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# Geochemistry and Acute Toxicity in Rat of Calabash Clay Consumed in Douala (Cameroon)

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## Abstract

**Background:** Calabash chalk geophagia commonly consumed in Sub-Saharan countries such as Cameroon may be a source of potential harmful elements such as lead and mercury. We sought to determine its geochemical composition on representative sample collected in the central market of Douala and evaluate their acute toxicity profile using rat.

**Methods:** The geochemical analysis of three prototypes of Calabash chalk samples collected in the Douala central market, their main place of supply in the town, has been performed using the X-ray Fluorescence (XRF) and the inductively Coupled Plasma-Mass Spectrometry (ICPMS) for major and trace elements respectively. Acute toxicity was assessed according to Organisation for Economic Cooperation and Development (OECD) guidelines test No 423.

**Results:** The geochemistry displays the $Al_2O_3$ as their main major component with iron, titanium, cerium and zinc as metal and metalloid trace elements. No acute toxicity was found in rat after administration of 5000 mg/kg body weight.

**Conclusion:** Calabash chalk belong to the kaolinite family which had showed evidence for digestive pharmacologic propriety. Their acute consumption may be less toxic while toxicity profile of chronic consumption should be investigated.

**Keywords:** Calabash chalk • Aluminium silicate • Heavy metal • Acute toxicity

## Introduction

Consumption of the Calabash chalk is a common and oldest practice in Sub-Saharan, South American and Asian countries. It is considered as the main type of geophagia which is defined as a deliberated consumption of earth, soil or clay [1]. The reason of the Calabash geophagia are multiple, including cultural and medicinal purposes. Some people consumed it for pleasure or for its supposed nutritional values. Pregnant women used it as a medicine for morning sickness and other for spiritual purpose. It is also claimed to increase women fertility, to cure diarrhea and stomach ache as well as effective antidote again poison [2,3].

Calabash chalk is considered as a mineral stone belonging to the kaolinite group. It naturally occurs a fossilized seashell clay material and extracted in underground mine. It can be prepared artificially by molding and heating mixed clay, mud, sand, wood and even salt. The chalk is available as powder, molder shape or block. The main component of Calabash clay is aluminium silicate hydroxide (Al2Si2O5OH4) associated with several oligo-element such as calcium, phosphorus, magnesium, zinc, copper, manganese and iron, especially the ones commonly ingested in Sub-Saharan Africa [4]. Calabash chalk is also reported as having a very high lead concentration and other harmful substances including heavy metals such as mercury, arsenic, titanium, chromium; and organic pollutants alike alpha lindane, endrin, endosulphan II [5]. Thus, there are safety concerns about Calabash chalk consumption. Many reports about health risk associated with their human consumption as well as in animals are available including increase risk of anaemia, alteration of growth rate and demineralisation in the femur bone, gestational complications as well as cerebral toxicity [6-11]. This study was sought to determine the geochemical composition on samples collected in the Douala (Cameroon) and evaluate its acute toxicity profile using rats.

## Materials and Methods

### Sample collection

Sample of Calabash chalk were collected in the Douala central market one of the main places of supply this chalk in the town. According to sellers, Calabash chalk found in the market mainly comes from Nigeria used for eating and cosmetic purpose. The Balengou mines located in West Cameroon provides the market with red and white chalks, mainly used for medicinal purpose. Each prototype of these 3 chalks was sampled.

### Sample analysis

The above samples were prepared and analyzed at the GEOLAB (Ontario, Canada) for their major and trace element abundances following the laboratory techniques and methods (Geoscience Laboratories 2015). Major elements were determined by X-Ray Fluorescence (XRF) by fusing 10 grams of the burned samples with a borate flux to produce a glass bead for the analysis and trace elements by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Sample preparation involved Agate Mill Preparation (SAM-AGM) techniques to reduce the size of rock particles to less than 90 lm (170 Mesh). After powdered, the samples were run for loss on ignition (LOI: 105 °C under nitrogen atmosphere, 1000°C under oxygen atmosphere). The calcined samples were fused with a borate flux to produce a glass bead for analysis. Total contents of each major elements detected are aluminium, chromium, iron, potassium, sodium, calcium, magnesium, manganese and phosphate and expressed as oxide.

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Concentration of elements was then determinated using gravimetric factors conversion. Ferrous irons were quantified by potentiometric titration with a standardized permanganate solution to bring more accurate data their determination. Concentration of Heavy metals such as mercury, arsenic, cadmium and lead were noted. Measurements of geochemical standards (DUP: Laboratory Duplicate) were carried out to guarantee the quality and accuracy of the analyses.

### Evaluation of acute toxicity in rat

The Balengou red calabash chalk since it had the lowest lead concentration, has been chosen for this study. The sample was grounded with a manual mortar and sifted twice with nylon sieve. The powder was then mixed with distilled water to obtain an emulsion of red calabash chalk. The emulsion was administrated in a single dose by force-feeding with a maximum of 2 ml/animal.

Six females Wistar rats of 8 weeks nulliparous and non-gestational were used for acute toxicity studies. Rats were obtained from the animal house, Faculty of Sciences, University of Douala.

They were handled according to the ethical guidelines of the National Institute of Health (NIH) of the United States. Acute toxicity was evaluated according to the test No 423 of the Organisation for Economic Cooperation and Development (OECD) guidelines for the testing of chemicals [12]. Animals were divided into 2 groups of 3 rats: Calabash treated group and a control group which receive distilled water. A single dose of 5000 mg/kg of aqueous red calabash was given orally to the first group. After administration, animals were observed for 4 consecutive hours and periodically until 48 h. Finally, all of the experimental animals were maintained under close observation for 14 days.

Modification of behaviour liked excessive cleaning, lethargy or hyperactivity after administration of the substance were recorded and compared between both groups. The weight of each animal was noted every day during the 2 weeks of observations. At the end of the 2 weeks, the number of rats that died within the study period was noted. The LD50 was predicted to be above 5000 mg/kg if three rats survived. The survived rats were sacrificed by clavicle dislocation. Macroscopic aspect and weight of liver, spleen, kidneys, lungs and heart were recorded during autopsy.

### Statistical analysis

Data were analysed with IBM SPSS 23. The student test was used to compare weight mean in Calabash chalk/distilled water group and p-value was 0.05.

### Ethical consideration

The study was approved by the Ethical board of the Douala University.

## Results

### Geochemistry composition of Calabash chalk

$SiO_2$ and $Al_2O_3$ are the main constituent of Calabash chalk among the 3 types of Calabash chalk collected (Table 1). The aluminium level (16.77 mg/kg) in the Nigerian chalk was higher than the Balengou one (red and white chalk 15.67 mg/kg). Iron was also found; especially among Balengou red chalk, with range from 1.748 to 2.256 mg/kg. All the Calabash chalk belong to per-aluminous bauxitic kaolinite stone.

| Variables | Balengou red chalk | Balengou white chalk | Nigeria chalk |
|---|---|---|---|
| | Wt% (mg/kg) | Wt% (mg/kg) | Wt% (mg/kg) |
| $SiO_2$ | 51.57 (24.105) | 51.52 (24.082) | 48.81 (22.815) |
| $Al_2O_3$ | 29.62 (15.676) | 29.62 (15.676) | 31.69 (16.771) |
| $Fe_2O_3$ | 3.23 (2.256) | 2.50 (1.748) | 2.59 (1.811) |
| $TiO_2$ | 0.33 (0.198) | 0.33 (0.198) | 1.56 (0.935) |
| MgO | 0.08 (0.048) | 0.05 (0.030) | 0.18 (0.108) |
| $Na_2O$ | 0.07 (0.052) | 0.07 (0.052) | 0.09 (0.066) |
| $K_2O$ | 0.02 (0.016) | 0.02 (0.016) | 0.05 (0.041) |
| $P_2O_5$ | 0.030 (0.013) | 0.030 (0.013) | 0.101 (0.044) |
| MnO | 0.018 (0.014) | 0.018 (0.014) | 0.008 (0.006) |
| CaO | 0.008 (0.005) | 0.006 (0.004) | 0.014 (0.01) |
| LOI | 15.354 | 16.166 | 16.456 |
| Total | 100 | 100 | 100 |

Wt%=weight percentage of oxide element; mg/kg correspond to element concentration not oxide; LOI=Loss of Ignition

**Table 1.** Major composition of Calabash chalk samples.

Arsenic was more abundant in Nigerian chalk (3.6 ppm) and upper the international toxic level of 1 ppm; while it was <0.4 ppm in both Balengou chalks. Cadmium level varied from <0.006 ppm (Nigerian chalk) to 0.015 in Balengou red chalk, and was under the toxic level of 0.3 ppm. Mercury was not detected in Balengou chalk (<0.006 ppm) and was found at 0.043 ppm in Nigerian chalk (toxic level 1 ppm). Lead level was found at 4.67 ppm, 6.780 ppm and 10.42 ppm respectively in Balengou red, Balengou white and Nigerian chalk (Toxic level 10 ppm). Copper was more abundant in Nigeria chalk (8.7 ppm) compare to Balengou red (2.5 ppm) and Balengou white (2 ppm) chalks. Selenium was also noted between 0.3 to 0.47 ppm and was more abundant in Balengou red chalk as well as zinc (Balengou red chalk 122.9 ppm, Balengou white chalk 112.3 and Nigerian chalk 102 ppm). Barium was found at high level especially in Balengou white chalk (86.5 ppm) considered the recommend level <0.21 mg/kg/day in adult (Figure 1).

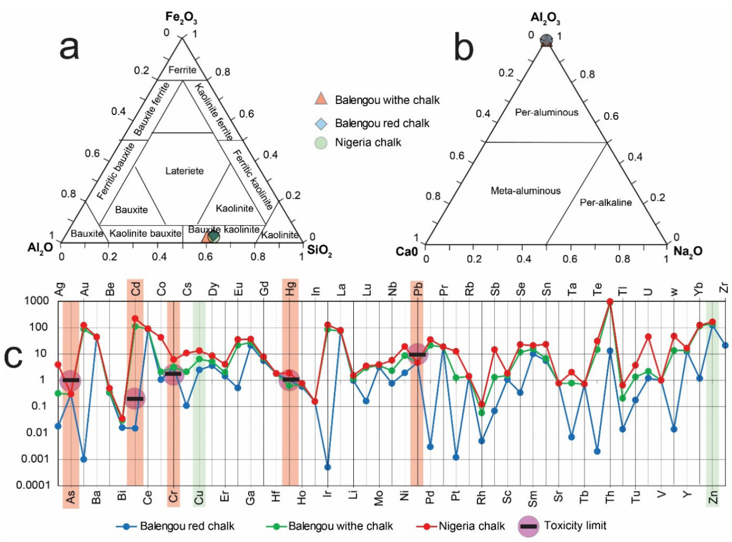


**Figure 1.** Geochemistry composition of Calabash chalk simple.

### Acute toxicity

**General behavior:** After 48 hours, there was no mortality recorded in treated group. On the 14th days after the treatment with the red Calabash chalk at a dose of 5000 mg/kg, all the animals in both groups survived. Cage side observations indicated behaviour changes such as lethargy or hyperactivity and excessive cleaning. No abnormality in stool was observed.

**Weekly body weight:** After 14 days of observation, a significant increase in body weight by 43.1% and 35.5% was observed respectively in the control and the red Calabash chalk-treated rats. However, the variation in body group between the two groups was not significant (Table 2)

**Organ relative weight:** A relative organ weight of rats was determined at the end of acute toxicity test period (14 days). There was no statistical difference in the organ's relative weight (liver, spleen, kidney, lung and heart) between treated and control groups (Table 3).

## Discussion

The objective of this study was to determinate the mineral composition of Calabash chalk sold in central market of Douala and its acute toxicity profile in rat. Geochemical analysis of the Calabash chalk sample collected establish the calabash chalk as per-aluminous bauxitic kaolinite. Lead and barium level were of concern, especially in Nigerian and Balengou white chalks respectively. Mineral nutrients such as iron, copper, selenium and zinc were also found. At 5000 mg/kg, modification of behaviour or weight was not found in rat treat with red calabash and control. The DL50 was >5000 mg/kg.

Geochemical analysis indicated that aluminium silicate (kaolinite) is the main component of all the sample collected, confirming that Calabash chalk belong to the family of kaolin. These three samples were characterized by the high contents of $Al_2O_3$, $SiO_2$, and low contents of MgO, Cl, $K_2O$, $P_2O_5$, CaO. Similar results have been reported in Egypt [13]. The depletion of alkali elements such as CaO, $K_2O$, and $Na_2O$ may be attributed to high kaolinite content in these samples [14-16].

| Parameter | Parameter | % Correlation | Pearson correlation (R) | Parameter not correlated |
|---|---|---|---|---|
| Total length | 13 | 100% | 0.429-0.872 | None |
| Standard length | 13 | 100% | 0.412-0.872 | None |
| Caudal peduncle Length | 13 | 84.62% | 0.209-0.728 | ED, PAL |
| Head length | 13 | 100% | 0.436-0.884 | None |
| Snout length | 13 | 100% | 0.343-0.811 | None |
| Head width | 13 | 92.31% | -0.199-0.670 | PAL |
| Eye diameter | 13 | 84.62% | 0.156-0.436 | CPL PAL |
| Pre-anal length | 13 | 100% | -0321-0.531 | CPL, ED, DFL, HW |
| Pre-pelvic length | 13 | 92.31% | -321.54 | None |
| Dorsal fin length | 13 | 100% | 0.219-0.613 | PAL |
| Pelvic fin length | 13 | 100% | 0.381-0.836 | None |
| Anal fin length | 13 | 100% | 0.253-0.690 | None |
| Pectoral fin length | 13 | 100% | 0.416-0.836 | None |
| Body depth | 13 | 100% | 0.301-0.816 | None |

**Table 2.** Effect of Calabash chalk on body weight in acute toxicity test.

| | Relative weight of organs (g/100g of body weight) | | | | |
|---|---|---|---|---|---|
| | Liver | Spleen | Kidneys | Lungs | Heart |
| Control (10 ml/kg) | 3.78 ± 0.5 | 0.29 ± 0.1 | 0.59 ± 0.0 | 0.66 ± 0.1 | 0.29 ± 0.3 |
| Calabash | 3.86 ± 0.4 | 0.27 ± 0.0 | 0.62 ± 0.1 | 0.80 ± 0.1 | 0.32 ± 0.1 |
| (5000 mg/kg) | | | | | |
| p | 0.45 | 0.78 | 0.5 | 0.12 | 0.19 |
| Data represent the mean ± standard deviation, n=3 | | | | | |

**Table 3.** Relative weight of organs in acute toxicity test.

Kaolin has been used in modern medicine as antacids, antidiarrheal, digestive aids and skin protectant [14]. According to the American Academy of science, the Food Chemicals Codex specifies limits of impurities for Kaolin as acid-soluble substances <2%; Arsenic <3 ppm or mg/kg) and heavy Metals <40 ppm with lead <10 ppm [14]. Indeed, our collected Calabash sample, especially, red Balengou chalk (arsenic <0.4 ppm, lead=4.67 ppm) could be used for pharmacology purpose. Mineral nutrients such as iron, copper, selenium and zinc were also found in the three Calabash chalks prototypes.

A common concern about Calabash chalk consumption is that Calabash chalk may be a source of potential harmful elements such as Heavy metals, including lead and mercury. On the other hand, Calabash chalk could also be a source of essential mineral nutrients such as iron, copper, or zinc. Abraham et al. [15], evaluate the solute content of some chemical elements of Calabash chalk in the gastrointestinal environment that is available for absorption. They found that most of the chemical, including copper, zinc and lead had concentration in leachate solution lower than the blanks, suggestive of adsorption of those elements by the geophagical materials. They concluded that Calabash chalk on the whole is not an important source of mineral nutrients or potential harmful elements to humans [15]. Other studies also noted limited digestive bioavailability of heavy metals of ingested clay including lead and arsenic [16,17]

As noted by Ekong et al. in Nigeria [4], Balengou Calabash chalk sample also contains metalloids elements such as barium, cerium, titanium or zirconium. The harmful or toxic nature of ingestion of these elements has not been well established. No health risk had been reported with some such as titanium, other like water-soluble barium compound may induced hypertension, arrhythmia or kidney damage, chromium may be caused liver and kidney damage; molybdenum may induce gout while other like cobalt or yttrium may be carcinogenic. However, the bioavailability of this component for intestinal absorption may not been important due to adsorption phenomenon [15-17]. Thus, these elements may have less impact on individual health. Although consumption of calabash may have biological activities that are beneficial to humans, the potential toxicity related to the related to their content of minerals or substances has not been well established. Thus, the safety and efficacy of these calabash must be studied thoroughly to maximize their benefits for mankind.

For the acute toxicity study, female Wistar rats were given single dose of 5000 mg/kg of aqueous red calabash orally and observed for

14 days. The rats were monitored closely for any signs of toxicity and mortality including general behavioural changes during the entire period of treatments. Calabash produced slight and no significant changes in behaviour. No deaths in the treated rats either during or after the treatment period were recorded. These results show that red calabash did not produce any mortality or alter slightly the behavioural patterns of the rats. This lack of any effect suggests that red calabash did not induce acute toxicity at the dose tested. No significant body weight gain as well as organ relative weight of treated rat was observed as compared to the control; therefore, the LD50 of the extract is estimated to be more than 5000 mg/kg.

## Conclusion

Analysis of Calabash chalk consume in our setting found aluminium silicate as the main constituents. Some of metals and metalloids found such as iron, copper or zinc with low proportions, are biological beneficial, while other may be harmful or unknown function on human health. Acute used of Calabash chalk may be safe. However, the effect of chronic usage should be study to clearly evaluate the risk of calabash geophagia.

## Conflict of Interest

none.

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