

## Copper and calcium concentrations in hair and fingernails of some Kano inhabitants

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### ABSTRACT

Copper and calcium concentrations in hair and fingernail were determined by Flame Atomic Absorption Spectrometry (AAS). The mean copper in hair and fingernail were  $0.19 \pm 0.11$  mg/g and  $0.62 \pm 0.46$  mg/g while the mean calcium in hair and fingernail were  $0.90 \pm 0.56$  mg/g and  $7.43 \pm 4.91$  mg/g respectively. There was a progressive increase in calcium concentrations in hair and fingernails with age. A significant difference ( $p \leq 0.05$ ) was indicated when their means were compared. Comparing the mean copper concentrations in hair with the fingernails a significant difference is indicated in the two tissues ( $p \leq 0.05$ ). Human hair and fingernails are therefore recording filaments that can reflect metabolic changes over long periods of time and hence furnish a print out of post nutritional event as dietary levels of some of the essential micro-elements

Keywords Copper, calcium, hair, fingernail, determination

### INTRODUCTION

The symptoms of copper poisoning include nausea, vomiting, diarrhea, hypotension, jaundice, haematuria, anuria, coma and death (Chuttani et al., 1965). Sources of copper include drinking water supplies running through copper piping (Barnes and Bradley, 1994; Bradley and Bennett, 1995). Cigarette smoking is also a source of excessive copper accumulation (Davidoff et al., 1978). Similarly oral contraceptives are implicated at raising the body's copper burden (Crews et al., 1980). Hair copper levels are indicative of body status, except that exogenous contamination may occur giving false results (Hull, 2003). Copper is an essential element that activates specific enzymes. Erythrocyte superoxide dismutase (SOD) is a Cu and Zinc dependent enzyme, lysyl oxidase which catalyzes crosslinking of collagen is another copper dependent enzyme (Hull, 2003). Deficiency signs include anaemia, thrombocytopenia, neutropenia, malabsorption symptoms and kinkhair (Baumgartner, 1993). Cardiovascular disorders are evident in severe copper deficiency whether genetic or nutritional in origin (Underwood, 1977).

A high hair and fingernail calcium with low hair potassium is associated with a copper imbalance. This is so even if the hair copper level is in the normal range. Copper imbalance is associated with acne and pre-menstrual tension. The copper level tends to correlate with the level of oestrogen in the body. Copper imbalance tends to accentuate emotions and can contribute to depression, mood swing and irritability (Wilson, 1998).

Calcium balance is determined by the relationship between calcium intake and calcium absorption and excretion. A striking feature of the system is that changes in calcium absorption and excretion can neutralize a high intake or compensate for a low one (FAO, 1990). There is a wide variation in calcium intake among nations, generally following the animal protein intake and depending largely on dairy product consumption.

However, high level of calcium in hair and fingernails does not mean one has an excess of calcium in the body. The phenomenon is called biologically unavailable calcium. In this case, calcium precipitates into the tissues, instead of

remaining in the blood (Wilson, 1998). A high tissue calcium level is associated with feelings of depression. Calcium stabilizes cell membranes and increases the voltage required for nerve cells. It depresses the functioning of the nervous system (Cranton and Passwater, 1983). When calcium is over 200mg per 100 grams it is called a calcium shell pattern associated with psychological withdrawal and at times a lack of awareness. Often these individuals are very sensitive to stress or not proficient at coping with stress (Wilson, 1998). Calcium hair levels correlate with long term dietary intake. The hair calcium level does not necessarily reflect current serum calcium or calcium ion concentrations and may not have a line or direct relationship with tissue deposition or bone density. The reported level of hair calcium may reflect external contamination from hair preparations, which contribute to the measured level. Hair is not particularly valuable for assessing calcium, it may be useful as part of ratios (Smith et al., 1998).

Different methods were used by different workers for the determination of metals in hair and fingernails (Ashraf et al., 1994; Bustueva et al., 1993; Nowak and Chmielnicka, 2000; Wilhem et al., 1991; Zhang et al., 2001). Wilhelm et al (1991) analyzing some metals in hair and toe nails of children using atomic absorption spectrometric method, reported that in toe nails all elements were positively correlated with each other while in hair, there was a close relationship only between cadmium and lead and concluded that for biological monitoring, toenail clippings may be less suitable than hair samples.

Employing flame AAS method for determining cadmium in hair, Bustueva et al (1993) reported higher cadmium levels for workers exposed to cadmium than for the unexposed populations. The age and sex dependence of some selected metals in scalp hair of urban population has been reported Ashraf et al. (1994). The observed order of metal concentrations in male hair as

Ca>Mg>Fe>Sr>Al>Cu>Mn>Cr>Ag>Cd and in female samples  
Ca>Mg>Cu>Fe>Sr>Al>Mn>Ag>Cr>Cd.

Using electro thermal AAS, lead in hair of children and adults from industrialized areas of Russia were reported with concentration range of 4.42 – 48.3µg/g and 2.0 – 14.50 µg/g in children and adults respectively (Boris, 1994). Estimating the level of arsenic in hair and nails of people from the districts of west Bengal, using flow injection hydride generation AAS to evaluate the

environmental exposure to lead and cadmium in hair, nails and teeth using atomic absorption spectroscopy, Nowak and Inicka (2000) observed that an increase in lead concentration in hair causes a decrease in iron and calcium, while in nail it decreases concentrations of copper and zinc.

Determining the levels of iron in hair and nails of some women from Saudi Arabia using electrothermal atomization atomic absorption spectrophotometer, Hashem and Othman (2001) reported a concentration range of  $0.94 \pm 0.11$  and  $18.64 \pm 1.01$ mg/g in hair and nails respectively.

Determining traces of cadmium in human hair, finger and toenails using flame atomic absorption spectrometry, Zhang et al (2001) observed the range for cadmium in hair as 0.0100 – 0.4100 mg/g, in fingernail 0.0295 – 0.5314 mg/g and 0.0899 – 1.274 mg/g in toenail respectively. To evaluate the nutritional status of zinc in children living in the municipality of Paraiba, Brazil, Sandra and Silva (2002) observed no correlation using the comparison between hair zinc concentration and enzymatic activity levels of serum alkaline phosphates.

Employing atomic absorption spectroscopic method for determining cadmium, lead, chromium, manganese, iron, nickel, copper and zinc concentrations in nails, Mehra and Jureja (2005) reported that the values obtained were correlated to the personal and medical history of the subjects. This paper reports the determination of copper and calcium in human hair and fingernails.

## MATERIALS AND METHODS

Copper and calcium were determined from various subjects resident in Kano for at least six months. 350 hair and 300 fingernail samples were collected from subjects in the age range of 1-55years. Nail samples were collected in polyethylene containers. Hair samples were cut at the occipital area of each subject. Surface contamination and grease were removed by washing the hair samples in teapot and distilled water after which the samples were kept in a 50% alcohol-ether mixture for 45mins and dried at 60°C for 72hr .

Each sample, 1.0g, was digested in 10cm<sup>3</sup> concentrated HNO<sub>3</sub> and the resulting solution was evaporated to dryness on a hot plate and redissolved in 0.1M nitric acid. Trace metal concentrations were determined by Flame Atomic Absorption on a Buck Model 210 VGP Spectrophotometer attached to IBM personal computer. The result of the absorbance of each

sample was the average of ten sequential readings. Background light absorption and scattering were compensated for either by deuterium hollow cathode lamp or by tungsten/halogen lamp. Distilled water was digested as blank using the same procedure previously described (Ayodele and Abubakar, 1998; Ayodele and Abubakar, 2001)

### Statistical Analysis

All statistical computations were carried out on the PC486 66MHZ microcomputer using either the integrated statistical package for windows from Umstat Ltd.(London) or dedicated micro instructions for the Excel spread sheets from Microsoft. The analyses of variance (ANOVA) were carried out according to described procedures (O'Mahony, 1986).

## RESULTS AND DISCUSSION

The concentration of elements in hair and fingernails vary widely among individuals, thus large number of samples from a population was analyzed and the results treated statistically for meaningful correlation. The copper and calcium concentrations in hair and fingernails were determined using atomic absorption spectroscopic method. The age, sex and occupation of the donors were noted where necessary.

The frequency distribution patterns for Ca and Cu in hair and fingernails vary widely among individuals, thus large number of samples from a population were treated statistically for meaningful correlation. The in these samples, their mean and coefficient of variation are employed in assessing their levels. The frequency distribution pattern for the age of donors (years) is as shown in (Fig1). The distribution is multimodal and is skewed towards high frequency of low age with a mean age of  $27.51 \pm 16.50$  years.

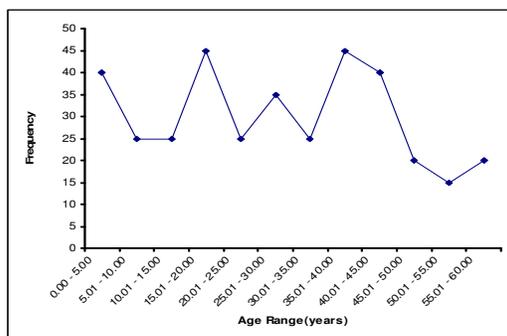


Fig.1 Frequency Distribution Pattern for Age (years) of Donors

The frequency distribution pattern for copper in hair as shown in Fig. 2. The distribution is unimodal and is skewed towards high frequency of low concentration with a mean and standard deviation of  $0.19 \pm 0.11\text{mg/g}$ . The mean copper concentration in hair is within the reference range of  $0.10 - 0.28\text{mg/g}$  (Hull, 2003). The frequency distribution pattern for copper in fingernails is as shown in Fig 3. The distribution is multimodal and is skewed towards high frequency of low concentration with a mean and standard deviation of  $0.62 \pm 0.46\text{mg/g}$ . The level in fingernails is above the reference range of  $4-56\mu\text{g/g}$  (Iyengar et al., 1978). A strong correlation exist between the copper content in hair and in fingernails ( $p < 0.01$ ) as shown in Table 2-4. The ANOVA has shown that the mean copper level in fingernails is significantly higher than in hair ( $p > 0.05$ ) as shown in Table 3. Fig. 4 represents copper concentration in hair and fingernails with respect to age. From Fig 4 copper concentration appears higher in adolescents Sources of copper in adolescents include dental alloys, smoking and the use of oral (Ross and Marion, 2001; Crews et al., 1980). Wilson (1998) reported that copper level tends to correlate with the level of estrogen in the body.

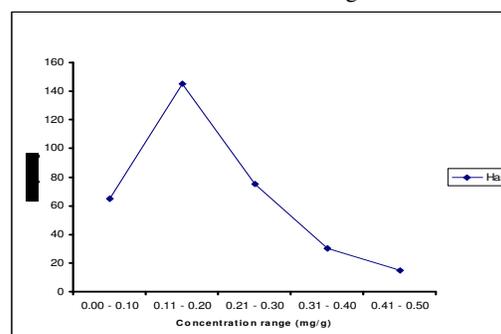


Fig. 2 Frequency Distribution Pattern for Copper in Hair

The frequency distribution pattern for calcium in hair is as shown in Fig.5. The distribution is bimodal and is skewed towards high frequency of high concentration with a mean and standard deviation of  $0.90 \pm 0.56\text{mg/g}$ . Fig.6 represents the frequency distribution pattern for calcium in fingernails. The distribution pattern is multimodal and uniformly distributed with a mean and standard deviation of  $7.43 \pm 4.91\text{mg/g}$ . A significant correlation exists between the calcium

content in hair and fingernails ( $p < 0.01$ ) as shown in Table 1. However, comparison between the mean concentration in hair and fingernails indicate no significance difference at  $p > 0.05$  (Table 3.). The high level of calcium obtained in nails indicates that calcium is playing some physiological functions in fingernails (Johnson, 2007). Calcium concentration in hair with respect to age is as shown in Fig 7. The distribution pattern is skewed towards high concentration in children and middle aged. Fig 8. represents calcium concentration in fingernails with respects to age of donors. The distribution pattern revealed that children have higher calcium level in their fingernails than adults indicating that calcium is playing some physiological functions in children such as the development of nail bed and nail plate as rapid growth occurs in children than in adults (Johnson, 2002). Calcium (Ca) levels in hair correlate with long term dietary intake, absorption from the gastro-intestinal tract and retention. The hair calcium level does not necessarily reflect current calcium ion concentrations and may not have a linear or direct relationship with tissue deposition or bone density. The reported level of hair Ca may reflect external contamination from hair preparations, which contribute to the measured level (Nowak, 1998; Miekeley et al., 1998; Rodushkin and Axelsson, 2000; Chojnacka et al., 2005 & 2006).

Hair copper (Cu) levels may be indicative of excess copper in the body. Medical conditions that may be associated with excess copper include: biliary obstruction (reduced ability to excrete Cu), liver disease (hepatitis or cirrhosis), and renal dysfunction. Symptoms associated with excess Cu accumulation are muscle and joint pain, depression, irritability, tremor, haemolytic anaemia, learning disabilities, and behavioural disorders. It is important to rule out contamination from dyes, bleaches, swimming pool/hot tub water, and washing hair in acidic water carried through copper (Barnes and Bradley, 1994).

Sources of excessive copper include contaminated food or drinking water, excessive Cu supplementation, and occupational or environmental exposures. Insufficient intake of competitively absorbed elements such as zinc or molybdenum can lead to, or worsen Cu excess. Confirmatory tests for copper excess are a comparison of copper in pre versus post provocation (D-Penicillamine, DMPS) urine elements tests and a whole blood elements analysis (Barnes and Bradley, 1994; Bradley and Bennett, 1995; Mehra and Juneja, 2005).

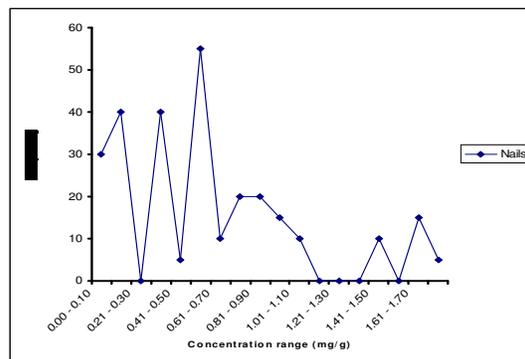


Fig: 3 Frequency Distribution Pattern for Copper in Nails

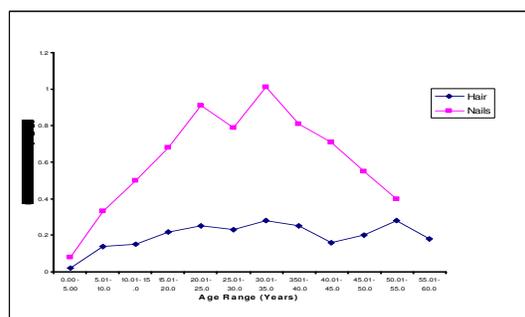


Fig. 4 Copper concentrations (mg/g) in Hair and Nails with respect to age

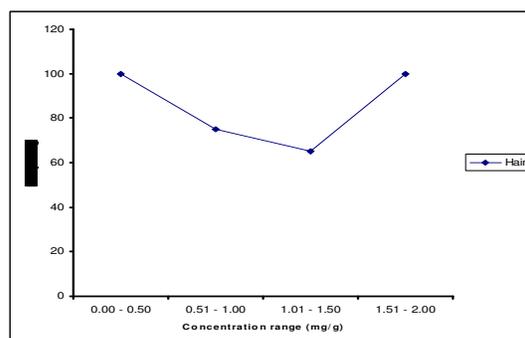


Fig. 5 Frequency Distribution Pattern for Calcium in Hair

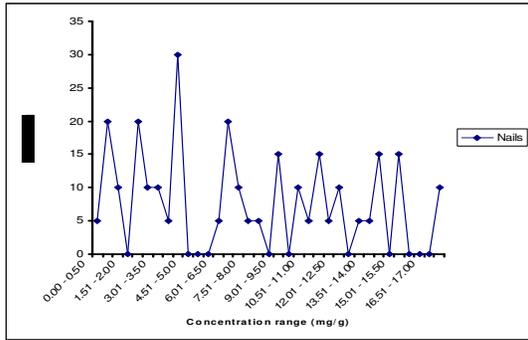


Fig. 6 Frequency Distribution pattern for Calcium in Fingernails

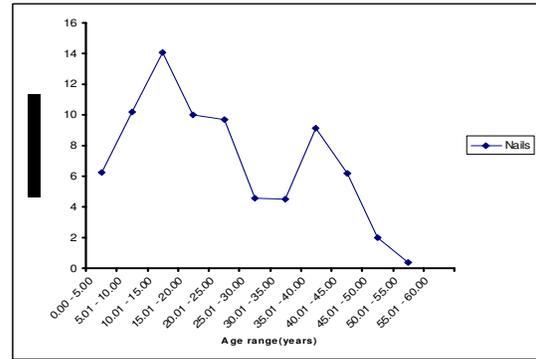


Fig. 8: Calcium concentration (mg/g) in Fingernails with respect to age

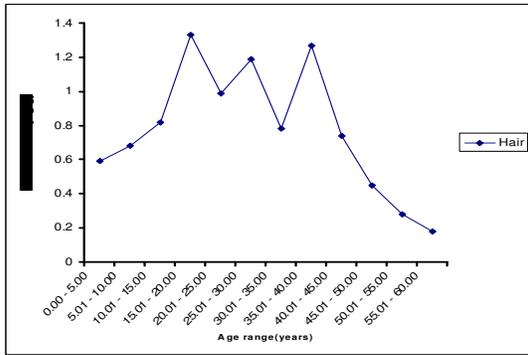


Fig. 7: Calcium concentration (mg/g) in Hair with respect to age

**Table. 1 Parametric Correlation Coefficients for Copper in Hair and Fingernails**

	Hair	Fingernails
Hair Pearson Correlation	1	.849**
Sig. (2-tailed)	.	.000
N	350	300
Fingernails Pearson Correlation	.849**	1
Sig. (2-tailed)	.000	.
N	300	300

\*\* Correlation is significant at the 0.0 1 level

**Table2 Analysis of variance for Copper in Hair and Fingernails**

Source of variation	SS	df	MS	F	P-valve	F crit
\$Between Groups	5.184131	1	5.18413091	46.096091	6.333E-10	3.9290115
Within Group	12.4607	108	0.11246357			
Total	17.3302					

**Table 3:Parametric Correlation Coefficients for Calcium in Hair and Fingernails**

	Hair	Fingernails
Hair Pearson Correlation	1	.577**
Sig. (2-tailed)	.	.000
N	350	300
Fingernails Pearson Correlation	.577**	1
Sig. (2-tailed)	.000	.
N	300	300

**Conclusion**

Human exposure to toxic trace elements has been the focus of increasing attention among researchers, formulators and managers of health and nutrition policies due to its damages to health. The levels copper and calcium in hair and nails vary and may be affected by various factors (Siedel et al., 2001). Age was observed to be a factor influencing their levels. The values of two elements recorded revealed sex dependence (Underwood, 1977; Oluwole et al., 1994; Siedel et al., 2001; Sandra and Silva, 2002). Hair colour, nutritional status, geographic, racial/ethnic and ecological can have a significant impact on the levels of these elements in hair and nails (Sandra and Silva, 2002) since the samples were collected from the same geographical location. The high trace element levels in hair and nails make analysis easy; slow metabolic turnover rate of hair; and its being a

reliable status of body without daily variation. The collection of samples were simple and non traumatic. Hair and nails are regarded as complementary to body fluids in biological monitoring. Comparing hair and nails as points of excretion the latter appear superior to the former. The former enables monitoring of elements accumulated over a time span up to several months. They are easily sampled, handled and transported, and less prone to post – sampling contamination because of higher elemental concentration. Therefore human hair and nails are recording filaments that reflect metabolic changes of many elements over long periods of time and hence furnish a print – out of post nutritional event (Strain et al., 1972) as dietary levels of some essential micro – elements have been reported corresponding to hair concentrations of the elements (Maugh, 1978; Hopps, 1974; Casey and Hambidge, 1980).

**Table 4: Analysis of variance for Calcium in Hair and Fingernails**

Source of variation	SS	df	MS	F	P-value	F crit
Between Groups	1162.135	1	1162.135	95.2727507	1.633E-16	3.9290115
Within Group	1317.382	108	12.197979			
Total	2479.517	109				

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2009

Ayodele and Bayero - *Copper and calcium concentrations*

121

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