DETERMINATION OF As, Cd, Cr, Cu, Ni, Pb AND Zn IN THE HAIR OF ADDICTED CONSUMERS OF EDIBLE CLAY -'NZU'- USING ATOMIC ABSORPTION SPECTROSCOPY

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ABSTRACT

Nzu is geophagious and of kaolin clay group. Hair has the advantage of long term memory of the history of the body chemistry compared to urine and sweat. This study reported the levels of As, Cd, Cr, Cu, Ni, Pb and Zn in the head hair of addicted consumers of 'Nzu' using atomic absorption spectroscopy. The metals in the hair of the Nzu consumers below 5 years (FB), those above 5 years (of consumption) (FA), and the non-consumers (NA), is in the order: Cr > As > Ni > Cd > Pb > Cu > Zn. The mean Cr in the hair of NA was 24.32 ± 6.45 mg/kg compared to 66.13 ± 7.23 mg/kg in FB and 92.45 ± 7.77 mg/kg in FA. The mean Cd in the hair of the FA was four-fold the level in the NA (1.09 ± 0.22 mg/kg). The study indicated that long-term consumption of Nzu significantly elevated the levels of Cr, Cd, Pb, Cu and Ni in the hair. The result further reiterated the health concern over the consumption of 'Nzu'. While canvassing for the enforcement of the ban on 'Nzu' consumption, while it's age-long medical benefits should not be dismissed. In this direction, our research team is currently investigating the remediation of toxic heavy metals and radioactive elements from 'Nzu'.

Keywords: accumulated metals, hair, kaolin, Nzu, remediation.

INTRODUCTION

The oldest evidence of geophagy practised by human came from the prehistoric site at Kalambo falls on the border between Zambia and Tanzania, where a calcium-rich white clay was found alongside the bones of *Homo habilis* (the immediate precussor of *Homo sapien*) [1]. Geophagy is nearly universal around the world in the tribal and traditional rural societies [2-4]. 'Nzu', also known as white clay and calabash chalk, is not a conventional food, though consumed by wide range of communities. It is said to be a West Africa remedy for morning sickness during pregnancy. In Nigeria, pregnant and breast- feeding women patronize it the most [5, 6].

A survey conducted using 120 people randomly selected (40 pregnant women, 40 non-pregnant women and ladies, and 40 children) in Zaria, Kaduna State in 2012 indicated that only 11.67% of the respondents do not eat 'Nzu'. The most frequent reason for eating 'Nzu' was the sweet taste and natural craving. About 53.33% of the respondents ate 'Nzu'at least once a day [7].

Apart from the fact that soil or clay serves as a reservoir for mineral elements (about seventy five), it contains chemical and biological agents. Among the chemical agents are heavy metals, radioactive gases and organic chemical [8, 9]. The heavy metals (Fe, Mn, Ni, Cu, Zn, Co, Cr, V, Ti, Cd, Hg, Mo, Pb, As) [10-13], radioactive elements [14] and trace metals [15] as well as Se and F occur naturally in soil. Some of these have been implicated in 'Nzu' and responsible for the clamour for its ban [8].

Despite the levels of some of these heavy metals of heath concern in 'Nzu', it has been asserted in some studies that the distribution of metals that followed dermal and systemic (through injection) administration *in vivo* led to Ni and Co being efficiently eliminated from the body through urine and faeces [16]. In contrast, Fe, Cr and even Co reveal a tendency to be stored in the body [17, 18]. For example, man absorbs 10% of ingested lead, and high level of Vitamin D increases absorption but absorption is inverse for high levels of iron and calcium [19].

Levels of released metal ions may be detected and determined in body fluids such as serum or plasma, urine and sweat [20, 21]. Urine and serum metal concentrations, however, are dependent on the excretory rate of the metal ions which is a highly individualised parameter and specie specific. On the other hand, hair has the advantage of long term memory. A 3-inch strand of human hair will give a 6-month history of the body chemistry [22]. Likewise, clinical research indicates that the hair levels of specific elements, particularly toxic elements such as cadmium, mercury, lead and arsenic, correlate with pathological disorders [23]. The levels of the elements in the hair provide a superior indication of body stores as opposed to blood or urine specimens [22, 24].

Omoniyi *et al* [25] used the fur of albino rat to quantify the amounts of metal ions accumulated in the blood of Wistar albino rat pre- and post-operative the implantation of stainless steel (SS) arch bar. The results showed that the concentrations of Co, Mn and Cr ions in the fur post-operative are indicative of the levels of these metal ions in the blood.

Therefore, this study is aimed at determining the concentrations of accumulated As, Cd, Cr, Cu, Ni, Pb and Zn in the hair of short- and long-term addicted consumers of 'Nzu'; and non-consumers of the clay using atomic absorption spectroscopy. This was undertaken towards assessing the short- and medium-term systemic bio-accumulation of the metals in consumers of 'Nzu'.

MATERIALS AND METHODS

Collection of Hair Samples and Sample Preparation

Hair samples were collected randomly from forty five volunteers within Zaria metropolis, Kaduna State, Nigeria in 2014. The samples were collected from pregnant and non-pregnant women of ages 27 - 48 years. Each subject filled a personal questionnaire indicating age, general health status, occupation, living habits (consumption of clay, type of clay consumed, smoking, non-smoking, alcoholic, non-alcoholic). Out of these results, the forty five that fit into the three groups needed were obtained.

Hair samples of the three groups: non-consumers of the clay (NA), consumers for less than 5 yr (FB), and consumers above 5 yr (FA) were obtained from the nape of the scalp by cutting 2 cm using a pair of sterilized stainless steel scissors washed with ethanol. The hair samples collected were sealed in plastic bags prior to analysis.

The hair samples of each group was first washed with deionised double distilled water to remove dust particles and then with acetone to remove organic impurities from the hair. Each sample was then taken into a 250 cm^3 pyrex beaker and oven-dried at 60 ^oC for 6- 8h.

Plate 1: Balls of 'Nzu'

Digestion of Hair Samples

The dried hair sample of each group was placed in a clean conical flask and acid digestion was carried out using 9 cm^3 of 3:1 mixture of concentrated trioxonitrate (V) acid and perchloric acid.

The mixture was heated on a hot plate at 100 ^{o}C until all the hair samples dissolved completely, giving a clear solution, void of evolution of white fume. The digest of each group was then cooled with deionised distilled water and made up to the 100 cm^3 mark of a volumetric flask in triplicate.

The levels of As, Cd, Cr, Cu, Ni, Pb and Zn in the digest of each hair group were then determined using an atomic absorption spectrophotometer (Model TAS990, Intec Co. Ltd., Rome) at the appropriate wavelength and lamp current for each analyte.

The quality assurance for the analyses was conducted through the spiking method, to evaluate the sample digestion process and effectiveness of the atomic absorption spectrophotometer [26-28].

Statistical analysis of data

The data obtained were analyzed and reported as mean ± standard deviation. The levels of each metal in the hair as a function of the extent of 'Nzu' consumption by the subject were compared using student's t-test at $P < 0.05$.

RESULTS AND DISCUSSION

From the study as depicted in Figure 1, the consumption of 'Nzu' generally increased the levels of As, Cd, Cr, Cu, Ni, Pb and Zn in the hair of the short- and long-term addicted consumers of 'Nzu'.The concentrations of the accumulated metals studied in all the head hair samples follow the ranking: Cr > As > Ni > Cd > Pb > Cu > Zn. From the study the levels of Cr was 24.32±6.45 mg/kg, Cd: 1.09±0.22 mg/kg, Pb: 0.63±0.25 mg/kg, As: 18.15±0.61 mg/kg, Zn: 0.11±0.05 mg/kg, Cu: 0.86±0.36 mg/kg and Ni: 1.77±0.61 mg/kg in the head hair of non-consumers of 'Nzu' (NA) (Figure 1). This finding is generally lower compared to the levels of the metals in normal head hair of some subjects aged 16 to 55 years in some towns in Nasarawa State, Nigeria, reported by Aremu *et al* [29]. However, the order of accumulated metals in the study by Aremu *et al* was Zn > Cr > Pb > Cu > As > Cd [29].

In the present study the concentrations of Cr, As and Ni in the head hair of normal human is high, and could point to the tendency of having exposure to heavy metals in the environment or household items, such as cosmetics. Therefore, awareness needs to be made in this respect, in view of the long- term implications to the subjects. For example, exposure to high levels of arsenic can create health symptoms such as memory loss, confusion and lethargy [30]. Zinc was the least in the head hair of the normal subject. It is known that specific metabolic cycle incorporates uptake and retention of this essential metal in the body as reported by Wein-Schwaitz and Oderda [22].

Previous reports on the level of Cr in 'Nzu' were 0.170 + 0.04 mg/g in edible clay obtained from Enyigba, Ebonyi State, Nigeria [31]. Cr concentration of 0.127±0.008 mg/kg was reported for 'ulo' obtained from Oil mill market Port Harcourt, Rivers State, Nigeria [32]. Omoniyi *et al.* [33] reported Cr level of 382±10 mg/kg for 'Nzu' obtained from Samaru market, Zaria, Kaduna State, Nigeria. However, the FAO/WHO safe limit for Cr in food is 2.3 mg/kg. The present study indicated that consumption of 'Nzu' resulted to bio-accumulation of Cr at 66.13±7.23 mg/kg in the hair of the clay consumers for less than 5 yr (FB). This was three-fold the level in the head hair of non-consumers of the clay (NA). Also, the study indicated that long-term consumption of 'Nzu' led to Cr in the hair of the consumers above 5 yr (FA) being about five-fold the level in the hair of non-consumers of the clay (NA) (Figure 1).

One way ANOVA using Student's t-test indicated that consumption of 'Nzu' significantly elevated the hair levels of Cr in FB and FA.

From the study by Ogah and Ikelle [31], Cd in edible clay obtained from Enyigba, Ebonyi State Nigeria was 1.930 ±1.23 mg/g. Likewise Ukwueze and Ochuba [32] reported 0.020±0.006 mg/kg for 'ulo' obtained from Oil mill market Port Harcourt, Rivers State, Nigeria; and Kelle *et al*. [15] reported the range 0.041±0.130 - 0.327±0.130 mg/kg for some edible clay types purchased from Onitsha markets, Nigeria. But the FAO/WHO safe limit for Cd in food is 0.2 mg/kg.

Cd in the hair of the clay consumers for less than 5 yr (FB) was 2.59±0.17 mg/kg which doubled the level in the hair of NA. Also long-term consumption of 'Nzu' in FA led to Cd being four-fold (4.35±1.83 mg/kg) the level in the head hair of NA (Figure 1). There was significant increase in the bio-accumulated Cd in the hair of the subjects FB and FA compared to NA.

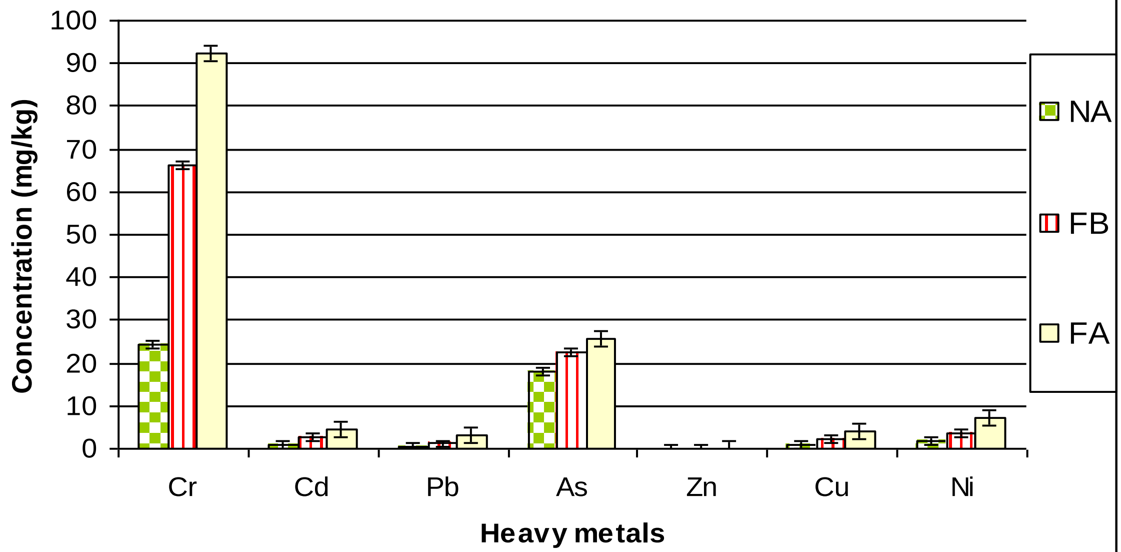

Figure 1: Mean concentration (mg/kg) of heavy metals in hair of female non-addict, addicts for below 5 yr and above 5 yr to 'Nzu clay'

NA - Non-addict; FA – Addicts 5 yr and above; FB – Addicts below 5 yr; Values are mean ± standard deviation of n = 15 determinations

As shown in Figure 1, Pb in the hair of FB doubles the value in the head hair of NA (0.63±0.25 mg/kg), while the hair of the subject FA had Pb being five-fold (2.99±1.52 mg/kg) than stored in NA. There was significant elevation in the concentrations of Pb in the hair of the subjects FB and FA compared to NA. High level of lead in normal head hair could index unhealthy living habit. High exposure of lead can cause problems in the synthesis of haemoglobin, damage to the kidney, gastrointestinal tract, joints, reproductive system and the nervous system. Studies have suggested that exposure to lead can cause up to a loss of 2 intelligent quotient (IQ) points [34].

It is worthy of note that the values of Pb in the hair of the addicts of 'Nzu' in this study are generally lower than the concentration of Pb reported for mechanic head hair with the value (457.9 mg/kg), and that in the academician head hair with the value (343.4 mg/kg) [29]. This points to the fact that consumption of 'Nzu' did not have health concern in terms of Pb bio-accumulation in its consumers even at 5 years of consumption.

The mean level of As was 18.15±0.61 mg/kg in the hair of NA, while it was 22.44±0.77 mg/kg in the hair of FB, and the level was 25.60±2.57 mg/kg in the hair of FA (Figure 1). There was no significant difference in the bio-accumulated As in the hair of the addicts to 'Nzu' compared to non-consumers of the clay. All the values obtained in this study are lower in As than those reported for As in the academician head hair with the value (28.8 mg/kg) and mechanic head hair with the value (19.6 mg/kg) by Aremu *et al* [29]. This study is have lower value of As than that reported in the head hair of normal human (2.90 mg/100g) by Onianwa *et al*. [35]. This infers that consumption 'Nzu' does not result to systemic accumulation of As.

From the study by Ogah and Ikelle [31], Zn in edible clay obtained from Enyigba, Ebonyi State Nigeria, was 0.830 ± 0.02 mg/kg. Also, Kelle *et al* [15] reported the range 2.737±0.181 - 10.332±0.067 mg/kg for some edible clay types purchased from Onitsha markets, Nigeria. The mean concentrations of zinc reported for 'Nzu'- kaolin- and 'ulo' -bentonite- were less than the permissible limit of 100 mg/kg of zinc in food substances [36]. The level of Zn was 0.11±0.05 mg/kg in the hair of NA, while it was 0.17±0.05 mg/kg in the hair of FA (Figure 1). There was no significant difference in the hair level of Zn as a function of 'Nzu' consumption ($P > 0.05$).

Edible clay contained 0.837±0.022 mg/kg Ni [32]; 89340 ± 34 mg/kg Ni [31]; but the FAO/WHO safe limit for Ni in food is 67 mg/kg [36]. The concentration of Ni was 1.77±0.61 mg/kg in the hair of NA, while it was 3.75±0.28 mg/kg in the hair of FB, and increased by six-fold (7.02±5.71 mg/kg) in the hair of FA (Figure 1). The study indicated that consumption of 'Nzu' beyond 5 years by addicts increased the Ni content of the head hair significantly ($P = 0.032$).

From Figure 1, Cu was 0.86±0.36 mg/kg in the hair of the subject NA, 2.25±0.19 mg/kg in FB, and doubles to 4.04±1.61 mg/kg in the head hair of FA. This indicated a significant elevation of Cu in the hair levels by consumption of 'Nzu' ($P = 0.036$).

CONCLUSION

From this study, the head hair of addicted consumers of 'Nzu' for less than 5 years and beyond 5 years accumulated these selected metals in the order: Cr > As > Ni > Cd > Pb > Cu > Zn. The mean Cd in the hair of the consumers beyond 5 years was four-fold the level in the non-consumers (1.09 ± 0.22 mg/kg). Pb in the hair of FB doubled the value in the head hair of NA

(0.63±0.25 mg/kg), while the hair of the subject FA had Pb being five-fold (2.99±1.52 mg/kg) that stored in NA. The long-term consumption of 'Nzu' significantly elevated the hair levels of Cr, Cd, Cu, Pb and Ni. From the level of As and Pb in the hair of normal adult human in literature, the consumption of 'Nzu' did not have health concern in terms of the systemic accumulation of As and Pb in its consumers even at 5 years of consumption. The concentrations of these heavy metals in the hair are an index of the systemic amounts. Thus, the long-term health concern of consuming 'Nzu' should not be dismissed, notwithstanding its long time acclaimed health benefits.

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