archives. Product warning: Excess lead in Calabash Chalk. DOI= www.food.gov.uk accessed on 16/06/2012.

Padilla, F. V. and de la Torre, A. M. (2006). La pica: retrado de unaentidadclinicapococonocida. *Nutricion Hospitalaria,*21, 557–566.

Parry-Jones, B. and Parry-Jones, L. L. 1992. Pica; symptom or eating disorder, a historical assessment. *British Journal of Psychiatry,* Vol. 160: 341-354.

Shakoor, I. T., Davids, T. R. and Sandeck, O. L. 2000. Cadmium, zinc, iron and arsenic concentration in biological samples. *Journal of Phamarcology, Pp*. 323-434.

Vermeer, D. E. 1971. Geophagy among the Tiv of Nigeria. *Annals of the Assoc. of American Geographers*, 56, 197-204.

WHO, (2010). Joint Expert Committee on Food Additives.