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## Comparative study of the brain and heart of rabbits after administration of edible clay for heavy metals

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### ABSTRACT

*This work involved the analyses of elemental content of edible clay and investigation of the kinetics of some of the detected elements in the brain and heart of rabbits. The clay sample was collected from Enyigbavillage in Abakaliki, Ebonyi State of Nigeria and only metallic elements were determined in the clay and the detected elements kinetically investigated. The analysis of edible clay obtained was carried out using Atomic Absorption Spectroscopy (AAS). They include Pb, Cd, Cu, Zn, Ba, Mn, Ca, Ni, Na, Mg, K, Fe, As, Co and Cr. The results showed the presence of essential metallic elements, trace elements and toxic heavy metals in the clay. The concentrations of all the other absorbed elements were within the WHO acceptable levels for human consumption per day. The levels of the trace heavy metals in the edible clay were low hence they were not absorbed into the body.*

**Key words:** Edible clay, Rabbit, Brain, Trace metal,

### INTRODUCTION

Clay is a naturally occurring aluminum silicate [4]. It is principally composed of ultra fine grain mineral. Clays are distinguished from other fine grain soils by differences in size and mineralogy. Silt is a fine soil similar to clay [1]. However, clay is finer (in fact ultra fine grain). It is so fine that it is almost impossible to identify crystal from it. The distinction between clay and silt grains varies by discipline. Geologist, soil scientist, sedimentologists and colloid chemist all draw their distinction based on particle size in  $\mu\text{m}$  which in all cases, clay has the finest particle. Geotechnical engineers distinguish between silts and clays based on soil plasticity [6].

Clay is widely eaten by children and pregnant women in eastern part of Nigeria. Some just pick up some clay particles from excavation sites and eat without any preparation while some others buy from the local market to eat. Some pregnant women can hardly do without it, while believing that it enhances the development of their babies and impart fair complexion to the babies.

### CLAY MINERALS AND USES

Clay minerals have common set of structural and chemical properties. They are members phyllosilicates that contain large percentage of water trapped within the silicate sheets [7]. There are four groups of clay minerals:

**(i) The Kaolinite Group:**

This is also known as white cosmetic clay. The members of this group are kaolinite, dickite and nacrite. It has the formula of  $\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$ . The different minerals are polymorphs. They are used in ceramics as fillers for paints, rubber and plastic and largely used in paper industry.

**(ii) The Montmorillonite/Smectite group:**

This group is also known as Bentonite clay. It is edible clay from naturally occurring volcanic ash sediments. Other minerals in this group include pyrophyllite, talc, vermiculite, sanconite, saponite, nontronite and montmorillonite. They differ in chemical content. They have the general chemical formula  $(\text{Ca}, \text{Na}, \text{H})(\text{Al}, \text{Mg}, \text{Fe}, \text{Zn})_2 (\text{Si}, \text{Al})_4 \text{O}_{10} (\text{OH})_2 \cdot X\text{H}_2\text{O}$  where X is variable amount of water. This group of clay has wide applications among other uses include facial powder, filler to paint and rubbers, an electrical, heat and acid resistant porcelain, in drilling mud and plasticizer in molding sand and other materials [7].

**(iii) The Illite or the clay mica group:**

This group is also known as French Green clay or sea clay. They are basically hydrated microscopic muscovite. It is rock forming mineral with the general formula  $(\text{K}, \text{H}) \text{Al}_2 (\text{Si}, \text{Al})_4 \text{O}_{10} (\text{OH})_2 - X\text{H}_2\text{O}$ . X is variable amount of water. It is used as filler and in some drilling mill.

**(iv) The Chlorite Group:**

This group is relatively large and common. The members include, Amesite, Baileychlorite, and a host of others. The general formula is  $X_4 - 6Y_4 \text{O}_{10} (\text{OH}, \text{O})_8$ , the X is either Al, Fe, Li, Mg, Mn, Ni, Zn, or Cr, Y is either Al, Si, B, or Fe [7].

**GEOPHAGY**

Eating of clay earth, soil or related material is generally referred to as geophagy, and such material referred to as geophagic material [3]. Geophagy is a worldwide practice. It is practiced by humans of all gender irrespective of age or race. Geophagy practices cuts across African continent from east Africa to West Africa including Nigeria, Cameroon and Ghana. Animals eat "dirt" clay to act as detoxins to protect them from poison arising from eating poisonous plants.

Geophagy is also known as soil paca or simply pica. It is a tradition in places like Philippines, New Guinea, Costa Rica, the Amazon and Orinoco basins of South America to use clay as part of food. Gerald Callahan called Pica a disease different from polio or smallpox [2]. Geophagy is more pronounced among pregnant women especially in sub-Saharan Africa including United States of America and children worldwide. The US Agency for Toxic Substances Committee (2000) identified 500mg of soil per day to be pathologic. Children under the age of two (2) eat different kinds of soil from different sources. Callahan (2003) was worried and felt debased that his children were what he called dirt eaters. According to him they ate dirt with surprising gusto, garden soil, road soil, leaf mash soil, sod soil, bug body soil and even gutter soil. While adult may be selective and consume clay deeper part of the earth, children are less selective and eat top part of the soil. Normal soil consumption falls between the ranges of 0-500mg per day per small mouth [2].

**HEAVY METALS**

Heavy metals are elements that exhibit metallic properties. It mainly includes the transition metals, some metalloids, lanthanides and actinides. Many different definitions have been proposed-some based on density, some on atomic number or atomic weight and some on chemical properties or toxicity. According to IUPAC the term heavy metal has contradictory meaning and lacks coherent scientific basis. The most likely term is toxic metal. Depending on context, heavy metals include elements lighter than carbon and exclude some of the heaviest metals. Heavy metal occurs naturally at various concentrations in the ecosystem. Heavy metal is a metal with a high relative atomic mass and a specific gravity greater than 5.0 especially one that is poisonous such as Pb or Hg. The term Heavy metal is usually applied to common transition metals, such as Cu, Pb, and Zn. Heavy metal is an inexact term used to describe more than a dozen elements that are metal or metalloids. Generally heavy metals have densities above  $5\text{g}/\text{cm}^3$ . Heavy metals generally refers to; Pb, Hg, Fe, Cu, Mn, Cd, As, Ni, Al, Ag, and Ba [5].

## MATERIALS AND METHODS

### Materials

Edible clay, Rabbits, Atomic Absorption Spectrometer, Kidney, Blood.

### Methods

Samples of clay were collected from different sampling points in the mining site of Enyigba village in Abakaliki, Ebonyi State of Nigeria.

### PREPARATION OF CLAY SAMPLE FOR ANALYSIS

The sample from the different sampling points were ground together and thoroughly mixed. The fine powder was dried in an oven at a temperature of 110°C and then cooled and heated to a constant weight. From this bulk sample 2000mg of the dried sample was weighed. The two grams was a representative sample of the bulk. This sample was placed in a beaker and 10cm<sup>3</sup> of distilled water added just to make paste of the clay. Sixty (60) cm<sup>3</sup> of freshly mixed aqua regia was added little at a time to dissolve the material. The mixture was allowed to stand overnight for complete digestion of the clay sample. Then the mixture was diluted with distilled water, and filtered. The filtrate was kept in refrigerator at 4°C and analyzed for the elements using Atomic Absorption Spectroscopy (AAS). The samples were run in triplicate. The elements whose concentration levels were determined include Pb, Cd, Cu, Zn, Ba, at the appropriate wavelength and lamp current for each analyte.

### EXPERIMENTAL ANIMALS

Clinically healthy rabbits of adult age of both sexes were used. They were kept in laboratory for 14 days to acclimatize. The weight of each animal was determined by use of a scale balance. The animals were clearly labeled and each body weight recorded. The animals were fed with grass and vegetables.

### TISSUE KINETICS OF THE CLAY CONSTITUENT IN RABBITS

The rabbits were separated in three equal groups, and placed in different cages. Group A was administered 2000mg/kg body weight of freshly made paste of clay while Group B was administered 4000mg/kg body weight. Group C, was control and was not dosed but fed with grasses and vegetables. The high and low doses were necessary to see if absorption and distribution into tissues would depend on the concentration of the constituent of the clay and hence whether the rate of disappearance in the tissue would depend on the concentration.

### CONCENTRATIONS OF METALS IN EDIBLE BRAIN AND HEART

The oral administration of the edible clay to rabbits at two varying doses of 2000mg/kg and 4000mg/kg resulted in detectable levels of most of the elements found in the clay in the brain and heart of the treated animals.

## RESULTS

**Table 1. Essential metallic nutrient elements in the edible clay obtained from Enyigba Abakaliki Ebonyi state Nigeria**

Metals	Mean ( ± SE) Concentrations mg/g
Na	71.960±3.63
K	84.330±2.73
Ca	249.000±1.73
Mg	198.570± 0.90

**Table 2. Trace metallic nutrient elements in edible clay obtained from Enyigba Abakaliki Ebonyi state Nigeria**

Metals	Mean ( ± SE) Concentrations mg/g
Cu	40.690±0.67
Zn	0.830±0.02
Mn	65.200±0.81
Ni	89.340±0.69
Fe	151.920±2.6
Co	1.190±0.51
Cr	0.170±0.04

Table 3. Toxic metals in edible clay obtained from EnyigbaAbakalikiEbonyi State Nigeria

Metals	Mean ( ± SE) Concentrations mg/g
Pb	0.570±0.08
Cd	1.930±1.23
As	3.920±1.90
Ba	8.680±4.91
Al	1.29 ± 0.24

Table 4. Mean (±SE) Ca Concentration (µg/g) in tissues of rabbits orally treated with doses of 2000 mg/kg body weight (low) and 4000mg/kg body weight (high)

Days/ Group	Brain	Heart
1. low	384.00±101.00 <sup>b</sup>	222.68±142.31 <sup>b</sup>
High	436.00±215.00 <sup>2</sup>	297.00±125.00
2. low	280.00±123.00 <sup>b</sup>	177.32±91.00 <sup>b</sup>
High	293.00±137.00 <sup>2</sup>	223.00±161.00
4. low	223.60±99.10 <sup>a</sup>	123.71±55.78 <sup>b</sup>
High	239.00±114.00 <sup>2</sup>	210.00±124.00
6. low	206.20±18.00 <sup>a</sup>	115.46±29.74 <sup>a</sup>
High	235.00±149.00 <sup>2</sup>	202.00±110.00
8. low	169.10±82.80 <sup>a</sup>	90.72±10.91 <sup>a</sup>
High	206.00±147.00 <sup>1</sup>	190.00±128.00
10. low	138.80±35.80 <sup>a</sup>	70.10±10.91 <sup>a</sup>
High	186.00±115 <sup>1</sup>	107.20±10.90

a, b, values for low dose along the same column differently superscript, differ significantly ( $p < 0.05$ ).

1, 2 value for high dose along the same column differently superscript, differ significantly ( $p < 0.05$ ).

Table 5. Mean (±SE) Mg Concentration (µg/g) in tissues of rabbits orally treated with doses of 2000 mg/kg body weight (low) and 4000mg/kg body weight (high)

Days/ Group	Brain	Heart
1. low	175.66±13.25 <sup>a</sup>	137.69±16.35 <sup>a</sup>
High	208.88±2.16 <sup>1</sup>	152.60±15.10 <sup>2</sup>
2. low	148.36±24.64 <sup>a</sup>	131.25±16.79 <sup>b</sup>
High	196.50±51.60 <sup>1</sup>	146.40±21.00 <sup>1</sup>
4. low	145.40±2.63 <sup>a</sup>	126.32±5.06 <sup>a</sup>
High	187.50±28.10 <sup>2</sup>	145.72±6.96 <sup>2</sup>
6. low	136.18±10.91 <sup>a</sup>	118.09±13.37 <sup>a</sup>
High	155.43±5.18 <sup>1</sup>	139.50±11.10 <sup>1</sup>
8. low	129.93±8.55 <sup>b</sup>	113.49±5.78 <sup>a</sup>
High	152.30±4.78 <sup>1</sup>	134.50±13.00 <sup>1</sup>
10. low	110.53±5.92 <sup>b</sup>	62.17±21.71 <sup>a</sup>
High	151.00±25.00 <sup>2</sup>	120.07±2.30 <sup>1</sup>

a, b, values for low dose along the same column differently superscript, differ significantly ( $p < 0.05$ ).

1, 2 value for high dose along the same column differently superscript, differ significantly ( $p < 0.05$ ).

Table 6. Mean (±SE) Fe Concentration (µg/g) in tissues of rabbits orally treated with doses of 2000 mg/kg body weight (low) and 4000mg/kg body weight (high)

Days/ Group	Brain	Heart
1. low	228.68±12.18 <sup>a</sup>	269.77±12.31 <sup>a</sup>
High	265.10±14.00 <sup>1</sup>	344.20±46.50 <sup>1</sup>
2. low	190.70±9.30 <sup>a</sup>	249.61±9.43 <sup>a</sup>
High	232.60±25.90 <sup>1</sup>	293.22±1.27 <sup>1</sup>
4. low	139.38±13.93 <sup>a</sup>	248.84±16.28 <sup>b</sup>
High	206.05±8.75 <sup>1</sup>	287.00±11.30 <sup>2</sup>
6. low	130.23±12.31 <sup>a</sup>	143.41±5.08 <sup>a</sup>
High	148.84±4.65 <sup>1</sup>	256.60±44.70 <sup>1</sup>
8. low	75.09±4.32 <sup>b</sup>	134.88±4.65 <sup>b</sup>
High	95.04±2.64 <sup>1</sup>	251.94±8.09 <sup>1</sup>
10. low	67.44±2.33 <sup>a</sup>	97.99±0.32 <sup>a</sup>
High	68.45±0.66 <sup>1</sup>	171.30±11.60 <sup>2</sup>

a, b, values for low dose along the same column differently superscript, differ significantly ( $p < 0.05$ ).

1, 2 value for high dose along the same column differently superscript, differ significantly ( $p < 0.05$ ).

## DISCUSSION

The essential metallic elements including Na, K, Ca, and Mg occurred in high concentrations in the edible clay. The concentrations of these elements in the edible clay were  $71.960 \pm 3.63$ ,  $84.330 \pm 2.73$ ,  $249.00 \pm 1.73$  and  $198.570 \pm 0.90$  mg/g respectively for Na, K, Ca, and Mg (Table 1). The concentrations of trace metallic nutrient elements in the edible clay are shown in Table 2. Iron (Fe) ( $151.920 \pm 2.6$ mg/g) occurred in a very high concentration, while Cr ( $0.170 \pm 0.04$ mg/g) was obtained in a very low concentration. The concentrations of Ni, Cu and Mn were  $89.340 \pm 0.69$ ,  $40.690 \pm 0.67$  and  $65.200 \pm 0.81$  mg/g respectively. Some toxic heavy metals were found in the edible clay (Table 3). The concentrations of these toxic heavy metals in the edible clay were Pb,  $0.570 \pm 0.08$ mg/g; Cd,  $1.930 \pm 1.23$ mg/g; As,  $3.920 \pm 1.90$ mg/g and Ba,  $8.680 \pm 4.91$ mg/g. The results of the tissue kinetics study showed that most of the elements present in the edible clay obtained from Enyigba, were readily absorbed following oral administration of the clay. The mean peak blood and tissues concentrations of these elements occurred twenty four hours (one day) after the clay administration to rabbit at 2000mg/Kg and 4000mg/Kg doses. The elements obtained in high concentrations in the sampled tissues include Ca, Mg, Ni, Fe, K and Na. Copper and Mn, occurred in low concentrations in the brain and kidney while Pb and Cr were absent. The high amounts of Ca, Mg, Ni, K and Na present in the brain and kidney may be due to increased uptake of these elements by organs and tissues involved. The high concentrations of these elements in tissues may also be due to the levels of these elements in the edible clay sample. The elemental analyses of the clay showed that Ca, Mg, Ni, Fe, K and Na were present in the clay at the following concentrations  $249.00 \pm 1.73$ ,  $198.57 \pm 0.90$ ,  $89.34 \pm 0.69$ ,  $151.92 \pm 2.60$ ,  $84.33 \pm 2.73$  and  $71.96 \pm 3.63$ mg/g respectively. It is therefore not surprising that these elements occurred in high concentrations in the sampled tissues. The low levels of Cu and Mn found in the various tissues following oral administration may be an indication of their low concentrations in the clay. Right from day 1 to day 10 post treatments with the clay (Table 4), Calcium occurred in the brain and heart of the treated rabbits. The concentrations of calcium obtained at day 1 post treatment were  $384.00 \pm 101.00$  and  $436.00 \pm 215.00$   $\mu$ g/g for the brain and  $222.68 \pm 142.31$   $\mu$ g/g and  $297 \pm 125.00$   $\mu$ g/g in animals treated with 2000 and 4000 mg/kg respectively. There was rather a decline in Ca concentrations as at 10 days post treatment the levels were  $138.80 \pm 35.80$  and  $206.00 \pm 115.00$   $\mu$ g/g respectively in 2000 mg/kg and 4000 mg/kg treated animals. The concentrations of Ca observed to be present in the heart, kidney, liver, lungs, skeletal muscle and blood were highest at day 1 post treatment, and these were followed by constant decline in Ca levels in the various tissues and blood, with the levels in the brain and kidney being the least on 10th day post treatment. The concentrations obtained with the 4000mg/kg dose in various tissues and blood were significantly ( $p$  less than 0.05) higher than those obtained with the 2000mg/kg dose (Table 4). The concentrations of Mg and Fe present in the various tissues of rabbits treated orally with 2000 mg/kg and 4000 mg/kg body weight of the edible clay are shown in Tables 5 and Table 6. The highest concentrations of these elements were obtained at day 1 post treatment, while the least levels were obtained at day 10 post treatment. The Mg and Fe concentrations were higher in the kidney and blood of animals treated with 4000mg/kg dose when compared to the level present in the tissues and blood of 2000mg/kg treated rabbits.

## CONCLUSION

The analyses of the clay sample obtained from Enyigba, showed the presence of essential metallic elements, trace elements and toxic heavy metals in the clay. The edible clay was observed to have low toxicity when administered orally, since the administration of 5000mg/kg to rabbits orally produced no ill-effect. The administration of the edible clay to rabbits orally resulted in the absorption and distribution of Ca, Mg, K, Na, Cu, Ni, Fe and Mn to the various brain and heart of the body. The absorbed elements were still present in the respective parts of the body 10 days post administration, showing that the elements were widely distributed in the body, with increased half-lives. The concentrations of all the other absorbed elements were within the WHO acceptable levels for human consumption per day. The levels of the trace heavy metals in the edible clay were low hence they were not absorbed into the body.

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