Microbiological Characterization of Dry White Clay, a Pica Element in Ghana

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Abstract: The consumption of clay, which is a soil, falls under what is known as 'soil pica' or geophagy. Geophagy has been a tradition in many cultures in different parts of the world. It is highly prevalent in Ghana, especially among pregnant women, for various reasons. Clay consumption is however expected to have some physiological and pathological effects owing to the fact that clay contains considerable amounts of organic material, including many live microorganisms that could have significant effects on consumers. In Ghana, clays consumed include adry white clay called *ayelo* or *shile*, specially mined and processed for local markets. This study characterized samples of *ayelo* sold in some markets in Ghana, to determine their microbial safety. Samples of *ayelo* were purchased from some common markets in Accra and analyzed using standard microbiological culturing procedures. Total viable counts, detection and identification of coliforms, yeasts, staphylococcal species, eggs of helminthes and cysts of protozoans were done. The moisture content and water holding capacity of the samples were also determined. The moisture content and water holding capacity of samples were very low, ranging from 0.59-0.82% and 31.2-35.1 g /g, respectively, suggesting that the commodity does not constitute a suitable menstruum for most microorganisms. The total viable count of samples ranged from 1.9 x 10^3 to 3.3 x 10^4 cfu/g. Samples of clay from the different markets were similarly contaminated. Coliform bacteria were isolated in all samples analyzed and the isolated species were identified as *Alcaligenes* spp. *Staphylococcal* spp and *Candida* spp were also isolated from all samples. Eggs of helminthes or cysts of protozoans were however not detected in any of the samples examined. Data obtained suggested that the product could be sources of microbial infection. [Report and Opinion 2010;2(6):77-81]. (ISSN: 1553-9873).

Key words: Dry white clay, microbiology of dry white clay, pica, geophagy

1. Introduction

Pica has been defined as the pathological act of eating nonfood items (Callahan, 2003). It is considered as a medical disorder (Ellis, 2009). There are several forms of pica, depicting the type of nonfood material that is eaten. The consumption of clay, which is a soil, falls under what is known as 'soil pica' or geophagy. Geophagy has been a tradition in many cultures in different parts of the world. It is reported that clays were used to remove toxins in aboriginal settlements, as condiments or spices in Philippines, New Guinea, Costa Rica, Guatemala, and parts of South America, and as food during famine (Johns 1990). The practice of geophagy is widespread in Africa and is variously associated with medicinal treatment and with spiritual ceremonial behaviour. Explorers and missionaries reported on clay eating, dating from the 18^{th} to the 20^{th} century. The practice is observed in Nigeria, Ghana, Sierra Leone, Swaziland, South Africa, Malawi, Zimbabwe, Zambia, etc. (Hunter, 1999). Geophagy is observed to be more common during pregnancy. Wiley and Solomon (1998), proposed reasons for eating clay during different periods of pregnancy in cultures of sub-Saharan Africa: For soothing stomach upset during morning sickness in first trimester, and supplementing nutrients (especially calcium) during the second and third trimesters. It is estimated that 30 g to 50 g of subsurface clay, such as kaolin and montmorillonite may be consumed in a day during pregnancy. Crosby (1976) also indicated that the practice of geophagy is more strongly connected to folk medicine, social customs and obsessive-compulsive behaviour. Bateson and Lebroy (1978), Morgan (1984), Hunter (1993) and Wiaz (1997), have also reported on the medicinal uses.

The consumption of clay is highly prevalent among women and children in Ghana. In a survey involving 330 respondents consisting of 186 pregnant and 144 lactating women, the prevalence of clay eating was observed to be about 17% and 22%, respectively (Twenefour, 1999). Tayie and Lartey (1995) also observed a prevalence of 28% among 502 pregnant women attending antenatal clinics in Accra. Twenefour (1999) estimated the average daily consumption of clay per respondent as 70g, ranging from about 3.5 g to 488 g. Some clay eaters in Ghana do so because of the flavour or just the feel for the clay, some to prevent excessive salivation and vomiting, and some to stop diarrhoea or treat heart problems (Twenefour, 1999).

The consumption of clay is expected to have some physiological and pathological effects. Nutritionists and other observers have tendered to view geophagy, and pica in general, as a compulsive craving and as a medicine to alleviate discomfort, yet it is not different from other treatments pursued in attempting to modify physiological and pathological states. The physiological and pathological effects may stem from the fact that soils, including clay, contain considerable amounts of organic material, including many live microorganisms, that could have significant effects on consumers. According to Callahan (2003), pica is considered to be a disease in certain cultures, and geophagy is associated with several psychological abnormalities in the USA. The U.S. Agency for Toxic Substances and Disease Registry is reported to have appointed a committee to review soil pica in June 2000 (Callahan, 2003). According to the report, the committee settled on pathological levels as consumption of more than 500 mg of soil per day. The practice has been implicated in a number of nutritional and physiological problems. Among these are the observed relationships with anaemia (Coltman, 1969; Mokhobo, 1986; Geissler *et al*, 1998), and lower hemoglobin and serum ferritin concentrations (Geissler *et al*, 1998). Gelfand *et al* (1975) associated geophagy to hyperkalaemia. In China also, low levels of zinc in hair and blood was associated with higher frequency of geophagy practice (Xue-Cun *et al*, 1985). Twenefour (1999) also observed more cases of constipation, abdominal pains and skin rashes among clay eating women, than those who have never eaten clay. Reports of lead poisoning and other toxicities in children eating contaminated soils are also available (Callahan, 2003). On the contrary, regular consumption of soil is reported to possess the potential of boosting a mother's secretory immune system. Monkeys that regularly eat soil have been noted to have lower parasite loads (Krishnamani and Mahaney, 2002). Clays which are rich in aluminum compounds are considered to pass as immunologic adjuvants which might act as vaccines (Gupta 1998). Hunter (1993) estimated that in populations of dietary deficiencies of minerals consumption of clay may help to meet the Recommended Dietary Allowance (RDA) for some essential mineral elements.

An important issue on clay eating which has not been extensively studied in Ghana is microbial safety of the clays consumed. Clays are soil materials and could naturally harbour microorganisms, which may include pathogenic and parasitic species. Pathogens such as those that cause tetanus and elephantiasis could be found in soil and consequently in the consumed clay. *Enterobacteriaceae*, *Staphyloccocus* spp. and spore-forming pathogenic species could also contaminate the processed clays. Torsvik *et al*, (1990) and Kent and Triplett, (2002) identified about 5000 to 7000 species of bacteria per gram of natural soil. A number of the associated nutritional problems may emanate from the presence of pathogens or parasites. Of the habitual parasites found in the soil, pinworm (*Enterobious vermicularis*) and four species of soil transmitted nematodes, the round worm *Ascaris lumbricoides*, the hookworms *Ancyclostoma duodenal* and *Necator americanus* and the whipworm *Trichuris trichuris*, are common causes of infections. Clinical features of some of these include asthma, dysnea, cough, substernal pain, abdominal pain, nausea and vomiting (Collier *et al*, 1998). In a cross-sectional survey in Western Kenya on the practice of clay eating, Geissler *et al* (1998) observed 48% of soil samples eaten by children to be contaminated with eggs of *Ascaris lumbricoides.* In the United States, toxocariasis, caused by the worm *Toxocara canis* is said to be the most common parasitic infection associated with geophagy (Laufer 2002). Problems of ascariasis are also known to be common among children in Nigeria who practice geophagy (Ozumba and Ozumba, 2002). In Ghana such data are not readily available as studies on clay eating in Ghana begun only recently, and has so far been on prevalence and toxicity (Tayie and Lartey, 1995; Twenefour, 1999; Koduah, 2000). Yet there is high prevalence of parasitic infection which could also be linked to geophagy.

The purpose of this work was to examine the microflora of clays eaten in Ghana. The objectives were to determine the presence of microorganisms in the clay, to isolate and identify the predominant types, to detect the presence of pathogens, and to detect the presence of parasitic helminthes and protozoans. The study was expected to contribute to the scanty literature on the subject matter and to provide information for the education of people who process and distribute the clays and those who consume clay.

2. Materials and Methods

Clay samples: Samples of dry clay lumps (sold on markets) were purchased from some markets in Accra (namely, Madina, Makola and Kaneshie markets). A total of 10 pieces (lumps) were purchased from different retailers at each market into sterile stomacher bags. The bags were carefully sealed and labeled and then transported to the laboratory for analysis.

Enumeration of microorganisms: Samples from each market were aseptically homogenized by crushing and about10 g aseptically transferred into new stomacher bags and blended with 90 ml of sterile diluent (maximum recovery diluent), in a Seward stomacher blender 80, for 1 minute. Aliquots of the homogenates were serially diluted and plated for the determination of total plate count and the detection of coliforms, *staphylococcal* spp. and fungi, using standard procedures.

Parasitological examinations were done using a combined external wash method and a floating method (Kaneko, 1987). In the former, 20 g of clay samples (lumps) were weighed into beakers and 10 ml normal saline added. Beakers were swirled gently for 2 minutes for the saline to wash the external surfaces of the samples. The saline was then poured into test tubes and centrifuged at 2000 g for 5 minutes. The supernatant was discarded and the sediment mixed with a drop of Lugol's iodine. The whole of the sediment was transferred in drops onto microscopic slides and observed under the light microscope. In the floatation method, the externally washed samples were soaked in more saline for about 30 minute to dissolve into thick slurry which was then transferred into 15 mm x 80 mm test tubes. More saline was added and vigorously shaken or stirred to completely dissolve or mix. Saturated NaCl solution was then added carefully to fill to the brim. The suspension was left to stand for about an hour to cause any egg or cyst to float. A cover slip was then placed, horizontally, on the surface of the suspension to pick possible floating eggs and cysts. The slip was transferred to a slide with a drop of iodine and examined under a light microscope.

Physico-chemical parameters: Moisture was determined using the Shimadzu moisture balance (Shimadzu, Tokyo Japan). Two gram samples were used and the heating temperature was set at 300 ˚C.

Water holding capacity was determined as follows: Approximately 10 g of sample was weighed onto a filter paper lined in a petri dish and covered with another filter paper. The filter papers were sprayed with water from a wash bottle until they were well soaked. The dish was then covered. The weight of sample was measured after 2 hours. The spraying of water onto filter papers was repeated after weighing the samples, and the samples weighed again after another 2 hours. The spraying of water and weighing were repeated until no further increase in weight of samples occurred. Samples were then dried in an oven to calculate the amount of water absorbed. This was expressed as gram of water per gram of sample that the sample could hold.

3. Results

Moisture content and water holding capacity of samples: Table 1 shows the moisture content and water holding capacity of the clay samples.

Table 1. Physico-chemical characteristics of clay samples

Sample Source	Moisture content (%)	Water holding Capacity (g/g)
Madina	0.61 ± 0.18	33.43 ± 3.21
Makola	0.59 ± 0.17	32.5 ± 2.56
Kaneshie	0.82 ± 0.19	35.07 ± 3.62

The moisture content of the samples ranged from 0.59% to 0.82% and the water holding capacities from 32.5 to 37.07 g/g sample.

Microbial counts: Data on the microbial counts of the samples in the different growth media are presented in Table 2. Growth occurred in all the media used, indicating that all the samples were contaminated. However, the counts were generally low, except the coliforms (isolates on EMB). The colony counts were higher in inocula prepared from external washes of the clay than in those prepared using inner portions aseptically taken. No growth was actually observed on EMB plates of the latter inocula. The levels of contamination in all the samples were statistically similar.

Characterization of the isolates from the various plates showed the predominant coliform as *Alkaligenes* (Table 3). *Staphyloccocu*s spp and some *Candida* spp. were also confirmed.

The parasitological analysis of the samples did not detect the presence of ova of helminthes and cysts of protozoa. Species aimed at finding were hookworm, t*richostrongylus*, *ascaris*, *trichuris* and tape worms.

4. Discussion

The clay samples showed low moisture content and water-holding capacity. Koduah (2000) using the air-oven method observed moisture contents of 0.18-0.19% for samples purchased from Madina and Makola markets. These values generally signify that the clay samples are extremely dry to support microbial growth. Microorganisms require high water activities to grow. Most bacteria do not grow at water activities below 0.91, including pathogens such as *Clostridium botulinum*. Below 0.60 no microbiological growth is possible. Though the relationship between moisture content and water activity is very complex and may not be linear, the moisture values recorded suggest water activities far below 0.10, which could definitely not support growth of microorganisms in the clay lumps.

Table 2. Microbial counts in/on clay samples from different markets

Source	Total Viable Count (cfu/g)		Faecal Coliform count (cfu/g)		Yeast & Moulds (cfu/g)		Staphylococcal count (cfu/g)	
	External	Internal	External	Internal	External	Internal	External	Internal
Madina	8x 10^2	7 x 10^1	2 x 10^2	nd	9 x 10^3	5x 10^1	6 x 10^2	2 x 10^1
Kaneshie	12x 10^2	7 x 10^1	10 x 10^2	nd	11 x 10^2	7x 10^2	6 x 10^2	5 x 10^1
Makola	12x 10^2	7 x 10^1	5 x 10^2	nd	17 x 10^2	5x 10^2	7 x 10^2	3 x 10^1

nd- not detected at 10^{-1} dilution.

Table 3. Types of microorganisms detected and their frequency of isolation in samples

Market source	Microorganism (cfu/g)	% of isolates
	Alkaligenes	44.7
Madina	*Candida* spp	51.1
	Staphylococcus spp	3.2
	Alkaligenes	36.7
Kaneshi	*Candida* spp	40.5
	Staphylococcus spp	22.8
	Alkaligenes spp	73.9
Makola	*Candida* spp	17.8
	Staphylococcus spp	7.4

Water holding capacity also indicates the porosity of materials, and indirectly the extent to which the internal part of the material is aerated. The values recorded are low, suggesting that the samples are very poorly porous and unsuitable as habitats for bacteria.

The results of the microbiological analysis suggest that the microbial contamination occurs during post-processing handling. However samples from Makola seemed more contaminated.

The detection of *Alcaligenes* spp. was no strange since the organism is generally found in soil and water, or exist as saprophytic inhabitants of vertebrate intestinal tract that regularly contaminate soils. Their presence in the clay may thus be by direct contamination from soil or faecal contamination owing to the unhygienic handling of the products or the insanitary conditions under which the clays are processed and distributed. The detection of *Staphylococcus* spp and *Candida* spp. also pointed to the unhygienic handling practices. The frequency of isolating these organisms from plates prepared from the samples of different sources varied.

The presence of *alcaligenes*, *staphylococcus* and *candida* spp. in the samples could pose some risks to consumers. *Alcaligenes* spp., though are not known to enter into either symbiotic or pathogenic association with animals, sometimes cause opportunistic infection in humans (Prescott, 1996 *et al*). As enteric microorganisms, their presence may also suggest the presence of other faecal coliforms which may be pathogenic. Species of *Staphylococcus* are known food intoxicants. Their presence could therefore implicate the clays in cases of cross-contamination. *Candida* spp. are known to occur in the small intestine, respiratory tract, vaginal area and mouth as harmless commensals. They do not cause diseases in healthy individuals because their growth is suppressed by other microbiota, but if the normal microbiota is upset, their numbers increase rapidly to cause candidiasis (Prescott *et al*, 1996). In recent times, *Candida* spp. have become important nosocomial pathogens.

The results showed no detection of ova of helminth and cysts of protozoa. The Council for Scientific and Industrial Research, Ghana has documented that the clay winning sites occur at locations far below the subsoil level (GTV, 1999). At such deep locations, the parasitic helminthes being part of the normal microflora of the clay might not be possible. The possible source of contamination could then be during the processing and marketing. The samples analyzed showed no contaminations. The moisture contents and the water holding capacities of the samples were very low. The values ranged from 0.59 to 0.85% and 31.2-33.7 cm^3/cm^3, respectively. The latter suggests poor porosity of samples and hence less spaces for containing heterotrophic microorganisms. Most of the isolates were therefore expected to be surface microflora and external surface swab cultures on PCA showed counts comparable to the cultures of the blended samples (data not presented).

Conclusion

The data obtained suggested that the clay samples consumed could be contaminated with microorganisms. Isolates may include coliform bacteria, staphylococcal species and yeast. Contaminants are generally concentrated on the surfaces, indicating unhygienic handling of the clays. Eggs and cysts of parasitic helminthes and protozoans were not detected in samples. Since the practice of geophagy is high in Ghana, it may be important to do a more comprehensive study to cover all other types of soil eaten in the country and also investigate the pathological and physiological impacts of the practice.

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