ANALYSIS OF KUALI AND ASSOCIATED BLOOD-LEAD CHANGES IN USERS

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ABSTRACT

Physico-chemical methods were used to analyse the commonly used kuali samples bought from Zaria and Kano local markets. Blood-lead concentrations in kuali users and non-useres were determined. All the kuali samples were opaque, brittle, greyish-black, stable to heat, insoluble in many inorganic and organic solvents and very unreactive towards chemicals. Metals and non- metals including blood-lead were detected by flame and spot tests and were determined by atomic absorptian spectroscopic and classical analysis methods respectively. Bromide, chloride, fluoride were estimated volumetrically and sulphur gravimetrically. The results of atomic absorption spectrometry and spot tests revealed the presence of antimony, arsenic, cadmium, copper, iron, lead, manganese, nickel, silver, tin and zinc. The concentration of lead in kuali samples was found to be low compared to other metals present in the samples. Sulphur has the highest concentration amongst the non-metals present in kuali samples. The mean blood-lead concentration in kuali users was 0.90 ppm compared with 0.29 ppm in non-users.

INTRODUCTION

A naturally occurring mineral called *kuali, tiro* and *otajele* in Hausa, Yoruba and Ibo languages respectively, is widely used by all age groups of Nigerians traditionally as a principle of hygiene, to relieve eye strain, cure stye or to increase visual acuity.

The relation between pollution and lead intoxication due to man made alterations in the environment, which may bring about changes in mineral balance and, as a consequence, in biological functions[1], has attracted considerable attention[2,3]. Literature review showed only few instances of lead intoxication due to cosmetics[4,5]. The effect of chronic exposure to lead on childhood development and pregnancy is of particular concern. There is no restriction on the sale of *kuali*. There is no report on the analysis of *kuali* and associated changes of minerals in the blood of users. This paper reports our preliminary findings.

EXPERIMENTAL

Reagents and instruments

All reagents used in this work were of analytical grade. De-ionized water was used for the preparation of all solutions. Pye-Unicam SP-1900 double beam atomic absorption spectrophotometer with automatic background correction operated on air-acetylene flame was used for metal determinations. 21 G X 1.5 Gillette sterile needles, 2ml disposable sterile syringes, Baird and Tatlock autobench centrifuge and Herbert Alexander grinder were used.

Kuali sample and sample preparation

Four different types of *kuali* samples were used in this study. Each of samples 1, 2 and 3 was a combination of a specimen bought in Zaria market with a similar one bought in Kano market. The fourth sample was bought from Kano local market only because it was not available in Zaria. The third sample was imported into Nigeria from Saudi Arabia while the others were native. All the samples used in this work were visually observed and are described later. For analysis, each solid sample was ground to powder and stored in a stoppered plastic bottle for use as required.

Solubility properties of kuali samples

All samples were tested for solubility in water, nitric, sulphuric, hydrochloric, perchloric and acetic acids, aqua regia, ethanol, chloroform and liquid paraffin with heating as appropriate. The results are discussed elsewhere in this paper.

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Table 1: Preparation of stock solutions

Metal	Source material (g mass)	Other reagents
Sb	Metal granules (1.000)	$10cm^3$ $HN0_3$
As	AS_20_3 (1.320)	$50cm^3$ HCl
Cd	$Cd(NO_3)_2$ (2.107)	none
Cu	$CuSO_4.5H_20$ (3.930)	none
Fe	$FeCl_3$ (4.830)	$1.5cm^3$ HCl
Mn	$MnSO_4.7H_20$ (4.069)	$1.5cm^3$ HNO_3
Ni	$Ni(NO_3)_2:6H_20$ (4.953)	$1.5cm^3$ HNO_3
Pb	$Pb(NO_3)_2$ (1.598)	$25cm^3$ HNO_3 (1:1 v/v)
Ag	$AgNO_3$ (1.575)	$1.5cm^3$ HCl
Sn	$SnCl_2.5H_20$ (2.954)	$1.0cm^3$ HCl
Zn	$ZnSO_4.7H_2O$(4.398)	none

Preparation of solutions

To prepare *kuali* sample solutions, 1.0g of each sample was digested with $40cm^3$ of concentrated perchloric acid in a hot water bath for 30 minutes in a fume cupboard; after cooling the volume was made up to $100cm^3$ in a volumetric flask with deionised water.

For each metal element to be determined by AAS, 1litre of a 1000 ppm stock solution was prepared using the reagents indicated in Table 1 and making up to mark with distilled water in a volumetric flask. Working standards were obtained from these stock solutions by appropriate dilution.

Qualitative analysis

Flame tests[6] and spot tests[7] were conducted on samples to qualitatively detect the metals and non-metals present in them. The results are shown in Table 2.

Table 2: Results of flame and spot tests.

Occurrence	Metals and Non-metals detected
All samples	Antimony, Arsenic, Iron, Lead Nickel, Silver, Tin, Bromide, Chloride and Fluoride
Sample 1 only	Cadmium and Sulphate
Sample 2 only	Zinc and Sulphate
Sample 3 only	Cadmium, Zinc, Copper and sulphite
Sample 4 only	Zinc and Sulphite

Collection and storage of blood

A survey was conducted for the collection of whole blood samples on 60 people mostly women attending University Health Services/Teaching Hospital, Ahmadu Bello University, Zaria, for various medical ailments. Blood samples were collected from 20 female *kuali* users and 10 male *kuali* non-users in the age group of 20-30 years. Care was taken to include only those cases where the possibility of lead ingestion was ruled out. Therefore, blood samples were not collected from painters, battery workers, traffic wardens and people living close to the main roads.

The whole blood samples were collected by venipuncture after cleaning the skin thoroughly and using sterile disposable needles and plastic syringes. The blood samples were stored in $2.5cm^3$ Pyrex sample bottles containing $0.2cm^3$ of 10% sodium-EDTA as anti-coagulant. The blood samples were stored in a freezer immediately on collection and were analysed within seven days[8].

Preparation of blood sample solutions

To separate lead from the blood matrix and extract it into an aqueous solution[9], $2.0cm^3$ of each blood sample was treated with $5.0cm^3$ of 5% trichloroacetic acid and the mixture extracted with $1.0cm^3$ of 2% ammonium pyrrolidine dithiocarbamate into $5.0cm^3$ of methylisobutylketone (MIBK) at pH 3 and the extracts made up to $100cm^3$ with de-ionized water. A blank solution was prepared in the same way but excluding the blood samples.

Determination of non-metals in kuali

Bromide, chloride and fluoride were determined by volumetric analysis while sulphur was determined by gravimetric analysis[10]. The concentration of bromides and chlorides in the four *kuali* samples were determined by titrating 0.1M silver nitrate solution against the sodium carbonate extract of the *kuali* samples by using eosin and fluorescein as indicators respectively. The method of fluoride determination involves the titration of the sodium carbonate extract of the *kuali* samples with thorium nitrate, at pH 3.35, using thymol blue as an indicator. Sulphur was determined as barium sulphate from the weakly acidic solution of the *kuali* sample. Sample results are presented in Table 3.

Table 4: AAS Instrument conditions.

Metal	Wave length (nm)	Lamp current (mA)	Acetylene flow rate (l min^{-1})	Air flow rate (l min^{-1})	Burner height (cm)
Antimony	206.84	15	1	5	12
Arsenic	193.70	8	1	5	12
Cadmium	228.80	6	1	5	12
Copper	324.75	5	1	5	12
Iron	248.33	15	1	5	12
Manganese	279.48	12	1	5	12
Nickel	232.00	15	1	5	12
Lead	217.00	6	1	5	12
Silver	328.07	4	1	5	12
Tin	224.61	8	1	5	12
Zinc	213.86	10	1	5	12

Table 3: Determination of non-metals.

Non-Metals	Sample concentration (mg/g)			
	Sample 1	Sample 2	Sample 3	Sample 4
Bromide	24.00 ± 2.00	43.20 ± 1.80	34.00 ± 2.10	48.00 ± 1.20
Chloride	5.60 ± 2.40	4.97± 2.90	9.50 ± 3.10	10.60 ± 4.20
Fluoride	23.40 ± 4.60	24.90 ± 5.30	21.90 ± 5.50	23.20 ± 4.00
Sulphur	126.00 ± 21.00	125.00 ± 15.00	122.00 ± 19.00	102.00 ± 9.40

Quantitative analyses for metals in kuali and blood

Antimony, arsenic, cadmium, copper, iron, manganese, nickel, lead, silver, tin and zinc were determined in *kuali* samples and lead also in the blood samples by atomic absorption spectrometry (AAS). The instrumental parameters for each element are shown in Table 4, while the results for metals in *kuali* are shown in Table 5 and those for the blood lead levels are shown in Table 6 for *kuali* users and non-users. For these results absorbance measured for sample solutions were interpolated on calibration graphs derived from working standard solutions to obtain sample solution concentrations. The lead blank levels were discounted from blood lead results in each case.

Table 5: Determination of metals detected in kuali samples.

Metals	Concentration (ppm)			
	Sample 1	Sample 2	Sample 3	Sample 4
Antimony	3.40 ± 1.80	4.80 ± 2.50	4.00 ± 2.10	8.00 ± 3.60
Arsenic	1.60 ± 0.20	1.80 ± 0.10	1.70 ± 0.40	1.80 ± 0.60
Cadmium	0.15 ± 0.02	0.10 ± 0.01	0.50 ± 0.03	0.10 ± 0.02
Copper	B.D.L.	0.10 ± 0.01	1.25 ± 0.20	0.25 ± 0.04
Iron	3.80 ± 0.60	2.80 ± 0.90	14.00± 2.20	4.00 ± 1.10
Manganese	B.D.L.	B.D.L.	0.35 ± 0.01	B.D.L.
Nickel	4.80 ± 0.40	4.60 ± 0.80	4.00 ± 1.10	4.20 ± 0.65
Lead	1.65 ± 0.10	2.00 ± 0.50	3.00 ± 0.40	1.15 ± 0.60
Silver	4.10 ± 1.90	3.40 ± 2.10	5.20 ± 1.90	4.50 ± 2.90
Tin	8.00 ± 2.10	11.30 ± 2.90	4.00 ± 1.20	6.80 ± 1.80
Zinc	0.10 ± 0.02	0.50 ± 0.04	35.00 ± 1.00	1.00 ± 0.05

B.D.L. = Below Detection Limits

Table 6: Blood-lead concentrations in kuali users and non-users.

Kuali Users		Non Kuali Users	
Case No.	ppm Pb	Case No.	ppm Pb
1	1.00 ± 0.05	1	0.18 ± 0.03
2	1.42 ± 0.06	2	0.34 ± 0.05
3	0.82 ± 0.04	3	0.42 ± 0.04
4	1.18 ± 0.04	4	0.25 ± 0.01
5	1.25 ± 0.03	5	0.34 ± 0.03
6	0.75 ± 0.03	6	0.18 ± 0.02
7	1.00 ± 0.01	7	0.34 ± 0.03
8	0.75 ± 0.03	8	0.25 ± 0.01
9	0.68 ± 0.01	9	0.25 ± 0.02
10	0.68 ± 0.02	10	0.42 ± 0.01
11	0.82 ± 0.01		
12	0.82 ± 0.01		
13	0.58 ± 0.02		Variance = 0.007
14	0.75 ± 0.02		
15	0.68 ± 0.05		
16	0.75 ± 0.05		
17	1.00 ± 0.06		
18	0.82 ± 0.08		
19	0.92 ± 0.07		
20	1.34 ± 0.04		

Variance = 0.054

F = 7.104

RESULTS AND DISCUSSION

Properties of kuali

All the *kuali* samples were crystalline, opaque and brittle; they were grey-black coloured lumps except sample 3 which was powdery already. The *kuali* samples did not melt or show any change in physical appearance or weight when heated in a flame. The *kuali* samples were insoluble in all the inorganic and organic solvents tested except concentrated HCl in which all the *kuali* samples were partially soluble and perchloric acid in which all the *kuali* samples were completely soluble. This reflects the chemical non-reactivity of the samples.

Qualitative analysis

The greenish and bluish colours observed in the flame tests on *kuali* samples gave an indication that more metals like antimony, zinc, arsenic and lead were probably present[6]. Spot test analysis[7] confirmed the metals and non-metals listed in Tables 2 and 3.

Determination of non-metals

Table 3 shows the non-metals determined in kuali samples including bromide, chloride, fluoride and sulphur. Sulphur had the highest concentration in all the samples with the maximum value detected in sample 1. Chloride had the lowest level with the least value in sample 2. The bromide level was higher than fluoride in all the samples. The concentration of fluoride was almost the same in all the samples. In the first sample the concentration of fluoride and bromide were nearly the same while in other samples they were different.

It appears that the major metals of the samples occur as sulphides, sulphites and sulphates. In addition to these, bromides, fluorides and chlorides of the metals are also expected. The action of concentrated HCl and perchloric acids on the samples with the evolution of H_2S gas confirms that some of the metals exist in the form of sulphides. The results of spot tests and classical analysis however, showed that sulphite/sulphate were present instead of sulphide. This may be because the sample solutions for spot test analyses were prepared with sodium carbonate and HNO_3, and during preparation the sulphide might have been oxidised to sulphite. The analysis could not be carried out on solid samples because of their inert nature towards the test reagents. Therefore, it was necessary to bring the non-metals into solution so that the test reagents could act on them.

Blood sample collection

Kuali users who were investigated used *kuali* for different reasons. Out of 20 *kuali* users, nine used it for cosmetic reasons, five used it traditionally to increase the visual acuity, five used it for relieving eye strain and one used it to cure stye. The belief that the use of *kuali* increases the visual acuity may not be entirely wrong because eyelid surfaces tinted with this shining powder will show more reflection of incident rays of light and therefore glare will be decreased. Use of *kuali* for prevention and treatment of eye infections may be due to the antibacterial action of organic substances, non-metal and metal ions present in *kuali*. However, due to the presence of lead and other hazardous ions its medicinal use is not recommended. All the persons included in the survey used *kuali* since their birth. Factors like age and sex which have important bearing on the trace metal status of individuals could not be taken into account during sample collection since the available *kuali* users were all adult females and non-users all adult males.

Metals in kuali samples

The metals determined by AAS in all the *kuali* samples include antimony, arsenic, cadmium, copper, iron, lead, manganese, nickel, silver, tin and zinc. The concentration of these minerals varied in all the samples (Table 5). The concentration of tin, nickel, silver, iron and antimony are appreciably high and arsenic, cadmium, copper and zinc are significantly low in all the *kuali* samples except in sample 3 which also has the highest concentration of tin and the lowest concentrations of cadmium and copper while manganese is below the detection limit in it. Nickel and antimony have quite similar concentrations in this sample. In sample 3 the concentration of tin, antimony and nickel are exactly the same and there are moderate levels of the other metals.

Sample 4 has the highest concentration of antimony and lowest concentration of cadmium while manganese was below the detection limit. This study shows that the *kuali* is very rich in minerals.

Lead in blood samples

Lead was determined in the blood of *kuali* users and non-users. Table 6 shows the blood-lead concentrations in *kuali* users ranging from 0.50 ppm to 1.42 ppm with a mean concentration of 0.90 ppm, whereas, blood-lead concentration in non-*kuali* users varied from 0.18 to 0.42 ppm with a mean value of 0.29 ppm. There is a significant difference of blood-lead levels between *kuali* users (0.90 ppm) and non-users (0.29 ppm). The F test[11] indicated that there was a significant difference between the lead levels of *kuali* users and non-users (Table 6). The lead might be absorbed across the conjunctiva from the drainage down the tear duct, or from rubbing the eyes and then licking the fingers; an appreciable amount of lead may be absorbed through the skin also[12], resulting in elevated blood-lead levels following the use of *kuali*.

A direct association between use of *kuali* and blood-lead level is indicated. Only those cases were studied in which *kuali* was the only additional source of lead in the users. Since clinical lead poisoning manifests at 0.80 ppm, it is expected that 12 patients among *kuali* users might have symptomatic lead poisoning. The results of this study show that the concentration of lead in four blood samples were low. Lead exposures at doses below those producing symptoms severe enough to be diagnosed clinically appear to be associated with neuro-psychological deficits that may interfere with mental performance[13]. Although in general blood-lead level is a reliable indicator of recent lead absorption, it may not be an accurate index of lead exposure since lead passes through plasma to be stored in bones and teeth resulting in low blood-lead concentrations[14], whereas, the body-lead status might be very high.

CONCLUSION

The use of *kuali* is associated with high blood-lead levels and may be dangerous to infants, young children and pregnant women. Therefore, there is a risk of lead poisoning from *kuali* and its application as eye liner.

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