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Assessment of Heavy Metals (Ni and Cr) and Fungal Contamination in Commonly Consumed Kaolin in Some Areas of Niger Delta of Nigeria

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Abstract: This study involved the isolation of fungi species and the determination of heavy metals (Ni and Cr) content in kaolin samples obtained from some areas of the Niger Delta Region of Nigeria. A total of 10 different kaolin samples were used in the experiment. Potato Dextrose Agar medium was used in the isolation of fungi species and the heavy metals determined using the atomic absorption spectrophotometer. The pH of the samples was measured using a pH meter. The samples were generally acidic ranging from 3.4 - 5.0. Fungi species isolated from the samples included; *Aspergillus flavus*, Mucor, Yeast, *Rhizopus* sp and Dermatophyte. From the result of analysis of heavy metals, only Ni and Cr were present in statistically significant amounts. However, the mean value of Ni (0.14 mg/kg) was higher than the maximum permissible limit of 0.05 mg/kg b.wt, while the mean level of Cr (0.73 mg/kg) was lower than the maximum permissible limit of 2.3 mg/kg b.wt recommended in food by FAO.

Key words: Chromium · Fungi · Heavy metal · Kaolin · Nickel · Niger Delta

INTRODUCTION

Kaolin is an odorless white to yellowish or grayish powder and contains mainly the clay mineral, kaolinite [1]. They are taken from termite mounds known to be rich in minerals, sold in the markets and generally consumed at an average rate of 30 g daily [2]. One of the most common human populations to engage in the deliberate consumption of earth based substances are pregnant women. In sub Saharan Africa, the level of consumption of kaolin by pregnant women is Kenya (65%), Nigeria (60%) and Tanzania (28%) [2]. No explanation for the historic persistence of geophagia; the habit of deliberately eating earthy substances. It is thought that Kaolin helps in the reduction or even stopping of nausea and vomiting during pregnancy [3]. In Southern Nigeria, geophagia is a phenomenon that has become a common habit mostly among females, however the level of microbial and heavy metal contamination in most commonly consumed kaolin is not well known and this could pose risk to humans, given the dirty and unhygienic environment associated with the areas. Heavy metals are environmental contaminants with the potential of causing human health problems if present in excess in food and food products. The analysis of heavy metals and microbial contamination is important for routine monitoring and risk assessment as kaolin is extracted from the ground by digging dry and even moist river beds and hills, whose hygiene status is frequently far from ideal. Therefore, the aim of this investigation was to determine the level of heavy metal contamination and fungi species associated with commonly consumed kaolin in some areas of the Niger Delta of Nigeria.

MATERIALS AND METHODS

Source of Samples: Smoked or sun-dried samples of kaolin were purchased from different markets in Akwa Ibom and Rivers States (Ikot Ekpene market, Uyo market and the two markets in Port Harcourt). The samples were either sun dried or smoked with or without salt. Five samplings each was made from the markets and bulked together. Also raw kaolin samples was also obtained from kaolin deposit in Ikot Ekpene, Akwa Ibom State. About 1 kg each of the different bulked kaolin samples was ground into powder and used for the experiment. The total number of different kaolin samples used in the experiment was ten.

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Experimental Site: The isolation and identification of fungi species and determination of heavy metals were carried out at the Crop Protection and Soil Science Laboratory of the Department of Crop and Soil Science, University of Port Harcourt, Rivers State, Nigeria (6.55° 0.2' N latitude, 4.54° 10.02' E longitude).

Isolation and Identification of Fungi Species: Potato Dextrose Agar (PDA) modified with 0.5 g streptomycin was prepared and used as a culture medium. 1 g of the ground sample of kaolin was put in a test tube and 5 ml of water added and then shaken for 2 minutes and then filtered. The filtrate was poured on the solidified PDA medium and incubated at 25±2°C with alternating cycles of 12 hr light and 12 hr darkness for 7 days. Sub-cultures were made from emerging colonies repeatedly until pure cultures were obtained. The identification was achieved by placing a drop of lacto-phenol cotton blue stain on a clean slide with the aid of a mounting needle where a small portion of the mycelium from the fungi culture was removed and placed in a drop of lacto-phenol. The mycelium was spread very well with the aid of the needle. A cover slip was gently applied with little pressure to eliminate air bubbles. The slide was mounted and the fungal isolates were identified using a microscope with reference to Illustrated Genera of Imperfect Fungi [4].

Heavy Metal Determination: For heavy metal determination, the kaolin samples were oven dried for 24 hours at a temperature of 105°C and ground using a mortar and 1 g of the sample was weighed using a weighing scale and then poured into a 250 ml Kheldal flask. Using filter and pipette, 2 ml of distilled water was then added to the weighed sample, 3 ml of ionic nitric acid (HNO_3) and 2 ml of concentrated hydrochloric acid (HCl) were added and the mixture was gently whisked.

The mixture was then transferred to the fume cupboard where the heating mantle is located. Using the heating mantle, the mixture was heated at a temperature of 80°C for 30 minutes. The round bottom flask was then removed from the heating mantle and allowed to cool. About 30 ml of distilled water was then added to the extract and stirred, using a filter paper. The mixture was then filtered into a 50 ml volumetric flask and made up to the mark with distilled water. The extract was then transferred into a sample container and stored in a refrigerator, then taken to the atomic absorption spectrophotometer for analysis.

pH Determination: About 20g each of the kaolin samples were weighed into 100 ml beakers and then 20 ml of distilled water was added with 0.01 ml $CaCl_2$ and stirred intermittently for 15 minutes. The pH of the samples was measured using a pH meter.

RESULTS

Table 1 shows the different fungi species isolated from the different kaolin samples. Five different fungi species were isolated namely; *Aspergillus flavus*, Dermatophyte, Mucor, *Rhizopus* sp and yeast. From the results, smoked kaolin with salt was least contaminated with a single fungus species which was either Mucor or yeast. Smoked kaolin without salt had two fungi species isolated from the samples. Yeast was commonly found in the samples and with either of the samples also containing either *Aspergillus flavus* or *Rhizopus* sp. However, the level of fungi contamination in sun-dried kaolin samples was higher when compared with the smoked kaolin samples. Three different fungi species were isolated from these samples, respectively. The fungi species were *A. flavus*, Mucor, *Rhizopus* and yeast.

Table 1: Fungi species isolated from different kaolin samples across sample locations.

Sample	Place collected	Fungi species
Smoked kaolin with salt	Uyo	Yeast
Smoked kaolin with salt	Ikot Ekpene	Mucor
Smoked kaolin with salt	Port Harcourt	Mucor
Smoked kaolin without salt	Port Harcourt	Yeast, *Rhizopus* sp
Smoked kaolin without salt	Uyo	*Aspergillus flavus*, Yeast
Sundried kaolin without salt	Uyo	*Rhizopus* sp, *Aspergillus flavus*, Yeast
Sundried kaolin without salt	Ikot Ekpene	Mucor, Yeast, *Rhizopus* sp
Sundried kaolin without salt	Port Harcourt	Mucor, *Aspergillus flavus*, Yeast
Sundried kaolin without salt	Port Harcourt	*Aspergillus flavus*, Yeast, *Rhizopus* sp
Unprocessed kaolin	Ikot Ekpene	*Aspergillus flavus*, Yeast, *Rhizopus* sp, Mucor, Dermatophyte

Table 2: Heavy metals present in different kaolin samples across sample locations

Sample	Place collected	Heavy metal	
		Ni (mg/kg)	Cr (mg/kg)
Smoked kaolin with salt	Uyo	0.13	0.13
Smoked kaolin with salt	Ikot Ekpene	0.20	1.18
Smoked kaolin with salt	Port Harcourt	0.16	0.77
Smoked kaolin without salt	Port Harcourt	0.08	0.01
Smoked kaolin without salt	Uyo	0.05	0.85
Sundried kaolin without salt	Uyo	0.13	0.78
Sundried kaolin without salt	Ikot Ekpene	0.11	1.44
Sundried kaolin without salt	Port Harcourt	0.26	0.29
Sundried kaolin without salt	Port Harcourt	0.19	0.43
Unprocessed kaolin	Ikot Ekpene	0.70	1.47
LSD		0.06	0.007
P. value		<0.001***	<0.001***
Grand Mean		0.14	0.73

*** Very highly significant.

Table 3: pH value of the different kaolin samples across sample locations

Sample	Place collected	pH
Smoked kaolin with salt	Uyo	4.8
Smoked kaolin with salt	Ikot Ekpene	4.5
Smoked kaolin with salt	Port Harcourt	4.6
Smoked kaolin without salt	Port Harcourt	3.7
Smoked kaolin without salt	Uyo	4.2
Sundried kaolin without salt	Uyo	4.2
Sundried kaolin without salt	Ikot Ekpene	5.0
Sundried kaolin without salt	Port Harcourt	4.1
Sundried kaolin without salt	Port Harcourt	4.3
Unprocessed kaolin	Ikot Ekpene	3.4

Samples from unprocessed kaolin had the highest level of fungi contamination. In addition to Mucor, *Rhizopus* and yeast, Dermatophyte was also found in these samples.

Of all the heavy metals assessed, only Ni and Cr were present in significant amounts (Table 2). The highest level of Ni was found in unprocessed kaolin samples, followed by sundried samples. Analysis of variance (ANOVA) revealed that there was a significant difference ($P<0.001$) in the level of Ni between samples. However, the mean value of Ni (0.14 mg/kg) was higher than the maximum permissible limit of 0.05 mg/kg recommended for food by FAO. Although, the level of Chromium in the samples was significant ($P<0.001$) between samples, the mean level of 0.73 mg/kg was lower than the maximum permissible limit of 2.3 mg/kg recommended for food. From pH analysis result, the different kaolin samples appeared to be acidic ranging from 3.4 - 5.0 in unprocessed kaolin samples to sundried kaolin without salt samples, respectively (Table 3).

DISCUSSION

It is clear that proper salting during kaolin processing could reduce the microbial load of the samples. In addition to proper sun drying and smoking, proper hygiene and sanitation at processing sites will also reduce the microbial load on future samples, as unprocessed samples had higher fungi loads than the processed kaolin samples. It is possible that the salt played a major role in reducing microbial load, since salt has been used as preservatives in food products and also known to have antimicrobial properties [5]. Exposure of kaolin at the time of sales could have aided in the disposition of the fungal spores. *Rhizopus* spp, *Aspergillus flavus,* Mucor, Yeasts and Dermatophytes are fungal species commonly associated with other products in the open markets. These pathogens could be source of contaminants during sample processing and sales in the open market. The presence of *Aspergillus flavus* in the kaolin samples is a source of concern as this pathogen is known to produce aflatoxin which poses considerable risk to humans [6, 7]. The incidence of *Rhizopus* sp and Mucor has also been reported [8, 9, 10] on other food products. *Rhizopus* sp are commonly found in the soil while the spores of *Aspergillus flavus* are commonly found in the natural environment. They form colonies in a wide range of colors ranging from yellow to green and can cause lung infection such as asthma and cough when ingested. Dermatophyte*s on their own are* responsible for skin infections due to their ability to utilize keratin. Infection caused by Dermatophyte includes ringworm, chickenpox and eczema and these diseases are common in the region. Furthermore, heavy metal contamination is largely due to environmental pollution and this seems to be high in the Niger Delta Region of Nigeria. High concentration of heavy metals have been determined in the analysis of water and concentration of Ni and Cr exceeding the WHO standard [11, 12], although in this study Chromium was below the permissible limit recommended in food by FAO. Chromium is an essential element that helps the body use sugar, proteins, and fat. Human ingestion of high doses of Cr can result in human health problems including gastrointestinal bleeding and necrosis of the proximal and distal tubules in the kidney. The possibility and the danger of Ni getting into human food chain through eating contaminated clay should be avoided. Such an exposure could lead to adverse health consequences especially in children, which may result in long-term health problems. Nickel is also an important cofactor for various enzymes and acts to accelerate normal chemical reactions occurring in the body [13], but high level of ingestion may aggravate vesicular hand eczema and possibly eczema arising on other parts of the body, even in the absence of skin contact with nickel [14]. Nickel mostly found in the soil, water, air and in the biosphere can easily contaminate food [15, 16]. [17] observed that high levels of exposure to Cd and Ni are associated with irritation of the eyes and respiratory passages, damage to brain, liver, bones and kidney, bronchitis, dermatitis, emphysema, hypertension, rickets and asthma. The kaolin samples regardless of the processing method had levels of Ni above FAO/WHO [18] tolerable limits of 0.05 mg kg^{-1} b.wt.

CONCLUSIONS

This study has shown high amount of microbial contamination and Ni level in the kaolin samples and when consumed could adversely affect humans. It is also clear from the study that treatment with salt during processing could reduce the microbial contamination of the samples. The reason for the unacceptable levels of these substances and the possible sources indicate the need for an investigation into the surrounding environment and processing methods that give rise to such contamination. The presence of fungi species such as *Aspergillus flavus* in the kaolin samples shows serious health implication as they are highly toxic and carcinogenic. Also as Ni is not easily eliminated by the human system, there is need for the serum investigation of individuals in those areas where this phenomenon is a habit.

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