World Academy of Science, Engineering and Technology
International Journal of Bioengineering and Life Sciences
Vol:7, No:12, 2013

Microbial Evaluation of Geophagic and Cosmetic Clays from Southern and Western Nigeria: Potential Natural Nanomaterials

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***Abstract*—**Geophagic and cosmetic clays are among potential nanomaterial which occur naturally and are of various forms. The use of these nanoclays is a common practice in both rural and urban areas mostly due to tradition and medicinal reasons. These naturally occurring materials can be valuable sources of nanomaterial by serving as nanocomposites. The need to ascertain the safety of these materials is the motivation for this research. Physical Characterization based on the hue value and microbiological qualities of the nanoclays were carried out. The Microbial analysis of the clay samples showed considerable contamination with both bacteria and fungi with fungal contaminants taking the lead. This observation may not be unlikely due to the ability of fungi species to survive harsher growth conditions than bacteria. 'Atike pupa' showed no bacterial growth. The clay with the largest bacterial count was Calabash chalk (Igbanke), while that with the highest fungal count was 'Eko grey'. The most commonly isolated bacteria in this study were *Clostridium* spp. and *Corynebacterium* spp. while fungi included *Aspergillus* spp. These results are an indication of the need to subject these clay materials to treatments such as heating before consumption or topical usage thereby ascertaining their safety.

***Keywords*—**Nanomaterial, clay, microorganism, quality.

I. Introduction

CLAY refers to a naturally occurring material composed mainly of fine grained minerals, which is generally plastic at appropriate water contents and will harden upon firing or drying [1]. The consumption of clay and other soil materials is widely practiced in certain parts of the world and this practice is called Geophagia [2]-[4]. The habit of deliberate eating of geophagic clay or soil is considered to be a deviant eating disorder, a sequel to poverty and famine which could also be observed in the absence of hunger [5]. Human geophagia is an ancient practice that has been sustained for thousands of years despite the purported health drawbacks. Human geophagic practitioners were reported to consume about 40-50 g/day of clay [6], [7]. More women than men engage in geophagic practice [8] and particularly during pregnancy [9]. The practice is also prevalent among lactating women, school children and people with psychiatric disorders [5].

Geophagia is a habit that has cultural acceptability in the societies where it is being practiced, though some deem it an abnormal practice. It has also been reported as a physiologic response to a deficiency of iron or calcium in the body [10]. The assumption of physiologic response might have arisen from the fact that non-human primates and various other animals including birds ingest soil in their normal lives [11], [12] Cultural, psychological, medicinal and nutritional needs have been advanced to justify the practice [13], [14]. Aside from deliberate consumption, clays are also used traditionally as cosmetics and in topical applications against some skin ailments. Clay materials have been reported to be used for ceremonial and religious occasions [15]. Also clay materials are applied to the body as anti-inflammatory and antiseptic agents [16] and are known to also detoxify [17].

Clay and clay minerals naturally constitute part of the geological structure of the earth, found along the river bed and examples are Aluminosilicate, Kaolinite, Montmorillonite clays [18]. They often have a glue-like property that promotes attachment of microorganisms and their nutrients. At the contact of clayey soils, bacteria will adhere to the surface through physical and chemical interactions facilitated by cell envelope structures and the diversity of mineral surface, functionality and crystallography. When bacteria and minerals are separated by some finite distance and by adhesion forces, the cumulative effects of interfacial forces determine the interactions [19]-[21]. Apart from the traditional practices involving the use of clay, nanoclays have found use in nanomedicine, essentially in the form of nanocomposite when exfoliated and organically modified. Nanocomposites have been shown to exhibit antimicrobial activities against bacteria, spores and viruses [22]-[25]. Nanomaterials are a combination of materials which have one or more dimensions in the nano range. The nanomaterials bridge the range of molecular and micro scales and allow connectivity of interactions which enhance the mechanical characteristics of composite [26]. Clay nanocomposites are potentially attractive for commercial use because of the wide availability and cheapness.

The probable presence in clay material of such highly toxigenic and parasitic organisms such as *Clostridium perfringens, C. tetani, C. botulinum, Ascaris, Trichuri*s spp. which occur in soil habitats have been inferred [5]. This may generate safety concerns, thereby counteracting the probable positive usage of clay as nanomaterial. The microbiology of geophagic and cosmetic clays is an area where there is relatively scarce information. With a paucity of literature that has documented the microbiological profile of geophagic and cosmetic clays in Nigeria and lack of data on the health

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implications, this study was embarked upon to evaluate the microbial quality of geophagic and cosmetic clays. This is to obtain background information on the safety implications of their consumption, topical and usage as nanomaterial.

II. Materials and Methods

A. Clay Sample Collection

Clay samples were collected from four sites, a river bank in Ozanogogo village, Orhiowhon Local Government in Delta state, a river valley village in Igbanke Local Government, Edo state, Hausa market and Main Market in Warri South Local Government, Warri Delta State. Clay samples were collected in sterile Ziploc bags, sterilized at 2450 Hz for 5mins [27]. Samples collected from river banks were taken using a sterile spatula that was flamed before and after collection and transferred aseptically into the sterile Ziploc bags and labeled. The clay samples were thereafter placed in an ice bag for transfer to the laboratory for analysis. Market samples were collected by asking the clay vendors to place the samples by themselves in the bag so as to maintain the microbial profile of the collected samples as usually sold. These were labeled appropriately and transported with ice packs to the laboratory for analysis.

B. Sample Preparation

The clay samples were divided out aseptically, the part for microbial analysis was stored at 4°C while the remainder was air dried and ground to achieve a smooth consistency. Thereafter the clay sample were sieved and ground repeatedly until no more filtrate was recovered.

C. Color Identification

The color of the clay samples were identified using the munsell soil color chart [28].

D. Microbial Soil Analysis

One gram of soil was measured and aseptically transferred into 9ml of sterile distilled water and homogenized by shaken until all the clay was dispersed. 1ml of dispersed clay was aseptically transferred to another 9ml of sterile distilled water using a sterile pipette to make a dilution of 10^{-1}. This was serially diluted up to 10^{-6} dilution. One ml aliquot of dilutions $10^{-3} – 10^{-6}$ was transferred aseptically into labeled sterile petri plates. One ml of Tetracycline, Penicillin, and Streptomycin (50 µg/ml) were added to the plates labeled for fungi. Molten nutrient agar and malt extract agar were then poured into the plates for bacteria and fungi culture respectively. The plates were swirled in a clockwise and anticlockwise motion, allowed to set, and then incubated in inverted position for 24h at 37°C for bacteria and 5-7 days at 25°C for fungi. After incubation the plates were checked for growth and the colonies were counted and recorded.

E. Isolation of Bacterial and Fungal Isolates

Colonies were identified and subcultured for purity on fresh plates. Representative colonies were also picked from the plates and subcultured on fresh agar plates. The plates were incubated again for 24 h at 37°C for bacteria and 4 days at 26°C for fungi. Colonies were picked from purity plates and stocked on corresponding agar slants for bacteria and fungi.

III. Results

The sources of the clay samples used in the study are as shown in Table I below. Whereas some samples 'source samples' were obtained directly by mining through the help of local miners, others tagged 'market samples' were obtained from vendors in the open market. The Munsell soil cooler chart indicated varying cooler classification of the sample as seen in Table II. The cooler ranges from pink, pale yellow, pale green to pale brown. The microbial loads of the samples are graphically represented in Fig. 1, while Figs. 2 and 3 represent the proportion of bacteria and fungi respectively.

TABLE I
SAMPLES AND SOURCES

Mining Source Samples		Market Sample	
Sample name	Location	Sample name	Location
Calabash Chalk	(Lower Ozanogogo, Delta state)	Efun	(Hausa Market, Warri)
Calabash Chalk	(Upper Ozanogogo, Delta state)	Eko	(Hausa Market, Warri)
Calabash Chalk Red	(Igbanke, Edo State)	Efun Ado	(Main Market, Warri)
Calabash Chalk Brown	(Igbanke, Edo State)	Atike Pupa	(Main Market, Warri)
Calabash Chalk Grey	(Igbanke, Edo State)	Nzu	(Main Market, Warri)
Nwanra	(Igbanke, Edo State)	Atike Ela	(Main Market, Warri)
Eko Black	(Igbanke, Edo State)		
Eko Grey	(Igbanke, Edo State)		

TABLE II
COLOUR IDENTIFICATION OF CLAY SUBSTANCES

Samples	Sample codes	Colour codes	Colour Interpretation
Atike Pupa	APm	10R8/4	Pink
	CCOU	10YR8/4	Very pale brown
	CCOL	10YR8/3	Very pale brown
Ca Brown	CCB	10YR8/6	Yellow
	CCR		
	CCI	2.5Y8/4	Pale yellow
Efun (Market)	Efm	2.5Y8/4	Pale Yellow
	EEm	7.5RP5/18	Deep pink
	EAm	10YR8/4	Very pale brown
Eko Black	EB	Gley 7/25G	Pale green
Eko Grey	EG	Gley 6/25G	Pale green
Eko (Market)	Em	5Y7/4	Pale Yellow
Nwanra	Nw	7.5R6/6	
Uzu (Market)	Um	10YR8/3	Very pale brown

To obtain the microbial load of samples, the number of colony-forming unit (cfu) per ml of the dilution was divided by the final dilution:

$$\frac{\text{X no of colonies}}{1mlX10^{-y}} = X \text{ cfu/ml x } 10^{-y} = X \text{ x } 10^{z} \text{ cfu/ml}$$

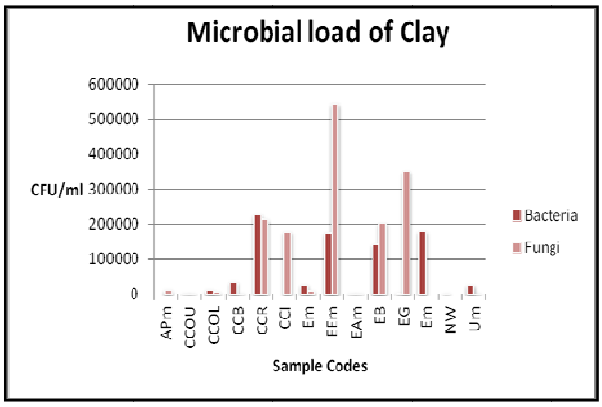

Fig. 1 Graphic display of the microbial load of Clay samples

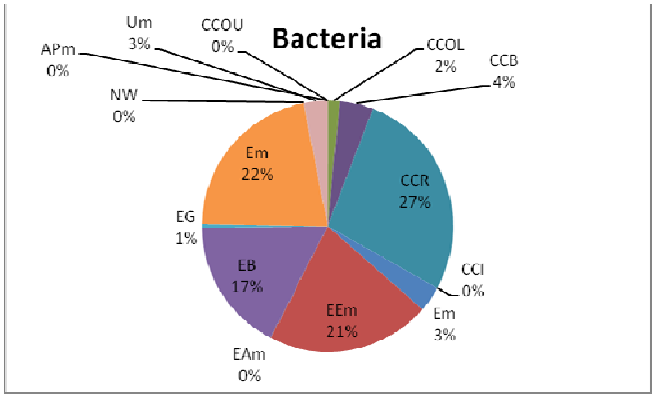

Fig. 2 Graphic representation of % bacteria in the clay samples

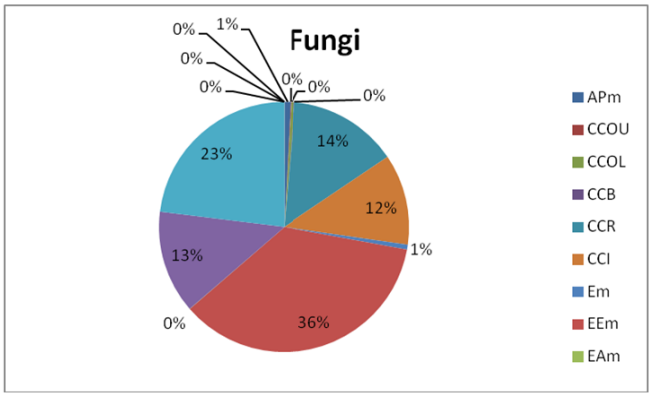

Fig. 3 Graphic representation of % fungi in Clay samples

IV. Discussion

The colors of the geophagic clay vary from yellow through pale green to pale brown, giving the consumers varying choices. Some of the hue values of the examined clay are consistent with the work of [29], and [30]. Cosmetic clays were usually pink in color whereas geophagic clays vary more in color depending on the source. A good reason for the practice of geophagia is nutrient supplementation, and red coloration in some clay samples were indicative of iron in those samples thereby serving as means of iron supplementation [31], [32]. Of the clay samples, 64% had low bacterial counts of between 10^3 - 10^5 CFU/ml and 71.42% of clay samples had fungal counts of between 10^3-10^5 CFU/ml, while some had very low counts with only 7% sample having no bacterial count and 14.28% having no fungal count. The presence of contaminating microorganism in a high percentage of the samples may be an indication of the samples not being safe for consumption without treatment either by heat or by other means. A similar high count of 2 X 10^4 CFU/g was reported by [33] as the average count of bacteria present in the clay samples examined.

The higher percentage of fungal counts in comparison to bacteria also indicates a poor adaptability of bacteria to existing in the clay environment and a better adaptability of fungi to clay soils. The most commonly isolated bacteria in this study were *Clostridium* spp. and *Corynebacterium* spp. while fungi included *Aspergillus* spp. This however was contrary to the report by [34] where the predominating bacteria identified from the high total count were *Staphylococcus aureus* and *Micrococcus acidophilus*. Although Kikouma et al. [35] reported that pregnant women and children consume clay for the purpose of obtaining beneficial bacteria, yet it is pertinent to be careful of opportunistic bacteria that can become infective. Some species of the microorganisms identified in this study such as the *Clostridium* and *Aspergillus* spp. are known to be pathogenic.

V. Conclusion

The practice of geophagia and application of cosmetic clays topically may have to be done with caution. There is a need to educate the traditional users on the necessity to heat-process and properly store the clay they consume, to avoid poisoning or infecting themselves with dangerous microorganisms.

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