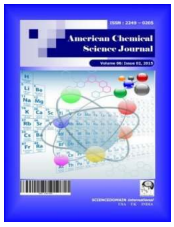

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Estimation of Some Essential Metallic Elements in Edible Clay from Enyigba in Ebonyi State of Nigeria Using AAS and Experiments with Rabbits

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Authors' contributions

This work was carried out in collaboration between all authors. Author SPIO designed the study, managed the data collection, presentations and preparation of the original manuscript. Authors TMA and EON were involved in checking the literature, statistics and general revision of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Edible clay from Enyigba in Abakaliki, Ebonyi State of Nigeria was analyzed for, Ca, Na, Mg and K, using AAS. The results showed that the sample contains 249.000±1.73 mg/g, 71.960±3.63 mg/g, 198.570±0.90 mg/g and 84.330±2.73 mg/g of the metals respectively. The analyses of the brain, heart, liver, kidney, lungs, skeletal muscle and blood of rabbits post administration of the clay showed that these metals were absorbed into tissues and organs. All the metals had highest absorption in the brain. There was exponential decrease in concentrations with time. The highest elimination rate was observed for Ca across the tissues and organs. The brain had the highest elimination rate with the blood having the least.

Keywords: Edible clay; rabbits; rate constant; kinetics; Ebonyi state-Nigeria; concentrations; AAS.

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1. INTRODUCTION

Food must satisfy certain requirement like free from microbes and lethal elements in addition to containing what is considered as essential for survival and development such as minerals and vitamins. In quest for these, man engages in various practices some of which might even endanger his life. Some other times the kind of thing man eats leaves one to begin to ask question as what does man mean by food? [1] The eating of earth called clay is one of such things. It is that part of the earth that man eats, processed or unprocessed, that this work calls edible clay. Clay is naturally and principally composed of ultrafine grain mineral. Clays are distinguished from other fine grain soils by differences in size and mineralogy. Silt is a fine soil similar to clay [2]. However, clay is finer (in fact ultrafine 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 μm which in all cases, clay has the finest particle. Geotechnical engineers distinguish between silts and clays based on soil plasticity [3]. 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. Even when a baby is born the same clay is used as lotion on the new born baby. There are so many local applications of clay with so many believes attached to it. Some clay has been noted to contain aluminum as principal metal. Since clay in most cases are formed as a result of sedimentation and weathering of rocks it is most likely that some other metals might be embedded in the clay and are consumed by same people that eat clay. It is of interest because while some metals at certain concentrations may be useful as part of diet others may be harmful and accumulative effect pose danger to health.

1.1 Health Uses of Clay

Generally, all the groups of clay have health uses as in medicine and cosmetics. Different people may have different reasons for eating clay or soil as sometimes called. However for whatever reason one has, there are definitely merits and demerits in the practice. Clay detoxifies by binding *alkalloids* in some poisonous plants and plant materials [4]. *Geophagy* (eating of clay) is mostly common among pregnant women and nursing mothers who have high demand for minerals and mineral supplements. Other Americans such as the indigenous Pomo of Northern California used clay in their diet mixed with ground acorn to neutralize the acid of the acorn [5]. Clay or soil consumption is believed to have relieving effects from ailments which includes supplementation of minerals and nutrients [6]. *Geophagic* materials are used orally to heal common ailments of gastrointestinal tract because of the medicinal properties [7]. Clay or earthy material is consumed to relieve hunger [8]. Just as almost natural in life that everything has merits and demerits, *geophagy* has its own demerits. There is health risks associated with clay consumptions. Contamination by animal or human feces, parasite eggs, such as round worm that can stay dormant for years can present a problem. Tetanus poses another risk [9]. Some clay may possibly contain toxic metals. There are reports of lead poisoning and other toxicities in children eating contaminated soils [10]. Millions of species of microorganisms were found in soil [11]. More species of *prokaryotic* microorganism were even found in the soil [12]. American Centers for Disease control and prevention reported infection of two children at separate sites with *raccoon* roundworm, *Baylisacaris procyoris,* due to eating of infected soil material [13]. One of the victims died of severe neurologic damage [10]. *Geophagy* can have dire consequences. Although geophagy has the potential to supply micronutrients, it has been reported that soils in the clay or earth do interfere with the bioavailability of micronutrients leading to micronutrients deficiency [14]. Additionally it has been shown that there was high prevalence of multiple micronutrient deficiencies amongst pregnant women [15]. Furthermore, it has also been suggested that geophagic soils can be highly contaminated with microbes and may contain high levels of lead [16]. More still, it has, similarly been shown that Ascaris lumbricoides infection, anemia and red blood cell characteristics suggestive of iron deficiency and lower hemoglobin concentration were very much associated with the prevalence of geophagy [17].

Most of the disease encountered through *geophagy* is childhood related because infected top soil is involved rather than deep clays [18]. Infection from geophagic material was reported in the United States with *toxocariasis* as the most common due to ingestion of soil contaminated

with dog or cat feces [19]. It has been reported that the most common parasitic infection associated with *geophagy* or dirt eating among Nigerian children is ascariasis [20]. The native pregnant women who eat clay claim that it helps to keep the baby in the womb healthy. Some say that it works as anti-vomiting in the early stage of pregnancy. Some also eat it because of its taste and others say they eat it because of its scent. All these are oral claims, but most importantly, clays are rich in essential nutrient such as Sulphur and phosphorous. The practice of clay eating is widely spread among animals and humans [21]. *Geophagy* is common in rural or pre-industrial societies among children and pregnant women [10]. It has been recorded that sick or injured animals such as mammals, birds, butterflies and even reptiles use clay for medicinal purpose [22]. *Geophagy* is practiced by members of all races, social classes, ages and sexes. In many parts of the developing world clay for consumption are displayed for sale. In some parts of Africa, rural areas of the United States and villages in India clay consumption is associated with pregnancy and some women eat clay to eliminate nausea, possibly because the clay coats the gastro intestinal tract and may absorb dangerous toxin. The clay rich in calcium may provide calcium for fetal development [23]. People seem to use *geophagy* to protect themselves from plant toxins. Indians that eat bitter and toxic wild potatoes capable of producing stomach pains and vomiting, usually eat them with clay to make them safe by binding the alkaloids [24]. In fact a lot of works are well documented on animal and human geophagy either for nutritious reasons or medicinal reason. It is recommended that a *paraquat* poisoned victim should swallow “dirt” clay even at the risk of salmonella, because *paraquat* will be deactivated upon contact with soil [25]. Chimpanzees in Kibale National Park, Uganda have been observed to consume soil rich in kaolinite clay shortly before or after consuming plants including *Trichilia rubescens*, which has anti-malarial properties. Simulated mastication and digestion has shown that clay helps to release active anti-malarial component from the leaves [26].

2. MATERIAL AND METHODS

2.1 Sample Area and Sample Collection

Samples of clay were collected from Enyigba village mining site in Abakaliki Ebonyi State of Nigeria. The samples were collected from five different points within the perimeter of the mining site at the depth of 0 to 15 cm with the location coordinate shown. This was within the area people collect clay particles for eating.

2.2 Preparation of Clay Sample for Analysis

Clay samples from different sampling points were thoroughly mixed and grinded together for a homogenous mixture. Thereafter, the fine clay powder was dried at a temperature of 100^0C in an oven for two hours, cooled in a desiccator. The process of heating and cooling was continued until a constant weight was obtained. Two (2.000) g of the dried clay was then weighed into a beaker and 10 ml of distilled water was added. Digestion of clay paste was then carried out by the gradual addition of 60 ml of Aqua regia solution (made by mixing concentrated nitric acid of 72% purity and specific gravity of 1.42 g at 20℃ with concentrated hydrochloric acid of 38% purity and specific gravity of 1.18 g at 20℃ in the ratio of 1:3 by volume respectively (both of Sigma-Aldrich product) and left overnight to Ensure complete digestion of the clay constituent. Thirty (30) ml of distilled water was added. The solution was filtered and made up to 100 ml. The filtrate was then refrigerated at 4℃ prior to elemental analysis for the elements Ca, Mg, K and Na at their appropriate experimental conditions (Table 2).

2.3 Experimental Animals

Fifty nine healthy New Zealand rabbits of ages (between 8 to 10 months old) of both sexes (40 females and 19 males) weighing between 0.5 kg and 1.5 kg were used. The animals were housed in standard rabbit cages in the Department of Veterinary Physiology, Pharmacology and Biochemistry Laboratory. They were kept in the laboratory for 14 days in Veterinary Teaching Hospital Federal University of Agriculture Makurdi Benue State Nigeria to acclimatize. All the animals were handled according to the International Guiding Principles for Biomedical Research Involving Animals [27] as permitted by Federal University of Agriculture Makurdi, Benue State Nigeria, Ethical Committee concerning the use of laboratory animals given the permit number (P/No:2012018). The laboratory temperature was between 30℃ and 33℃ throughout the period of the experiment which invariably was the environmental temperature. Each experimental rabbit was accurately weighed and labeled for correct identification. They were also well fed with *Imperata cylindrica* and *Amaranthus hydradus*.

Table 1. Geographical description of the sample collection points

Sample	Sample depth (cm)	Location description	Location coordinates	
			Lat (N)	Long (E)
01	0-15	Perimeter fence of the mining site	5°57 ' 43.43"	7°26 ' 30.61"
02	0-15	500 m away from the 1st point	5°55 ' 45.39"	7°29 ' 27.09"
03	0-15	500 m away from the 2nd point	5°55 ' 45.39"	7°29 ' 27.09"
04	0-15	500 m away from the 3rd point	5°55 ' 58.44"	7°29 ' 50.94"
05	0-15	500 m away from 4th point	5°56 ' 09.69"	7°30 ' 03.42"

2.4 Acute Toxicity Study

The acute toxicity of the clay on the rabbits was studied using up and down method as revised by Dixon [28]. The up and down method aims to estimate the LD_{50} value by testing individual animal sequentially, the dose for each animal being adjusted up or down, depending upon the outcome on the previous animal.

The rabbits were fasted overnight but allowed to drink water prior to the study. Five adult rabbits of 1.5 kg body weight each were randomly selected and used for the study. 5000 mg /kg body weight of freshly made paste of clay in distilled water was given orally to one of the rabbits using stomach tube. The dosed animal was observed for 48 hours for signs of toxicity or death. The same procedure was adopted until all the five animals were treated. The dosed animals were observed for 14 days.

2.5 Tissue Kinetics of the Clay Constituent in Rabbits

This was achieved by diving 54 experimental animals (Rabbits) into three groups: A, B and C. 2000 mg/kg and 4000 mg/kg freshly made clay paste was given to groups A and B respectively. Group C was used as the control hence they were not treated, but continued to be fed with *Imperata cylindrica* and *Amaranthus hybridus*. The reason for a low and high dose was to study the kinetics of the absorption process. The dose for each animal was calculated according to its weight for each group. All the groups were fasted prior to the dosing but water was provided *ad-libitum* in plastic bowels. The clay was in the form close enough to that which it is eaten by humans. The fine weighed powder was made into paste by adding 10 ml of distilled water just "mobile" enough to allow easy administration. After dosing all the animals were continuously fed with grass *Imperata cylindrica* and vegetable *Amaranthus hybridus* and water *ad libitum*, but in different containers in different cages. Group C was administered with distilled water only from the source used for paste making of the clay sample. The animals were observed for any clinical behaviour.

2.6 Collection of Tissue and Blood Samples

Three animals from each group were sacrificed each day of sample collection. Samples of brain, heart, liver, kidney, lungs, skeletal muscle and blood were collected from the sacrificed animals. Samples were collected on the following hours post treatment with clay; 24 h, 48 h, 96 h, 144 h, 192 h, and 240 h. The work area and instruments were thoroughly cleaned between sacrifice to avoid contamination from the previous collection. The tissue samples were put in plastic bags and stored in refrigerator below -10℃ until analyzed. Heparin was used as anticoagulant for blood sample.

2.7 Digestion of Tissue Samples

The tissues were dried to a constant weight in an oven at a temperature of 60℃. One gramme of the dried tissue was weighed into a conical flask. Fifteen (15) ml of freshly prepared aqua-regia was added followed by gradual addition of 20 ml

Table 2. Experimental conditions for AAS determinations

In terms of:	Calcium	Magnesium	Potassium	Sodium
Type of lamp	Hallow cathode	Hallow cathode	Hallow cathode	Hallow cathode
Type of gas	Air/Acetylene	Air/Acetylene	Air/Acetylene	Air/Acetylene
Burner slot	0.5 mm *100mm	0.5 mm *100mm	0.5 mm*100mm	0.5 mm *100mm
Flow rate: support gas	10 L/min	10 L/min	10 L/min	10 L/min
Flow rate: Fuel gas	2.5 L/min	2.4 L/min	2.5 L/min	2.4 L/min
Spect. Buffer	Skl sln 20982	Skl sln 20982	Skl sln 20982	Skl sln 20982

N/B Skl sln = Schinkel's solutions

of 20% H_2O_2. The aqua-regia was employed to solubilize the metal while the H_2O_2 was to oxidize the tissue. The mixture was placed on hot plate at 80℃ for two hours. The solution was allowed to cool, then filtered and made up to 100 ml in a volumetric flask. The filtrate was refrigerated until further analysis.

2.8 Determinations of Metals in Tissue and Blood Sample Using AAS

The sample solutions were in turn aspirated for each suspected element in triplicate. The concentration of the analyte in the sample was recorded from read out device of the bulk AAS.

2.9 Blank Preparations

The Blank were made by taking 15 ml of aqua-regia with 20 ml of 20% H_2O_2 in 100 ml volumetric flasks and made up to the mark with distilled water. This solution was aspirated into the flame of AAS and concentrations recorded at wave length of each suspected analyte. Solutions of control were in turn run for each suspected element just like the samples of group A and B. The concentrations were recorded. The values, where applicable, were effectively eliminated by subtracting such values from the values of those tissues whose control showed trace amounts of such elements as shown in the expression below;

$$\text{Metal Concentration in dry sample} = \frac{AAS\ Reading \times Sample\ Volume}{Weight\ of\ sample} - Metal\ Conc.\ in\ control\ tissue$$

2.10 Calculation of Kinetics Constants

The kinetic analysis of experimental data obtained from blood and tissue was performed using a mean value by standard procedures [29]. A program for linear regression analysis was performed on concentration time data to determined elimination rate constant k from the slope (i.e. change in concentration/change in time). Half-life $t_{1/2}$ was obtained using the formula,

$$t_{1/2} = \frac{\ln 2}{K} = \frac{0.693}{K}$$

Where k is the elimination rate constant and $t_{1/2}$ is the half life

2.11 Statistical Analysis

The data collected are presented as mean ± standard error mean. One way analysis of variance (ANOVA) was used to analyze the differences between the means. P values less than 0.05 were considered significant [30]. Graphpad instant (R) version 3.0(2003) statistical computer software was used.

3. RESULTS AND DISCUSSION

3.1 Elemental Analyses of Edible Clay from Enyigba Ebonyi State Nigeria

The results of the analyses of the elements present in the edible clay obtained from Enyigba Abakaliki, Ebonyi State Nigeria are presented in Table 3.

3.2 Acute Toxicity Study

The administration of the edible clay to rabbits at the dose of 5000 mg/kg for LD_{50} determination did not produce any mortality in the treated rabbits using the up and down method.

The first animal survived the LD_{50} test and so did all the treated five animals. There was no loss of animal even after 14 days post LD_{50} test.

Table 3. Essential elements in clay sample from Enyigba, Abakaliki, Ebonyi state, Nigeria

Metals	Concentrations mg/g
Calcium	249.00 ± 1.73
Magnesium	198.57 ± 0.90
Potassium	84.33 ± 2.73
Sodium	71.96 ± 3.63

3.3 Essential Metal Concentrations in Tissues and Organ

Table 4. Ca was detected in all the tissues from 24 hours post administration of the clay to the rabbits up till 240 hours of the period of experiment. The peak values occurred 24 hours post administration. Thereafter there was steady decrease and least values for all the tissues were observed 240 hours post administration. In all the tissues analyzed the blood maintained the highest concentration of Ca followed by the brain. Earlier work had also obtained high concentration of Calcium in the blood and kidney [31]. The high concentration of Calcium in the brain is worthy of note because of its lingering high level up to 240 hours post administration. High deposition of Calcium in the brain is attributed to high or excess circulation of Calcium or Phosphorous in the blood [32]. The high

concentration of Calcium in the blood informs the high concentration in the brain. The blood is the vehicle of transportation in animal body.

Table 5. Mg was well distributed across all the tissues and organs 24 hours post administration of the clay. The level of Mg 24 hours after administration has the highest values in all the tissues. This gradually decreased with time and the least values observed 240 hours post administration. Right from 24 hours post administration, the kidney maintained the highest concentration of Magnesium up to 240 hours post administration. Previous workers recorded appreciable high level of magnesium in the blood and kidney of rabbits orally dosed with clay sample [31]. Beyond 24 hours post administration the liver became the organ with highest concentration of Magnesium up to the last period of the experiment. Throughout the period under investigation, the skeletal muscles maintained the lowest concentration of magnesium. Generally, the oral administration of the edible clay to rabbits at two varying doses of 2000 mg/kg body weight and 4000 mg/kg body weight resulted in detectable levels of all the elements found in the clay in various tissues and blood of the treated animals. From 24 hours to 240 hours post treatments with the clay Calcium occurred in the brain of the treated rabbits. The concentrations of calcium obtained 24 hours post treatment were 384.00±101.00 µg/g and 436.00 ±215.00 µg/g in brain of animals treated with 2000 and 4000 mg/kg respectively. Thereafter, there was a decline in Ca concentrations and at 240 hours post treatment the levels in the brain were 138.80±35.80 µg/g and 186.00±115 µg/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 24 hours post treatment, and these were followed by steady decline in Ca levels in the various tissues and blood, and the least observed 240 hours post treatment. The results of statistical analysis between the concentrations in various tissues and organs show that there are significant difference at $p<0.05$. Similarly a comparison within any given tissue and organ with respect to time (hours) also shows significant different at $p<0.05$.

The levels of K and Na in the various tissues and blood of rabbits treated orally with edible clay are presented in Tables 6 and 7 respectively. The brain, heart, kidney, liver, lungs, skeletal muscle and blood contained 141.00± 36.76 µg/g, 78.00± 15.93 µg/g, 94.67±7.26 µg/g, 114.00±10.50 µg/g, 109.00± 18.33 µg/g, 177.67± 12.72 µg/g and 46.90±5.18 µg/g of K in rabbits treated with 2000 mg/kg of the edible clay 24 hours post treatment. The rabbits treated with 4000 mg/kg of the clay were observed to contain 152.70± 19.10 µg/g, 124.70± 16.20 µg/g, 106.32± 12.50 µg/g, 114.30 ± 10.20 µg/g, 113.30± 10.30 µg/g, 181.67± 8.28 µg/g and 51.50± 6.34 µg/g of K 24 hours in the brain, heart, kidney, liver, lungs, skeletal muscle and blood respectively. These initial high concentrations obtained 24 hours were observed to decrease with time and 240 hours post treatment substantial amounts were still present in the various tissues and blood (Table 6). Sodium concentrations were observed to be high in the tissues of rabbits treated with 2000 mg/kg and 4000 mg/kg of the clay (Table 7). In general the Na levels in the tissues and blood of the animals treated with 4000 mg/kg body weight of clay were higher than in those treated with 2000 mg/kg dose for p less than 0.05 level of significant.

3.4 Elimination Rate Constant and Half Lives of Essential Metallic Nutrient Elements from Edible Clay in Various Tissues

The elimination rate constants of the elements present in the edible clay in the sampled organs and tissues are presented in figures 1 to 4. The rate of elimination of Ca from the brain of rabbits treated with the 2000 mg/kg and 4000 mg/kg edible clay was higher than the rate of its elimination from other tissues and blood (Fig. 1). The kidney had the lowest rate of Ca elimination with elimination rate constant of 9.15 µg/day and 16.80 µg/day in animals treated with 2000 mg/kg and 4000 mg/kg edible clay respectively. Magnesium was slowly eliminated from the tissues and blood of the clay treated animals. The rate of elimination from the brain of rabbits treated with the clay at 2000 mg/kg and 4000 mg/kg were 5.53 µg/day and 6.85 µg/day respectively, (Fig. 2). The elimination rate constant for the blood were 17.6 µg/day and 17.2 µg per day in rabbits given the clay orally at 2000 mg/kg and 4000 mg/kg body weight.

The rate constant of elimination of K and Na are presented in Figs. 3 and 4. The rate constants of elimination of these two metals were higher at $p<0.05$ in 2000 mg/kg treated rabbits for the following tissues; kidney, liver, lungs, skeletal muscle and blood, compared to those treated with 4000 mg/kg.

Table 4. Concentrations of calcium in (µg/g) in sampled edible tissues and blood of orally dosed rabbits

Time/ Dose	Brain	Heart	Kidney	Liver	Lungs	Skeletal Muscle	Blood
24h.							
2000 mg	384.00± 101.00^{b}	222.68±142.31^{b}	185.57±111.57^{a}	325.77±138.74^{b}	227.00±173.00^{b}	296.91±121.84^{b}	424.74±25.08^{b}
4000 mg	436.00±215.00^{2}	297.00±125.00^{1}	235.00±161.00^{1}	425.00±178.00^{2}	260.00±143.00^{1}	408.20±70.30^{2}	486.60±93.20^{2}
48 h.							
2000 mg	280.00±123.00^{b}	177.32±91.002^{b}	107.22±10.91^{a}	152.58±46.47^{b}	119.60±27.00^{a}	131.96±57.73^{b}	416.49±71.90^{b}
4000 mg	293.00±137.00^{2}	223.00±161.00^{1}	206.00±152^{1}	210.00±130.00^{1}	223.00±118.00^{1}	309.00±125.00^{2}	482.50±24.70^{2}
96 h.							
2000 mg	223.60±99.10^{b}	123.71±55.78^{b}	103.09±10.91^{a}	107.20±33.00^{b}	107.22±27.00^{a}	127.83±90.72^{a}	305.15±8.25^{b}
4000 mg	239.00±114.00^{2}	210.00±124.00^{1}	198.00±112.00^{1}	148.45±106.66^{1}	156.70±51.70^{1}	219.00±157.00^{2}	437.10±55.50^{2}
144 h.							
2000 mg	206.20±18.00^{b}	115.46±29.74^{b}	86.60±18.90^{a}	90.72±14.87^{a}	107.22±27.04^{a}	90.72±17.97^{a}	280.41±71.54^{b}
4000 mg	235.00±149.00^{2}	202.00±110.00^{2}	115.50±18.00^{1}	107.20±10.90^{1}	148.50±35.70^{2}	190.00±134.00^{2}	392.00±111.00^{1}
192 h.							
2000 mg	169.10±82.80^{a}	90.72±10.91^{a}	86.60±0.00^{a}	74.23±7.14^{a}	107.20±14.87^{a}	78.35±4.12^{a}	243.30±39.34^{b}
4000 mg	206.00±147.00^{2}	190.00±128.00^{2}	107.20±10.90^{1}	74.23±7.14^{1}	127.80±47.60^{2}	127.80±43.60^{2}	268.00±28.90^{2}
240 h.							
2000 mg	138.80±35.80^{b}	70.10±10.91^{a}	74.23±7.14^{a}	70.10±4.12^{a}	74.23±7.14^{a}	65.98±4.12^{a}	243.30±39.34^{b}
4000 mg	186.00±115^{2}	107.20±10.90^{2}	90.70±16.50^{1}	70.10±8.25^{1}	119.60±45.90^{2}	78.35±4.12^{1}	268.00±28.90^{2}

The superscript letters (a and b) and numbers (1 and 2) show the significant differences among low and high doses respectively. High Dose (4000 mg) of clay with the different superscript numbers are significantly different from one another while low dose (2000 mg) with different superscript letters are significantly different from one another at 95% level of confidence ($p<0.05$)

Table 5. Concentrations of magnesium in (µg/g) in sampled edible tissues and blood of orally dosed rabbits

Time/ Dose	Brain	Heart	Kidney	Liver	Lungs	Skeletal muscle	Blood
24 h.	175.66±13.25^{a}	137.69±16.35^{a}	241.63±27.86^{b}	207.24±26.11^{a}	160.53±25.33^{a}	188.49±35.61^{a}	179.28±58.41^{a}
2000 mg	208.88±2.16^{2}	152.60±15.10^{1}	250.30±15.10^{2}	226.60±19.90^{2}	196.05±3.66^{1}	268.40±27.60^{2}	230.80±37.00^{2}
4000 mg							
48 h.	48.36±24.64^{a}	131.25±16.79^{b}	171.05±21.38^{b}	185.51±5.69^{b}	157.24±10.84^{a}	149.67±1.65^{a}	171.88±8.59^{b}
2000 mg	96.50±51.60^{1}	146.40±21.00^{1}	204.30±14.30^{1}	192.40±15.90^{1}	175.66±5.22^{1}	249.00±19.50^{2}	215.50±16.70^{1}
4000 mg							
96 h.	145.40±2.63^{a}	126.32±5.06^{a}	164.47±29.14^{a}	166.45±17.77^{a}	150.00±2.96^{a}	120.07±3.29^{a}	159.54±3.79^{a}
2000 mg	87.50±28.10^{1}	145.72±6.96^{2}	198.68±8.00^{2}	180.92±0.87^{2}	173.00±13.80^{2}	167.80±15.90^{2}	203.80±46.50^{2}
4000 mg							

Time/ Dose	Brain	Heart	Kidney	Liver	Lungs	Skeletal muscle	Blood
144 h.	36.18±10.91^{a}	118.09±13.37^{a}	146.38±16.67^{b}	152.30±17.07^{b}	146.38±19.52^{b}	112.34±8.04^{a}	138.32±3.83^{b}
2000 mg	155.43±5.18^{1}	139.50±11.10^{1}	191.45±8.39^{2}	165.40±18.60^{2}	161.40±11.50^{2}	160.20±14.70^{2}	180.90±18.10^{1}
4000 mg							
192 h.	129.93±8.55^{b}	113.49±5.78^{a}	144.41±18.11^{b}	139.80±10.77^{b}	132.24±13.03^{b}	31.84±0.72^{a}	41.48±17.80^{a}
2000 mg	152.30±4.78^{2}	134.50±13.00^{1}	160.86±8.62^{2}	155.90±26.00^{1}	146.40±15.40^{1}	150.00±19.30^{2}	161.35±5.15^{2}
4000 mg							
240 h.	110.53±5.92^{b}	62.17±21.71^{a}	141.45±16.45^{b}	139.47±11.97^{b}	107.73±7.84^{b}	31.22±5.42^{a}	35.86±11.68^{a}
2000 mg	151.00±25.00^{2}	120.07±2.30^{1}	157.70±11.40^{2}	145.60±16.10^{1}	146.40±19.50^{1}	134.20±37.00^{1}	47.30±12.30^{1}
4000 mg							

The superscript letters (a and b) and numbers (1 and 2) show the significant differences among low and high doses respectively. High Dose (4000mg) of clay with the different superscript numbers are significantly different from one another while low dose (2000mg) with different superscript letters are significantly different from one another at 95% level of confidence (p<0.05)

Table 6. Concentrations of potassium in (µg/g) in sampled edible tissues and blood of orally dosed rabbits

Time/ Dose	Brain	Heart	Kidney	Liver	Lungs	Skeletal muscle	Blood
24h.							
2000 mg	141.00±36.76^{a}	78.00±15.93^{a}	94.67±7.26^{a}	114.00±10.50^{a}	109.00±18.33^{a}	177.67±12.72^{a}	46.90±5.18^{a}
4000 mg	152.70±19.10^{2}	124.70±16.20^{1}	106.30±12.50^{1}	114.30±10.20^{1}	113.30±10.30^{1}	181.67±8.28^{2}	51.50±6.34
48 h.							
2000 mg	128.00±21.73^{a}	72.33±7.69^{a}	88.67±14.75^{a}	89.67±2.90^{b}	94.00±6.00^{b}	153.33±8.67^{b}	37.07±7.09^{a}
4000 mg	143.70±37.80^{1}	69.00±15.60^{1}	105.00±4.73^{1}	96.00±12.50^{1}	110.00±7.21^{1}	179.00±10.40^{1}	50.12±5.53^{1}
96 h.							
2000 mg	115.67±7.12^{b}	68.00±8.74^{a}	88.33±4.63^{b}	81.33±14.84^{a}	93.33±6.33^{b}	142.00±2.31^{b}	36.97±7.24^{a}
4000 mg	141.67±7.51^{2}	64.70±15.90^{1}	95.30±13.40^{1}	94.00±6.81^{1}	97.33±8.33^{1}	159.30±32.00^{2}	48.77±6.25^{1}
144 h.							
2000 mg	92.67±11.68^{b}	54.67±1.45^{a}	84.33±1.76^{b}	77.67±1.86^{b}	76.33±5.90^{b}	141.33±6.00^{b}	31.63±5.68^{a}
4000 mg	120.00±1.73^{2}	57.33±9.02^{1}	89.33±7.69^{1}	90.67±7.31^{1}	93.70±15.50^{1}	159.30±11.60^{2}	37.80±1.30^{1}
192 h.							
2000 mg	42.33±22.52^{a}	49.00±4.00^{b}	75.67±6.17^{a}	75.00±8.96^{a}	69.67±12.55^{a}	120.00±14.73^{b}	31.43±6.77^{a}
4000 mg	114.70±13.40^{2}	54.00±5.69^{1}	87.69±3.28^{1}	89.00±6.03^{2}	90.00±5.57^{2}	153.30±10.50^{2}	37.30±0.36^{1}
240 h.							
2000 mg	19.33±11.57^{a}	38.67±6.17^{a}	67.67±6.23^{b}	69.00±10.07^{b}	66.67±2.33^{b}	115.33±19.10^{b}	17.87±1.41^{b}
4000 mg	100.00±10.00^{2}	48.67±1.45^{1}	86.67±0.88^{2}	85.30±12.70^{1}	70.33±6.97^{1}	151.00±5.77^{2}	35.13±2.81^{1}

The superscript letters (a and b) and numbers (1 and 2) show the significant differences among low and high doses respectively. Values with a, b and 1, 2 in the same column with different superscripts differ significantly at confident limit of p<0.05 for 2000 mg/Kg and 4000 mg/Kg body weight respectively

Table 7. Concentrations of Sodium in (µg/g) in sampled edible tissues and blood of orally dosed rabbits

Time/ dose	Brain	Heart	Kidney	Liver	Lungs	Skeletal muscle	Blood
24h.							
2000 mg	110.00±28.87^{a}	66.67±12.02^{a}	116.67±17.64^{a}	86.67±6.67^{a}	96.67±14.53^{a}	97.00±18.68^{a}	85.67±6.30^{a}
4000 mg	110.70±20.30^{2}	85.70±34.90^{1}	116.70±24.00^{2}	90.00±11.50^{1}	130.00±10.00^{2}	120.30±42.00^{2}	89.30±7.42^{1}
48 h.							
2000 mg	110.00±15.28^{a}	66.67±18.56^{a}	110.00±11.50^{a}	73.33±12.02^{a}	94.67±17.94^{a}	95.00±13.23^{a}	84.40±3.65^{a}
4000 mg	110.00±28.90^{2}	77.00±8.50^{1}	113.33±12.02^{2}	80.00±0.00^{1}	116.67±8.82^{2}	113.30±16.70^{2}	9.00±6.00^{1}
96 h.							
2000 mg	90.00±11.55^{b}	63.33±28.48^{a}	100.00±15.30^{b}	66.67±3.33^{a}	90.00±23.09^{b}	93.33±33.33^{b}	83.57±5.70^{b}
4000 mg	103.33±3.33^{2}	73.30±12.00^{1}	110.00±15.28^{1}	80.00±11.50^{1}	110.00±10.00^{1}	106.70±23.30^{1}	88.83±8.99^{1}
144 h.							
2000 mg	80.00±55.08^{a}	63.33±6.67^{a}	80.00±0.00^{a}	66.67±6.67^{a}	86.67±8.82^{b}	90.00±5.77^{b}	76.17±7.00^{a}
4000 mg	90.00±5.77^{2}	73.30±20.30^{1}	100.00±10.00^{2}	76.70±17.60^{1}	103.30±18.60^{2}	100.00±15.30^{2}	86.60±10.70^{1}
192 h.							
2000 mg	76.67±8.82^{b}	60.00±17.32^{a}	76.67±12.02^{b}	63.33±18.56^{a}	70.00±10.00^{a}	86.67±6.67^{b}	63.17±11.59^{a}
4000 mg	90.00±5.77^{2}	63.33±8.82^{1}	93.67±3.18^{1}	66.67±6.67^{1}	100.00±17.30^{2}	100.00±17.30^{2}	76.73±1.32^{2}
240 h.							
2000 mg	30.00±10.00^{a}	36.67±3.33^{a}	70.33±11.84^{b}	45.00±5.00^{a}	69.00±5.86^{b}	66.687±12.02^{b}	61.60±15.15^{b}
4000 mg	66.67±3.33^{1}	46.70±12.00^{1}	83.33±3.33^{2}	63.30±14.50^{1}	83.33±8.82^{2}	96.70±20.30^{1}	69.30±4.84^{1}

The superscript letters (a and b) and numbers (1 and 2) show the significant differences among low and high doses respectively. High Dose (4000mg) of clay with the different superscript numbers are significantly different from one another while low dose (2000mg) with different superscript letters are significantly different from one another at 95% level of confidence ($p<0.05$)

3.5 The Half Life ($T_{1/2}$) of Elimination of Metals Present in the Edible Clay in the Various Organs and Tissues of Treated Rabbits

The half-life ($t_{1/2}$) of elimination of metals present in the edible clay in the various organs and tissues of treated rabbits are presented in Figs. 5 to 8. The half-life of elimination of Ca was higher at $p<0.05$ in the tissues of animals given the clay at 2000 mg/kg compared to those given the clay at 4000 mg/kg body weight, (Fig. 5). Fig. 6 shows the half-lives of Mg in the various tissues of treated rabbits. Skeletal muscle, lungs and heart have higher half lives in the animals treated with 2000 mg/kg body weight dose, than those treated with 4000 mg/kg dose.

The half-lives of K and Na in the various sampled tissues of rabbits treated with the edible clay are shown in Figs. 7 and 8. The half-lives of these two metals were higher in the kidney, liver, lungs, skeletal muscle at 2000 mg/kg compared to the one given at 2000 mg/kg. The brain of rabbits treated with the clay at 2000 mg/kg had the least half-life of Na.

The edible clay from Enyigba in Abakaliki, Ebonyi State Nigeria contains different types of metal elements from the observations made in the study. The analyses showed that the elements found in the clay occurred in varying concentrations. These elements may be grouped based on human nutritional requirements or health implications as essential metallic nutrients in animal and human nutrition. In this group, contains such elements as Na, K, Ca and Mg. Ogah and Ikelle obtained similar results as in table 1 [33, 31,31[a]]. The elements are required for growth and maintenance of health [34]. That elements such as Ca, Mg, Na and K play essential roles in human and animal health and diseases have been previously highlighted [35, 36,37]. Calcium apart from its importance in the maintenance of the skeletal structure of the body is also involved in blood coagulation, functioning of the heart, muscle and nerves and permeability of cell membranes [38]. Sodium is the major cation of the extracellular fluid and is responsible for 50% of the osmolality of the plasma or serum. Potassium is the major cation of the intracellular fluid, and is very important in the maintenance of life [39]. Nutritionists have long recognized magnesium as an essential nutrient. Severe deficiencies of this element result in neuromuscular signs similar to those of eclampsia. Magnesium is also known to regulate calcium transport and so can play a significant role in bone metabolism [40]. The concentrations of these essential elements found in the edible clay are within the safety limits reported by World Health Organization. The recommended daily allowances are 3,510 mg, 2,000 mg, 1,300 mg and 400 mg for K, Na, Ca and Mg respectively [41].

The high levels of Ca and Mg in the clay may suggest that it may be compounds of calcite ($CaCO_3$) or dolomite $CaMg(CO_3)_2$ or a combination of the two compound to form lime stone.

Kinetically all the essential metals in the edible clay were distributed in all the tissues and organs analyzed 24 hours up to 240 hours of the period of the experiment. Some of the elements occurred in higher concentration than others probably due to enhanced absorption of such elements. It was also observed that the concentrations in the tissues were related to the proportion of such element in the analyzed clay for example Sodium concentration in the clay was 71.96±3.63 mg/g and Ca was found to be 249.00±1.73 mg/g being the least and highest concentrations respectively. The distributions in the tissues and organs followed the same trend with sodium concentration in the tissues being relatively less than that of Calcium. The investigation showed that Calcium Magnesium, Potassium and Sodium in the clay have the concentrations of 249.00±1.73 mg/g, 198.57 ± 0.90 mg/g, 84.33±2.73 mg/g and 71.96±3.63 mg/g respectively. Ogah and co-workers obtained 249.000±1.73 mg/g, 198.570±0.90 mg/g, 84.330±2.73 mg/g and 71.960±3.63 mg/g concentrations of Calcium, Magnesium, Potassium and Sodium respectively from sampled clay from same location [33, 31, 31[a]]. Though the control samples also showed few of these elements, their concentrations were however not reflected in the results. This was because the emphasis was on the result of high and low doses of the animals and any additional amount of the metals as observed in the control must have been present in the water given to the control rabbits or any other source which was eliminated by subtraction from the observed concentrations in groups A and B. It is therefore not surprising that those elements occurred in high concentrations in the sampled tissues. Biological systems are known to possess mechanisms for absorption, transportation, storage and excretion of elements. The administration of the edible clay orally may have resulted in the following within the gastro-intestinal tract. (i) The dissolution of the clay, (ii)

Absorption of elements into the systemic circulation and (iii) Transfer of particles via the lymphatic system to lymph nodes or blood [42, 43]. Elements transported by the blood are deposited in the body organs and tissues. The liver, kidney, brain, heart, skeletal muscle, and lungs constitute likely recipients of such elements [44].

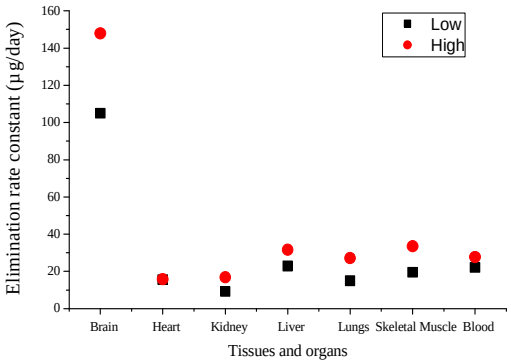

Fig. 1. Elimination rate constant of Ca in tissues of rabbits treated with doses of 2000 mg/kg body weight (low) and 4000 mg/kg body weight (high)

This study has shown that the liver acted as the organ with the most maintained high concentration of the elements. The observed high concentrations of these elements in the liver are probably related to their effective absorption from the gastro-intestinal tract and the ability of the liver to store them. Other studies have reported the capabilities of most organs, notably liver and kidney, to store large quantities of elements in the biological systems. The mechanisms entailed interactions between these elements with the intrinsic proteins in the organs and tissues, [45]. The high concentrations obtained in the liver and kidney should be expected since the kidney is the primary organ of elimination and the liver the main organ of biotransformation [46,47]. The presence of the sampled elements in the brain, heart, and skeletal muscle is interesting. Extensive amounts of elements such as Ca, Mg, K and Na were obtained in these tissues. The presence of these elements in the brain and skeletal muscle may be an indication of the ability of these elements to cross physiological barriers. The organs of the body, which are moderately supplied with blood such as heart and lungs, also contain high levels of Ca, Mg, Na, and K. This may be an indication of rapid distribution of these elements to the tissues which resulted in the increased uptake of these elements by these organs.

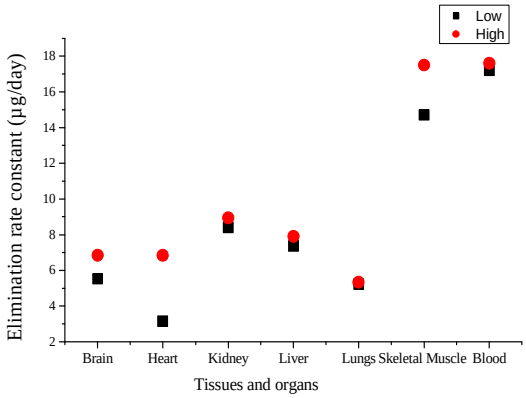

Fig. 2. Elimination rate constant of Mg in tissues of rabbits treated with doses of 2000 mg/kg body weight (low) and 4000 mg/kg body weight (high)

The presence of Ca, Mg, Na and K in the blood after 24 hours post administration of the edible clay may be due to absorption of the elements from the stomach and or small intestine [48]. However, absorption may not explain the continued presence of these elements in the blood at 240 hours post administration.

The elements after absorption into the blood were distributed to organs and tissues of the body. Re-distribution of these elements may account for the prolonged presence of the elements in the blood, since the blood is the medium of transport of these elements in the body either to the tissues or to the excretory organs. There has been reported persistence of Ca and Mg in the kidney and blood of rabbits up to 240 hours post administration of substance containing these metals [31].

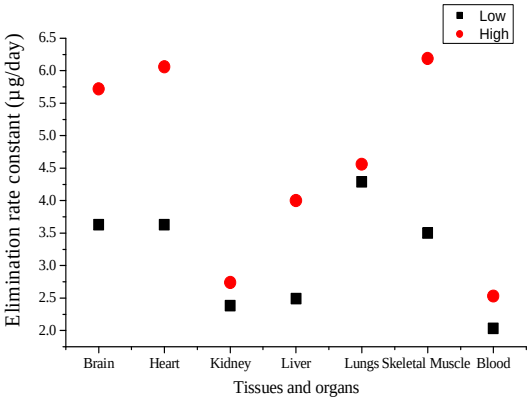

Fig. 3. Elimination rate constant of K in tissues of rabbits treated with doses of 2000 mg/kg body weight (low) and 4000 mg/kg body weight (high)

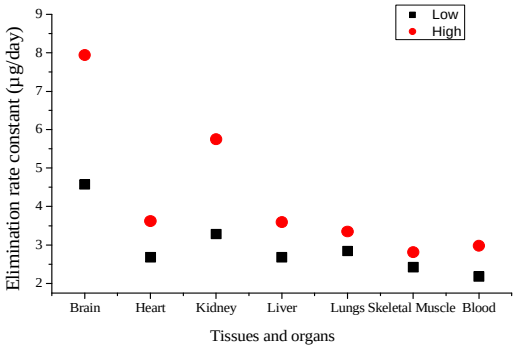

Fig. 4. Elimination rate constant of Na in tissues of rabbits treated with doses of 2000 mg/kg body weight (low) and 4000 mg/kg body weight (high)

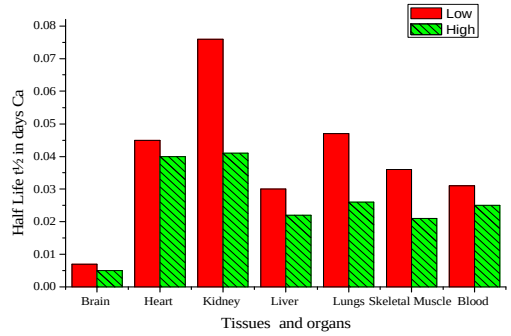

Fig. 5. Elimination half-life of Ca in days in tissues of rabbits treated with doses of 2000 mg/kg bodyweight (low) and 4000 mg/kg body weight (high)

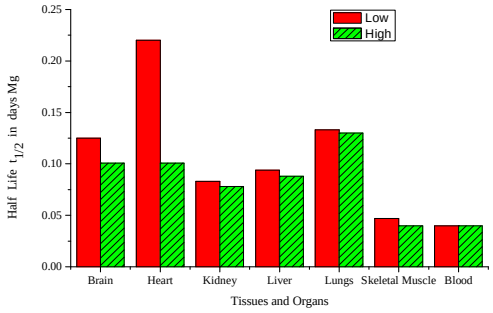

Fig. 6. Elimination half-life of Mg in days in tissues of rabbits treated with doses of 2000 mg/kg body weight (low) and 4000 mg/kg body weight (high)

Plasma protein binding of the elements may also contribute to the prolonged presence of these metals in the blood. Although the study did not involve the plasma protein binding of these metals, the elements are known to bind to plasma albumin. High degree of plasma protein binding generally makes substances long acting because the bound fractions are not available for elimination, unless actively extracted in the liver or kidney. *Enterohepatic* circulation of these elements could also prolong their presence in the blood. These elements distributed to the liver may be metabolized or eliminated through the bile into the intestine and thereafter reabsorbed into the blood thereby prolonging the duration of presence of the elements in the blood [49]. The high concentration of calcium in the brain of clay treated rabbits is worthy of note since in an earlier study it has been shown that elevated level of calcium in the brain decreases learning ability and also decreases brain serotonin turn over [50]. However, with the recommended dietary calcium level of 1,300 mg/day for human adults, the concentration of Ca in the clay may be considered to be low when given at the doses 2000 mg/kg and 4000 mg/kg. The half-lives of the elements found in the clay sample were indications of the lengths of time each of these elements will be retained in a particular organ or tissue. The higher the half-life of an element, the longer the persistence of that element in a particular tissue [51], for example Ca with a half-life of 0.076 day in kidney of rabbits treated with 2000 mg/kg of the edible clay orally will persist in the kidney more than Ca found in the liver of same rabbits with a half-life of 0.03days.The enhanced half-life of elements may be a reflection of increased tissue sequestration, prolonged absorption and or presence of complexing compound in the clay. It also indicates how widely the elements were distributed or increased serum protein or tissue binding of the elements which limits their distribution to excretory organs.

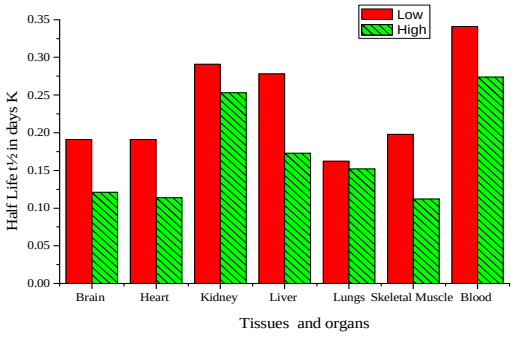

Fig. 7. Elimination half-life of K in days in tissues of rabbits treated with doses of 2000 mg/kg body weight (low) and 4000 mg/kg body weight (high)

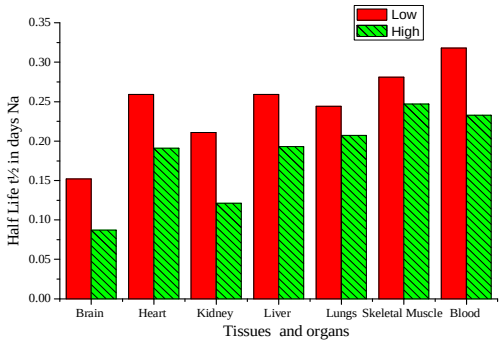

Fig. 8. Elimination half-life of Na in days in tissues of rabbits treated with doses of 2000 mg/kg body weight (low) and 4000 mg/kg body weight (high)

4. CONCLUSIONS

The clay from Enegba Ebonyi State Nigeria contains essential electrolyte in concentrations within the world Health organization limit. The low level of Sodium in the sample is worthy of note positively since too much sodium has been associated with hypertension and kidney malfunction. The adequate distribution of these elements in the analyzed tissues and organs pose no danger since the kinetic study showed that they are optimally eliminated without accumulation at least within the limit of this study. Consumption of Clay from Enyigba in Ebonyi State poses no danger from the essential metallic element stand point.

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COMPETING INTERESTS

The authors do hereby declare that there is no competing interest in this research work.

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