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Microbial and helminthic quality OF EDIBLE CLAY (BENTONITE) sold in Port Harcourt

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ABSTRACT

This work was aimed at evaluating the heavy metal, microbial and helminthic quality of edible clay (bentonite Samples were procured at different markets in Port Harcourt, bentonite contains some trace elements with concentrations such as zinc (0.152mg/kg), copper (<0.001-1.15mg/kg), lead (0.005mg/kg), cadmium (<0.005mg/kg) and nickel (0.02-12.7mg/kg). The THB counts ranged from 3.5 x 10^6 to 5.5 x 10^6, while Total Fungal culture ranged from 2.9 x 10^3 to 3.3 x 10^3. The bacterial species isolated include *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia*, *Serratiasp* and *Aeromonas sp*. While. Fungi cultures include: *Aspergillusniger Saccharomyces sp*, *Aspergillusoryzae*, *Fuscarium sp. Cladosporiumsp*, *Penicilliumsp* and *Aspergillussp* Edible clay has no antimicrobial effect against tested isolates n *S.aureus, E coli,K. puemonia, P. aeruginosa and C .albican* using well agar and disk diffusion method.. Edible clay was also observed for the helminthes, the eggs of *Ascarislumbricoides* and *Ancylostomaduodenale* found to be associated with the clay. Benthonite harbours pathogenic microorganisms and parasites. probably because of the source and handling or packaging.

Key words: Antimicrobial, bentonite, Heavy metals, Agar well diffusion

INTRODUCTION

Clay is a fine-grained natural rock or soil material that combines one or more minerals with traces of metal oxides and organic matter. Clays are plastic due to their water content and become hard, brittle and non–plastic upon drying or firing (Guggenheim, *et al.*, 1995). Geologic clay deposits are mostly composed of phyllosilicate minerals containing variable amounts of water trapped in the mineral structure. Depending on the content of the soil, clay can appear in various colors, from white to dull gray, ash or brown to a deep orange-red. Clay is a widely distributed, abundant mineral resource of major industrial importance for an enormous variety of uses (Ampian, 1995).

In both value and amount of annual production, it is one of the leading minerals worldwide. In common with many geological terms, the term "clay" is ambiguous and has multiple meanings: a group of fine grained minerals — i.e., the clay minerals; a particle size (smaller than silt); and a type of rock — i.e., a sedimentary deposit of fine-grained material usually composed largely of clay minerals (Patterson & Murray, 1993; Bates & Jackson, 1997). In the latter definition, clay also includes fine-grained deposits of non-aluminosilicates such as shale and some argillaceous soils.

The role of clays in human health has experienced a revival in interest due to advances in modern instrumentation [e.g., transmission electron microscopes (TEM), field emission scanning electron microscopes

(FESEM), atomic force microscopy (AFM), and secondary ion mass spectrometers (SIMS)], that allow us to study surfaces of nano-scale minerals *in situ* within their natural environmental matrix. By identifying the special characteristics that make a particular clay antibacterial, we may elucidate some of the reasons these common nano-minerals not only have potential applications in medicine, but may also contribute to the general understanding of antibacterial mechanisms lending insights to potential cures.

The purpose of this study was to examine the microflora of clays eaten in Port Harcourt, Nigeria. The objectives were: (1) to determine the heavy metal content in edible clay; (2) to detect the presence of pathogens in edible clay; (3) to detect the presence of parasitic helminthes and protozoans; (4) to test for antimicrobial; susceptibility of edible clay;

MATERIAL AND METHOD

Samples Collection

Samples of dry clay lumps (sold in markets) were purchased from fivemarkets: Mile 3, Mile 2, Aluu, Choba and Rumuokoro market in Port Harcourt. A total number of 40 pieces (lumps) were purchased from different retailers at each market and transferred into sterile stomacher bags. The bags were carefully sealed and then transported to the laboratory for analysis.

Physicochemical parameters Heavy Metal Analysis

The samples were analysed for Nickel, Chromium, Lead, Zinc and Cadmiu The bentonite sample was washed in a muffle furnance at a temperature of 630°C for 3hrs The ash sample was dissolved in l0ml concentrated Hydrochloric Acid and was heated on an electro-thermal heater hotplate The solution of the ash was diluted to 50ml with distilled water the sample was analysed for metal ion by atomic Absorption Spectrophotometer

Lead Ion

Lead ion was analysed by an Atomic Absorption Spectrometer at 383nm wave 1ength. The wave length was selected with a narrow slit with, air and acetylene gas flow was adjusted other Hallow Cathode lamp was given adequate time to stabilize before aspirating standards for equipment Calibration After calibrating the equipment with standard lead concentrations, the aspiration tubing and system were flushed with distilled water severally before aspirating the test sample solution on the sample experimental condition used for the standard The concentration of lead in the sample displays on the AAS machine and was recorded.

Zinc Ion

A 2138 nm wavelength was selected, air gas pressure flows was adjusted Slit width and other setting as was recommended were adjusted Hallow cathode lamp was allowed to stabilize. Standard Zinc concentration was aspirated to calibrate the equipment and to obtain a standard graph of the ion tested Aspiration system was flushed with dc-ionized water occasionally before further use. The sample solution was aspirated and the concentration of Zinc ion in the sample was extrapolated and recorded.

Copper Ion

5248nm wavelength selected air and gas pressure flow was adjusted slit width and other settings as recommended for the instrument was adjusted Hollow cathode lamp was energized and adequately allowed to stabilize The instrument was calibrated with standard copper ion concentrations. The sample solution was aspirated into the equipment and the concentration in the same was extrapolated from the standard copper graph.

Cadmium Ion

229nm wavelength was selected; air and acetylene gas was adjusted Other m,settings as recommended for the instrument, Hollow cathode lamp gas allowed adequate time to stabilize Sample standard and was aspirated respectively and the result were extrapolated from the standard graph.

Nickel

Three quantitative methods for the determination of nickel are: The first is an absorptiometry method using dimethyiglyoxime, the second is an atomic absorption method, whilst the third uses a polarography.

Enumeration of Microorganisms

Samples from each market was aseptically homogenized by crushing, 10g was aseptically transferred into new stomacher bags and blended with 90 ml of sterile diluent (maximum recovery diluent), for about a minute. Aliquots of the homogenates was serially diluted and plated for the determination of bacterial, total plate count and the detection of coliforms and fungi, using standard procedures.

Enumeration of microbes associated with ready to eat clay samples was carried out using pour plate method. Ten-fold serial dilution of each baked clay was done using 0.85% normal saline as diluents and 0.1ml aliquot of dilution was inoculated on duplicate plates of sterile plate count agar, Salmonella Agar, TCBS for Vibrio,MacCconkey agar and Potato Dextrose Agar. The plates were incubated at 37°C for a period of 24hours for bacteria and 96hours to l20hours *(*5 *days* for fungi).

Each colony was counted and purified by sub culturing in a plate using streaking method. The isolates were identified and characterized morphologically and by biochemical appearances and reactions.

Procedures For Microbiological Analysis

a. Total Heterotrophic Bacteria (THB)

Ten gram (10g) of clay sample was weighed into 90ml sterile diluents (0.85% NaCl) under aseptic condition. It was then shaken vigorously to homogenize and serially diluted. Then 0.1ml aliquot of the inoculums was collected using a sterile pipette, inoculated on Nutrient Agar (NA) surface. The inoculum was spread evenly with a sterile hockey stick. Plates were incubated at 37^0C for 24 hours. Thereafter, colonies are counted to obtain colony forming unit (cfu) value per gram of the clay sample. Distinct colonies with different morphological patterns were subcultured on freshly prepared nutrient agar medium to obtain pure culture after 24 hours incubation at 37^0C. The pure culture was gram stained for microscopic examination. It was also used to carry out biochemical tests for characterization and identification of the isolates.

b. Total Heterotrophic Fungi (THF)

Ten grams (10g) of clay sample was weighed into 90ml sterile diluents (0.85% NaCl) under aseptic condition. It was then shaken vigorously and serially diluted, 0.1ml aliquot was inoculated on Potato Dextrose Agar (PDA) acidified with 0.1% lactic acid to inhibit growth of bacteria and allow for only the growth of fungi. Inoculated plates were incubated at ambient temperature for 5-7 days. Cultural characteristics of isolates were observed and subcultured for purification. Microscopic examination was done using lactophenol cotton blue stain with x40 magnification.

Salmonella Shigella Agar

Salmonella Shigella Agar was prepared according to manufacturer's directions, poured into sterile petri dishes and allowed to set. Serial dilution was done by weigh 10 grams of sample in to 90ml normal saline. Samples were diluted to 10^{-3} and 10^{-4}dilutions respectively. Thereafter, 0.1ml aliquot of insculum was inoculated on agar surface and spread evenly with a sterile glass rod. The plates were incubated at 37^0c for 24 hrs. A black coloration indicates the presence of Salmonella while a red coloration indicates the presence of Shigella

TCBS/ MCA (Thiosulphate Citrate Bile Salt/ MacConkey Agar)

Media were prepared according to manufactures specification. Serial dilution was done by weighing 10g and clay samples and dissolving in 90ml dilution (normal solution). The samples were diluted respectively. Thereafter, 0.1ml aliquot of inoculums was inoculated on ager surface and spread evenly

using a glass rod and incubated at 37^0c for 24 hrs.

Biochemical Tests

a. Citrate uti1zation test

This test used in the identification of enterobacteria. It is based on the ability of an organism to utilize citrate as its only source of carbon, and ammoniurm as its only source of nitrogen. The medium used were DNAse Agar and 1N HCL. The medium was inoculated by spot inoculation or streak across the plate. Inoculate at 37^oC overnight. The plate was flooded with 1N HCL which precipitated the DNA. The excess acid was tipped off. A clear zone around the colony indicated that *K. pneumonia* was present.

b. Oxidase test

This test was employed in the identification of Pseudomonas, Neisseria, Vibrio or other groups. The enzyme oxidase will oxidize a redox dye such as tetremethylene diamine dihydro chloride (TMPPDH) to deep purple colour. This enzyme is produced by some aerobic bacteria as part of their respiratory oxidation mechanism. A few drops of the oxidase reagent were added to a few colonies on the culture plate. The colonies were observed to change from blue to deep purple within 5-10 sec which indicates the presence of *Pseudomonas aeruginosa.*

c. Coagulase test

This test is used to differentiate *Staphylococcus aureus* from other 13 inoculum. The enzyme coagulase causes plasma to clot by converting fibrinogen to fibrin. Coagulase is produced by S. *aureus.* There are two types of coagulase; free coagulase and bound coagulase. Human or rabbit plasma, from EDTA (ethylene diamine-tetra acetic acid) was used. Two separate drops of saline were placed on a slide. One or two colonies of the organisms were emulsified in each of the drops to make a thick suspension. The tip of a straight wire was dipped into the undiluted plasma and the adhering traces of plasma were mixed into one of the bacterial suspensions. Immediate coarse clumping was checked from the mixture (within 5-10 sec).

Agar in Well Diffusion Method

The agar well diffusion method as described by Lino Deogracious (2000) was used. Standardized inoculums (0.5 Mcfarland standard turbidity standard equivalent to 5 x 10^8cfu/ml/NCCLS, 1999) of each test bacterium was spread onto sterile Muller Hinton Agar plates so as to achieve even growth. The plates were allowed to dry. Wells of 6mm in diameter were made on the plates with the aid of a cork borer. The samples were prepared by double dilution method this is done by adding 1 ml of the stock solution to the test-tube containing 9 ml of distilled water to achieve a concentration of 200 mg/ml. Subsequently, 0.1 ml of each sample was introduced in well earlier bored in the agar plate cultures. Ciprofloxacin and fluconazole were used as control for bacteria and fungi respectively. The plates were incubated at 37^0C for 24 hours, and resulting diameters of inhibition zone were measured with a transparent rule.

Disc Diffusion Method

This method of antibiotics susceptibility testing is the most practical method for determining antibiotic susceptibility/resistance of microorganism to different antimicrobial agent. The disc diffusion method was carried out using paper disc measuring about 6mm in diameter, which was made using a perforator to perforate the whatmann No:1 filter paper. The paper disc was wrapped in a foil and sterilized at 121^0C for 15 minutes to prevent contamination. The concentration of the edible clay was 100mg/ml. using a sterile pipette, 1ml of the edible clay was transferred into a sterile plate containing the sterilized perforated disc and allowed to diffuse and 24 hrs old culture was transferred in a fresh prepared nutrient agar and was incubated for 6-8 hrs. The 13 inoculum was adjusted to 0.5 McFarlands units. After the adjustment a sterile swab was dipped into the test tube containing the 13 inoculum. The swab was pressed against the wall of the test tube to allow the excess to drop.

The swab was spread on the freshly prepared Muller Hinton Agar (MHA) and allow for some minutes. The plates were divided into partitions after which a sterile forceps was used to pick the perforated whatmann filter paper containing 0.05mg/ml of the clay and impregnated one part of the plate and the antibiotic disc to the other part. The procedure was repeated in duplicate for each organism, incubated for 18 hrs at 37^0C after which the zones of inhibition were measured using a transparent ruler.

RESULTS

Table 1 Results for heavy metals

Paramater	Method	Choba	Aluu	Mile 1	Mile 3	Rumokoro
Copper (ppm)	APHA 3111B	<0.001	0.04	<0.001	0.58	1.15
Lead(ppm)	APHA 3111B	<0.001	0..02	<0.001	0.001	<0.001
Nickel(ppm)	APHA 3111B	0.28	4.84	0.02	6.29	12.7
Cadmium(ppm)	APHA 3111B	<0.001	<0.001	<0.001	<0.001	<0.001
Zinc(ppm)	APHA 3111B	2.05	1.08	1.001	0.02	3.001

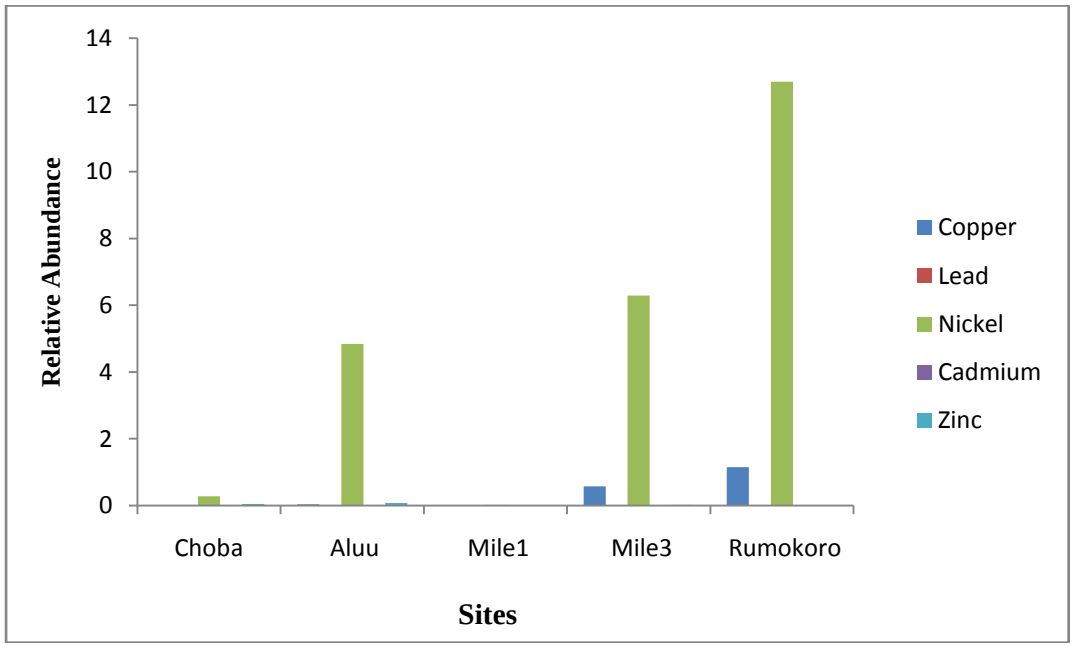

Fig. 3.1 Chat for heavy metal content in edible clay from the different sites.

Table 3.2: Result of Total Heterotrophic Bacteria and Total Fungi count

Location	THB(10^6cfu/ml)	SSA(10^1)	MCA(10^1)	TCBS(10^1)	TF(10^3sfu/ml)
Mile I	3.5	Nil	Nil	Nil	3.3
Mile III	3.5	Nil	Nil	Nil	3.3
Rumuokoro	5.5	Nil	Nil	Nil	3.1
Aluu	3.6	Nil	Nil	Nil	3.1
Choba	4.1	Nil	Nil	Nil	2.9

Table 3.3: Incidence of Bacteria isolated from clay sample

Sample	*Staphylococcus sp*	*E. coli*	*Klebsiellasp*	*Serratiasp*	*Bacillus sp*	*Aeromonassp*
Mile 1	-	-	+	-	+	-
Choba	+	+	-	-	+	-
Rumokoro	+	-	+	-	+	-
Aluu	+	+	+	+	-	+
Mile 3	-	-	+	-	+	-

Table Incidence of Fungi isolated from clay sample

Fungi	Mile1	Mile3	Rumokoro	Choba	Aluu
Aspergillusniger	+	-	-	-	-
Aspergillusoryzae	+	-	+	+	+
Cladosporiumsp	+	-	-	-	-
Fusariumsp	+	-	+	+	+
Saccharomyces Cerevisiae	-	+	-	-	-
Penicilliumsp	-	+	-	-	+

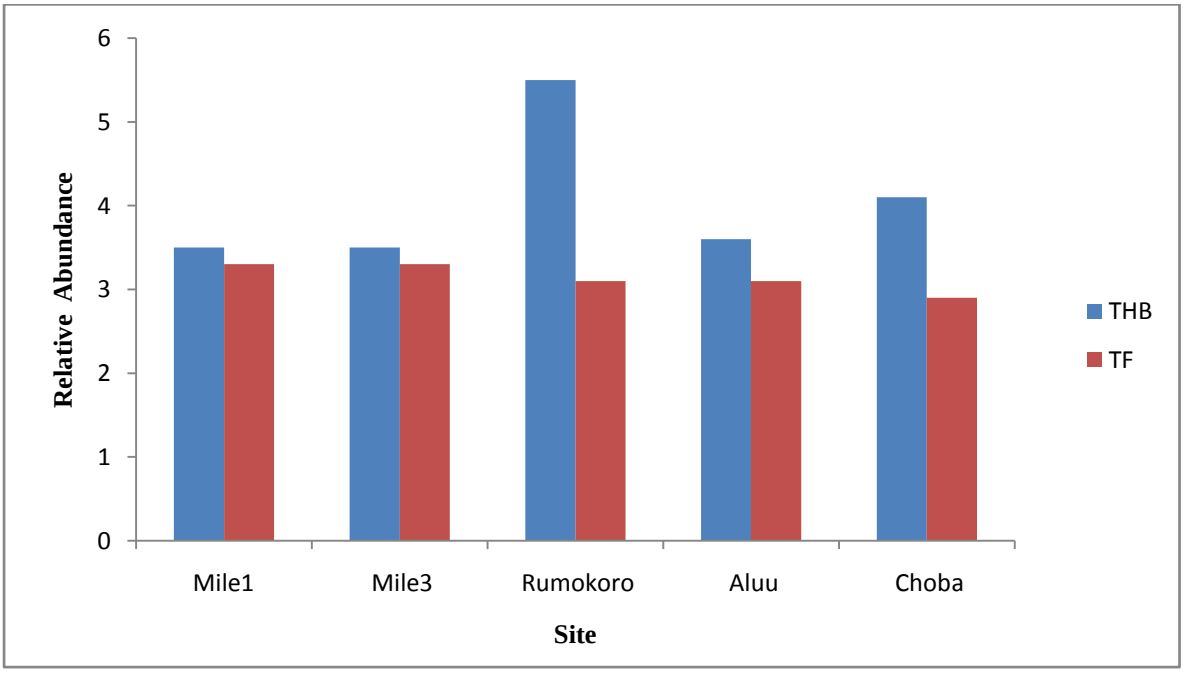

Fig 3.2 Chat for total heterotrophic bacteria and total fungi

Table 3.5 Frequency of eggs of worms

Sample	No examined	No infected	Ascaris	Hookworm
Mile1	5	0	0	0
Mile3	5	0	0	0
Rumokoro	5	1	1	0
Aluu	5	1	1	2
Choba	5	0	0	0

Table 3.6 Relative abundance of microorganism found in edible clay

Organism	Percentage (%) Abundance	Organism	Percentage (%) Abundance
Bacillus	26.67	*Aspergillusniger*	7.69
Klebsiella	26.67	*Aspergillusoryzae*	7.69
Staphylococcus	20	*Cladosporium*	7.69
E.coli	13.33	*Fusarium*	30.77
Serratia	6.67	*Saccharomyces*	7.69
Aeromonas	6.67	*Penillium*	15.38
		Aspergillussp	23.10

Table 3.7: Bacteria isolate of cm,lay sample using well in agar

Organism	Mile 3	Aluu	Mile 2	Choba	Rumuokoro	Control
S.aureus	0	0	0	14	0	35
C.albican	0	0	0	0	0	32
P.aerugirosa	0	9	12	0	0	40
E.coli	16	0	0	0	0	35
K.pneumonia	0	0	10	0	0	35

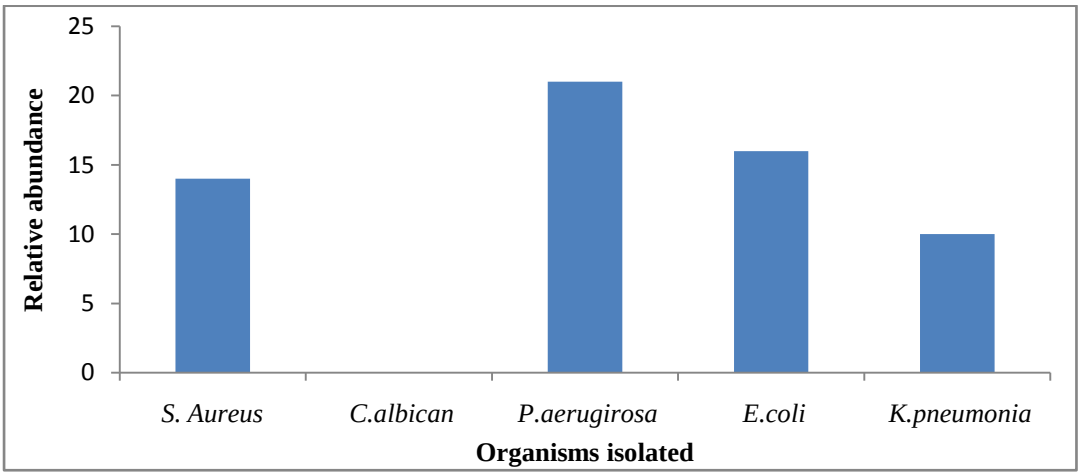

Fig. 3.3: **Diameter of zones of clearing obtained when the clinical isolate were exposed and edible clay samples using Agar well diffusion method.**

Table 3.8 showing fungi isolate of clay sample using disc diffusion

Organism	Mile 3 (mm)		Aluu (mm)		Mile 2 (mm)		Choba (mm)		Rumokoro (mm)		Control (mm)
S.aureus	Neat	(10^{-4})	Neat	(10^{-4})	Neat	(10^{-4})	Neat	(10^{-4})	Neat	(10^{-4})	
	0	0	0	0	0	0	8	4	0	0	32
C.albicans	0	0	0	0	0	0	0	0	0	0	35
P.aeruginosa	0	0	7	2	9	3	0	0	0	0	40
E.coli	9	5	0	0	0	0	0	0	0	0	35
Kpneumonia	0	0	8	2	0	0	0	0	0	0	30

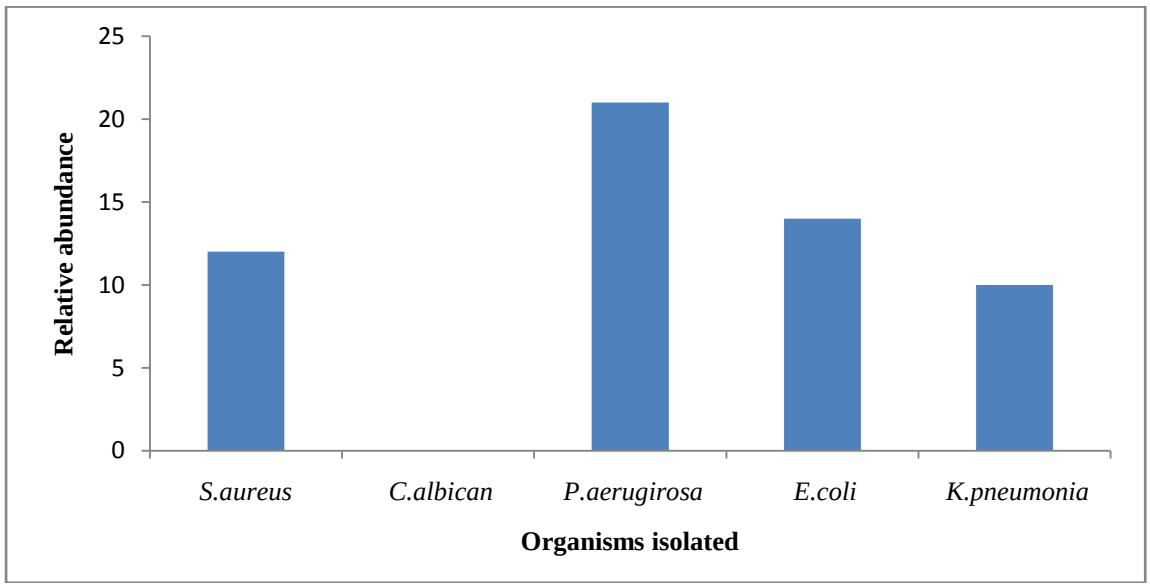

Fig. 3.4: Fungi isolate of clay sample using disc diffusion

Table 3.9: Antihelminth and susceptibility of clay samples (*Ascarislumbricoid*/*Trichuristrichiura*)

Clay samples	Count (0 hours)	(24 hours count)
Mile 3 market	200 eggs/ml	0 eggs/ml
Aluu market	200 eggs/ml	100 eggs/ml
Mile 2 market	200 eggs/ml	0 eggs/ml
CHoba market	200 eggs/ml	0 eggs/ml
Rumuokoro market	200 eggs/ml	0 eggs/ml

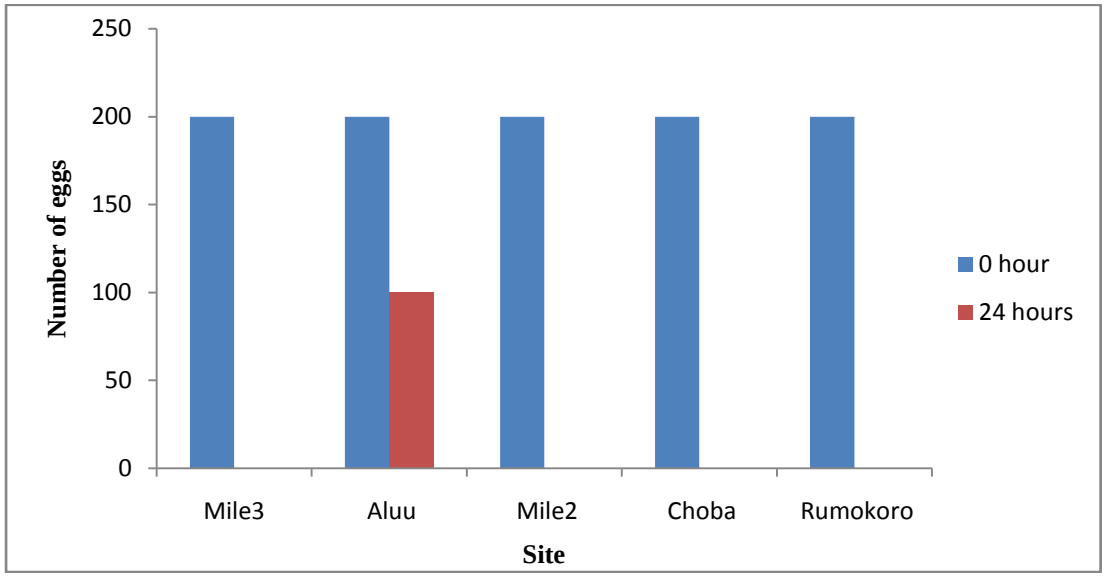

Fig 3.5: Antihelminth and susceptibility of clay samples(*Ascarislumbricoid*/*Trichuristrichiura*)

4. DISCUSSION

The result of heavy metal analysis showed the presence of some heavy metals in edible clay samples bought from Aluu, Choba, and Rumuokoro markets Nickel, Zinc, Copper, Lead and Cadmium were found in bentonite samples used. The result indicates that Nickel had the highest concentration which is

24.13mg/kg while cadmium had the lowest which is < 0.005mg/kg.Heavy metals, if present even in very low concentrations in foods, have the capabilityto cause human health problems. Information about the dietary intake of such metalsis important to assess risks to consumers. Dietary intake could account for some fractions of the toxic metals found in the blood and urine of many Nigerians

The idea of microbial analysis is to ensure a check on edible clay, considering the numbers of microorganisms isolated which showed a remarkable result. The result on examination of edible clay showed that the clay sample contains microorganisms. The bacterial species found include *Staphylococcus aureus* 20 %, *Bacillus subtilis* 26.67 %, *Escherichia coli* 13.33 %, *Klebsiella pneumonia* 26.67 %*Serratiasp* 6.67 % and *Aeromonassp* 6.67 %.

Fungi species found include: *Aspergillusniger*7.69 %, *Saccharomyces sp* 7.69 %, *Aspergillusoxyz* 7.69 %, *Fuscarium sp.* 30.77 %, *Cladosporiumsp* 7.69 %, *Penicilliumsp* 15.38 % and *Aspergillussp*23.10 %. Antimicrobial activity of edible clay were used against clinical isolates these includes *S.aureus, E coli,K. puemonia, P. aeruginosa and C .albican* using well agar method and disk diffusion method

Helminths isolated were *Ascarislumbricoides* and *tricuristricursa*. The antihelmith properties of the sample from the result showed that the edible clay sample has an effect on these helmiths listed above except for the clay sample from Aluu market which was not effective against these helmiths Ascarislumbricoides and tricuristricuria, possibly because of the soil type where the sample was collected and how it was collected and also as a result of handling.

This observation showed that those who eat clay are not prone to worms infestation, but it could be dangerous when handled without safety measures or poor handling and exposure of beakers in the environment.

CONCLUSION

Considering the number of organisms isolated, the frequency of its occurrence and population of these organisms as a result of the level of growth population sample and the quality of organisms isolated due to the pathogen and the antiheimith properties of the sample (edible clay), consumption of edible clay should be discouraged. Also further research should be done to ascertain if eating clay is healthy or not.

RECOMMENDATION

In view of this research on antimicrobial effect of edible clay (bentonite) it is recommended that edible clay is checked before use.

- Edible clay has to be used when confirmed health safe
- If it must be used by the traditional users the necessity to heat and proper processing and storage is advisable to avoid contamination with microorganisms

Pelotherapy is the use of natural clay from the earth for therapeutic purposes, most are familiar pelotherapy in the form of a facial mud mask, when activated with moisture clay has a unique drawing capacity which provides its detoxifying benefits such as skin balancing and should be done with caution**.**

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