

Co-effect of Leached Metals and pH of Simulated Gastric Fluid on the Survival of Microorganisms in Geophagic Clays

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Abstract: *In this study, the bacterial activity was investigated following exposure of geophagic clays to simulated gastric fluid. The inhibitory effects of either acidity or metals released were considered. The geophagic clay samples were characterized using XRD and XRF techniques; the bacteria were identified using 16S rDNA sequencing and their survival in simulated gastric overtime was monitored using plate count. The results showed that heavy metals were released in the gastric fluid overtime while the pH remained acid over the same period of time. The mobility of the metals from the geophagic clays decreased in the order Pb>Ni>Co>Fe>Cr>Cu>Zn. The count of cell number under such conditions showed that both acidity and metal concentrations had contributed to the decrease of cell number in the simulated gastric fluid. Total metal concentration increase from 25 to 35 mg/L contributed to a 50% decrease of bacterial number after 150 min; a decrease of bacteria count overtime was observed in samples containing mostly Gram-negative bacteria; while no significant change was observed in samples dominated by Gram-positive bacteria. The results have shown the decrease of bacterial activity and increase of metals released at low pH. The acidity inhibits the growth of bacteria and also increases metal release which further enhances bacteria inhibition.*

Keywords: Geophagic clay, heavy metals, micro-organisms, simulated gastric fluid, health risk

1. Introduction

The consumption of non-food materials is generally called pica and there are different forms of pica, examples of pica as described in [1] include geophagia (dirt or clay eating) and pagophagia (ice eating). Geophagia is the most encountered form of pica, which is defined as the deliberate consumption of soil and related materials [2]. It is a very extensive practice in all continents of the world, and has been reported in Africa, Asia, South America, North America [3, 4]. Geophagia has been reported commonly in pregnant women and children [5]. Soil ingestion can be anything, however, eating dropped food, contacting dirty hands and mouthing cannot be considered as geophagia since they are accidental. Study has reported the prevalence of pica among urban and rural South Africa women to be 38.3% and 44% respectively compared with 2.2% and 1/6% among Indians and white women respectively [6]. Geophagic individuals believe that geophagia provides health benefits as supplementation of mineral and nutrients, ailment of diarrhea, reduce nausea and reduce pregnant sickness [7; 8; 9; 10]. The consumption of soil and clay may cause mineral deficiency [5, 11] because geophagic materials are capable to adsorb elements such as iron [12]. Geophagia is associated with dirt because of the natural source of the clay [13]. Geophagic clays could be contaminated with microorganisms, metals and other contaminants from collection sites or resulting from exposure to the polluted atmosphere at the markets. Studies have shown that the geophagic clays contained harmful materials such as heavy metals, pathogenic bacteria, viruses and parasites [7, 13]. The regular ingestion of highly

contaminated soil could result in brain and renal system impairment in the unborn baby due to lead poisoning [14]; apart from lead poisoning other toxicities have been observed in children eating contaminated soils [15]. Several studies have linked geohelminths infection with soil consumption, for the eggs of parasitic worm (geohelminths) consumed with soil [2; 16] and moreover, geohelminths infection has also been linked to iron deficiency among HIV-infected women who consumed soil [17]. Studies have shown that geophagic pregnant women from Kenya are at risk for geohelminth infection and especially *A. lumbricoide* re-infection [18, 19]. Bacteria tend to thrive in the environment where they can either get nutrients or be away from the predators [13] and can survive under harsh conditions [20]. For the bacteria to survive in any environment they need nutrients, energy and organic carbon that will be converted to new cells [13]. Many microbes can enter the stomach, but some of them do not survive the low pH acidity of the stomach in human. In the study of [21], micro-organisms *Klebsiella*, *Salmonella*, *Shigella flexneri*, *Proteus*, *Enterobacter*, *Enterococcus faecalis*, *Enterococcus faecium*, *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Candida albicans*, did not survive pretreatment at pH 1.0 or 2.0, whereas at pH 4.0, all bacteria tested survived. *Helicobacter pylori*, and *Clostridium botulinum* are microbes that can survive the acidic environment of the stomach of the host [21]. Microorganisms in ingested clays are exposed to adverse conditions in the stomach such as acidity and toxic metals leached from the clays; When heavy metals are present in excessive concentration they can cause toxic effect in the microorganisms [22]. When the soil ingested goes through the mouth being chewed or smoothed

and then to the gastrointestinal tract there are so many possible reactions that happen. It is however uncertain what could be the combined effect of acidity and metal toxicity on the load of microorganisms in geophagic clays ingested. In this study, there is an attempt to predict the survival of microorganisms following concurrent exposure to acidic gastric fluid and leached metals.

2. Methodology

Materials

Samples were purchased from markets in Pretoria and Potchefstroom and mined from the respective sources. The collected clay samples were packed into sterile plastic bags. The bags were sealed and labelled and then transported to the laboratory for analysis. Samples were kept in the refrigerator at 4°C until analyzed.

DNA sequence

Samples from the market and mining sites were ground and homogenized into fine powders using mortar and pestle. Genomic DNA was extracted from ± 250 mg of each of the six clay samples using the ZR Soil Microbe DNA Mini Prep kit™ according to the manufacturer's instruction. DNA concentrations were determined spectrophotometrically with a Nano-Drop spectrophotometer (Thermo Scientific). Then extracted DNA samples were sent to a commercial biotechnical company for sequencing using Next Generation Sequencing (NGS).

Leaching of metals using simulated gastric fluid

The simulated gastric fluid was prepared by dissolving 60.06 g glycine in 1.9 L of deionized water and adjusting the pH to 1.54 by adding concentrated HCl. The solution was then brought to a volume of 2 L and incubated at 37°C for 30, 60, 90, 120 and 150 mins. Hundred milliliter (100 mL) of the simulated gastric fluid was added into a bottle containing 1 g of clay sample, the mixture was placed in an incubator with shaker set at a speed of 120 rpm at 37°C. Subsequently, 5 mL samples were collected at 30, 60, 90, 120 and 150 mins intervals using a 45 μ m nitrocellulose syringe filter. The pH of the mixture was simultaneously determined at each sampling period. The filtered solution was diluted by adding 15 mL of deionized water prior to analysis. The metal concentrations in the leachates were then determined using an inductively coupled plasma optical emission spectrometer (ICP-OES).

Characterization of clay

Mineralogical studies of the geophagic clays were carried out using an X-ray diffractometer (XRD). The samples were ground and homogenized to a fine powder and small amounts of powdered samples were loaded on sample holders and mounted in the Philips PW 3710 XRD X-ray diffractometer system for identification of the mineral

phases. The XRD equipment, which operated at 40 kV and 45 mA, was equipped with a Cu-K α radiation tube and a graphite monochromator. Samples were scanned at a speed of 1°2 θ /min; at a covering range of 2°2 θ to 70°2 θ . A PW 1877 automated powder diffraction (APD). X'PERT Data Collector software package was employed to capture raw data and a Philips X'PERT Graphics and Identity software package was used for qualitative identification and semi-quantitative analyses of the minerals. Concentrations of major and trace metals was determined using an X-ray fluorescent spectrophotometer.

Microbial Analysis

To determine the subsistence of microorganisms in the simulated gastric fluid, 1 mL of solution was collected from the mixture of clay samples and simulated gastric fluid after 30, 60, 90, 120 and 150 mins then aseptically transferred into a test tube containing 9 mL of sterile distilled water and homogenized using a vortex. Serial dilution was done up to 10⁻⁵ dilution. Hundred μ L of the diluted samples as well as the undiluted samples was inoculated to the freshly prepared Brilliance *E. coli*/coliform medium (Oxoid, SA) and incubated at 35°C for 48 h; the distinctive colonies identified by color or shape were counted and expressed as CFU/mL. Individual colonies were sub-cultured in the fresh Brilliance *E. coli*/coliform medium (Oxoid, SA) and incubated at 35°C for another 48 h and then finally sub-cultured in the nutrient agar plate ("Lab-Lemco" powder 1.0; yeast extract 2.0; peptone 5.0; sodium chloride 5.0; agar 15.0; pH 7.4 \pm 0.2 at 25°C; gram per one liter; Merck Chemicals, SA) under the above conditions. The isolated cultures were sent to a commercial biotechnical laboratory for the identification of microorganisms through the sequencing of the 16S rDNA.

3. Results and Discussion

Chemical composition

Results obtained for the major oxides (Table 1) show the average SiO₂ values ranged from 15.56% - 55.62% with the highest value recorded from samples collected at Ikageng source (sample A) while the samples from Pheramindi source (sample E) exhibited the lowest value. The concentrations of Al₂O₃ ranged from 5.17% - 33.15% in the clay samples. Oxides of Si, Al, and Fe made up the greatest percentage of major element oxides in all the geophagic clay samples (Table 1). Results presented in Table 1 indicate that the concentration of Al₂O₃ was lower in sample E than other samples. The concentrations of oxides of Ca, Mg, and Na, were relatively low in all the geophagic clay samples and were less than 0.6%. Low abundance of MgO and K₂O shows a lack of expandable clays [7]. A high ratio of SiO₂/Al₂O₃ is related to the quartz content of the sample.

Table 1: Results of major oxides (mass %) analysis of samples

Oxide concentration (wt %)	Samples					
	Ikageng source	Ikageng market	Phelandavha source	Phelandavha Market	Pheramindi source	Pheramindi market
SiO ₂	55.62	55.37	51.79	47.83	15.57	50.72
TiO ₂	1.04	0.96	0.89	0.92	0.076	5.76
Al ₂ O ₃	26.07	25.55	28.32	29.56	5.18	33.15
Fe ₂ O ₃	12.6	13.45	15.23	18.55	58.35	9.03
MnO	0.008	0.01	0.1	0.03	0.012	0.04
MgO	0.41	0.39	0.45	0.21	n.d	0.21
CaO	0.12	0.11	0.02	0.01	0.17	0.09
Na ₂ O	0.53	0.52	0.19	0.2	0.5	0.06
K ₂ O	3.13	3.02	2.5	1.98	0.74	0.46
P ₂ O ₅	0.11	0.13	0.24	0.31	0.08	0.11
Cr ₂ O ₃	0.03	0.03	0.04	0.03	0.25	0.08
Co ₂ O ₃	0	0	0.01	0.013	0.044	0
NiO	0.009	0.009	0.009	0.011	5.5	0.01
CuO	0.006	0.008	0.013	0.009	0.011	0.01
ZnO	0	0	0.008	0.008	0	0.007
PbO	0	0	0	0	1.12	0.01

Mineralogical studies of clays

X-ray diffraction patterns were obtained for representative geophagic clay samples (Table 2). The most abundant of the non-clay minerals was quartz (Ikageng samples and Phelandavha samples) and corresponded closely with SiO₂ values from major trace elements and oxides results. The sample from Pheramindi source contained the lowest amounts of quartz. The results show that kaolinite is a dominant clay mineral in the geophagic clay samples (except the samples from Ikageng). Kaolin minerals are used as medicines to treat diarrhea [2]. Others minerals in low abundance present in the samples were muscovite, gypsum, anatase and illite. The presence bentonite minerals in the sample from Ikageng market and not in the sample from the source might be due to contamination.

Table 2: XRD results on geophagic clays

Sample ID	Minerals			
	Quartz	Kaolinite	Muscovite	Bentonite
Ikageng source	X		X	
Ikageng market	X		X	X
Phelandavha source	X	X		
Phelandavha market	X	X		
Pheramindi source	X	X		
Pheramindi market	X	X		

pH of extraction fluid over specified time

The mobility of metals can be affected by certain factors such as pH, soil physical properties, mineralogical and chemical composition and fluid composition [12]. However, pH is the most essential parameter affecting the mobility of heavy metal in the soil. Geophagic clays have chemical properties which include pH, cation exchange and ion exchange which affect the interaction in the gastrointestinal tract [23]. Constant shaking was used in the present experiments to simulate the movement within the stomach. After two and a half hours, there was no difference in the pH of the mixture compared with the initial pH of the extractive fluid (Table 3). The noticeable change of pH observed in the leachate was from pH 1.32 to 1.67, which could be ascribed to the electrostatic neutralization of protons in solution by the negatively charged groups in the clays [24]. When there

is a decrease in soil pH, metal solubility and availability increases [12]. The variation of the pH of the extracted fluid overtime was inconsistent and negligible (Table 3). The change of pH could be also explained by the mineralogical composition of clay.

Table 3: pH variation during exposure of geophagic clays to simulated gastric fluid

Samples ID	Time (minutes)					
	Initial pH	30 min	60 min	90 min	120 min	150 min
M1A	1.39	1.66	1.67	1.55	1.55	1.56
M2B	1.39	1.53	1.65	1.59	1.56	1.57
S1A	1.46	1.5	1.58	1.51	1.5	1.48
S2B	1.48	1.49	1.56	1.48	1.49	1.56
I1A	1.35	1.39	1.36	1.4	1.34	1.35
I2B	1.37	1.38	1.36	1.38	1.39	1.4
S11A	1.41	1.44	1.38	1.37	1.34	1.43
S12B	1.4	1.41	1.42	1.41	1.42	1.42
P1A	1.44	1.44	1.45	1.44	1.4	1.39
P2B	1.45	1.4	1.44	1.47	1.43	1.44
SP1A	1.36	1.4	1.41	1.39	1.41	1.4
SP2B	1.34	1.34	1.42	1.42	1.43	1.48

Results are expressed as mean ±SE (n=2). I1A=Ikageng market; S11A= Ikageng source; M1A=Phelandavha market; S1A= Phelandavha source P1A=Pheramindi market; SP1A=Phelandavha source

Dissolution of geophagic clays in simulated gastric fluid and subsequent behavior of metals

Previous studies [25, 26] showed that metal bio-accessibility under low pH (acidic conditions) is consistent with an increased metal mobility in soils at low pH. Metals such as Pb, Ni, Co and Fe showed a high bio-accessibility with an increased mobility. The mobility of the metals was at a different pace during the leaching period and decreased in the order Pb>Ni> Co>Fe>Cr>Cu>Zn Zn (Fig 1a, 1b,1c, 1d, 2a, 2b,2c, 2d, 3a, 3b, 3c and 3b).

At the solid-liquid 2:100 higher concentration of metals compare to the solid-liquid 1:100 where there was the low amount of metals leached. For example, Fe concentration

ranged from 2.44 to 6.04 mg/L at the solid-liquid 1:100, while an increase of the amount of Fe leached (4.85- 13.75 mg/L) was recorded at the solid-liquid 2:100 from the sample collected at Pheramindi field. The solid-liquid ratio therefore has a significant effect on the quantity of metal release. The average amount reported consumed by the pregnant women is 20g/day [27]. It ensues from the above that a once off ingestion of large amount of geophagic clays may expose the consumer to relatively high concentration of heavy metals.

Metals were released in various concentrations at different time periods of the leaching experiment. In all samples, Iron (Fe) was released in higher amounts as time increased. The effect of time on the release of metals from the geophagic clays shows that there is a relative increase in the release

over time (Figures 1, 2 and 3). Most metals were released from the first 30 minutes while Cr was initially released after an hour in the Pheramindi samples.

Higher metals concentrations were released from the samples collected in Phelandavha (1A) and Pheramindi (P1A) as compared to the samples collected from Ikageng (I1A). For example, the average concentration of Fe and Cu released from 1A was 5.13 mg/L and 0.51 mg/L respectively, while from P1A the average concentration was 5.20 mg/L and 0.60 mg/L for Fe and Cu respectively. Comparatively lower amounts of metal was released from the I1A; this was likely due to the presence of bentonite clay which has a high binding affinity for the metals and tends to immobilize the metals.

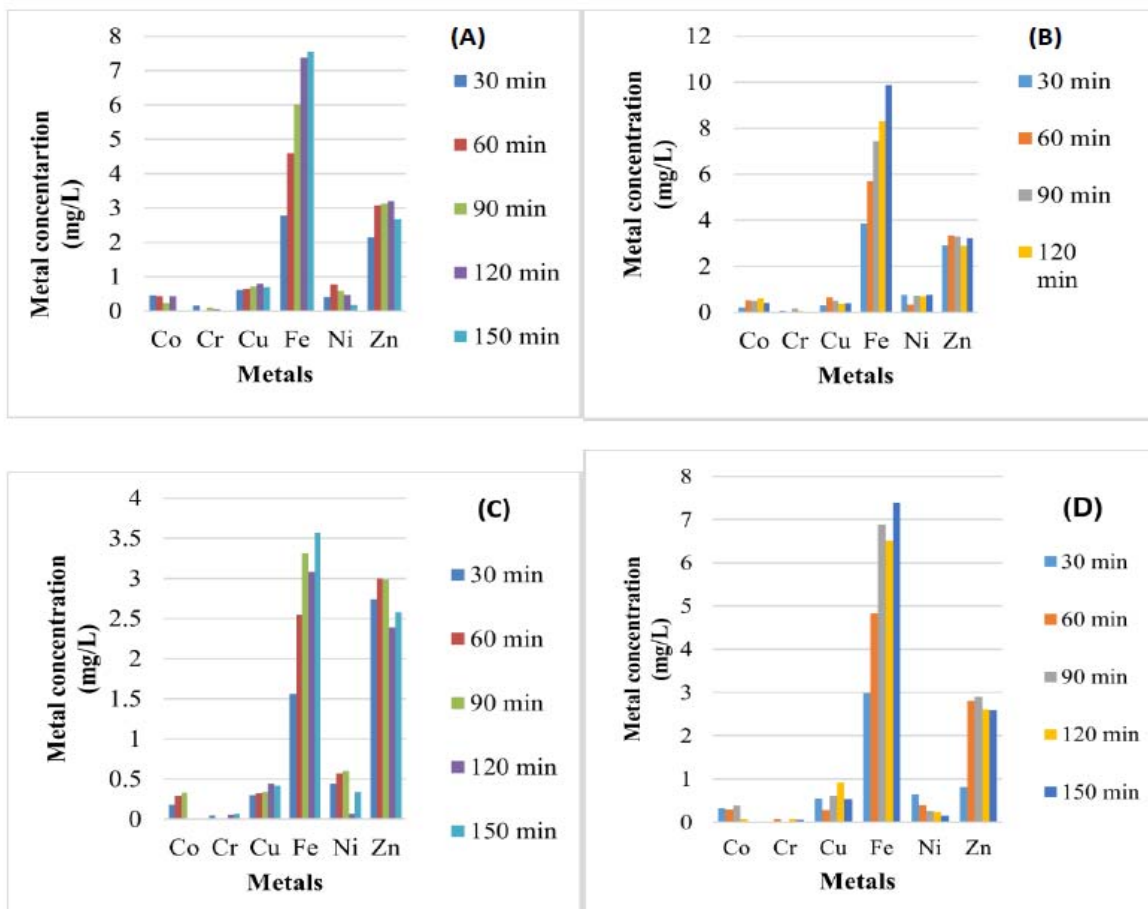


Figure 1: Amount of metal (mg/L) leached from the geophagic clays of Phelandavha: (A) Market sample S/L of 1/100; (B) Market sample S/L of 2/100; (C) Source sample S/L of 1/100; (D) Source sample S/L of 2/100.

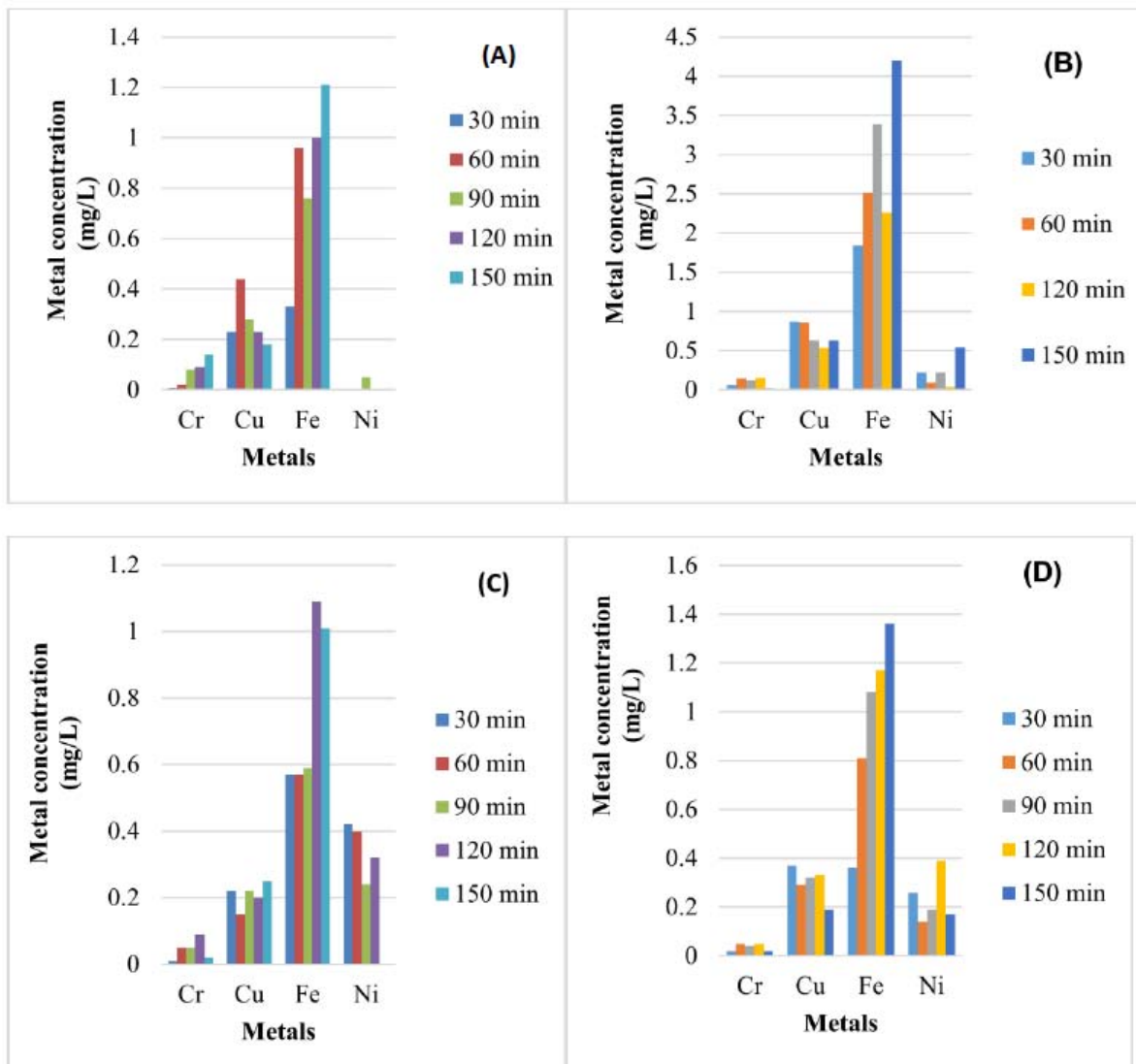


Figure 2: Amount of metal (mg/L) leached from the geophagic clays of Ikageng: (A) Market sample S/L of 1/100; (B) Market sample S/L of 2/100; (C) Source sample S/L of 1/100; (D) Source sample S/L of 2/100.

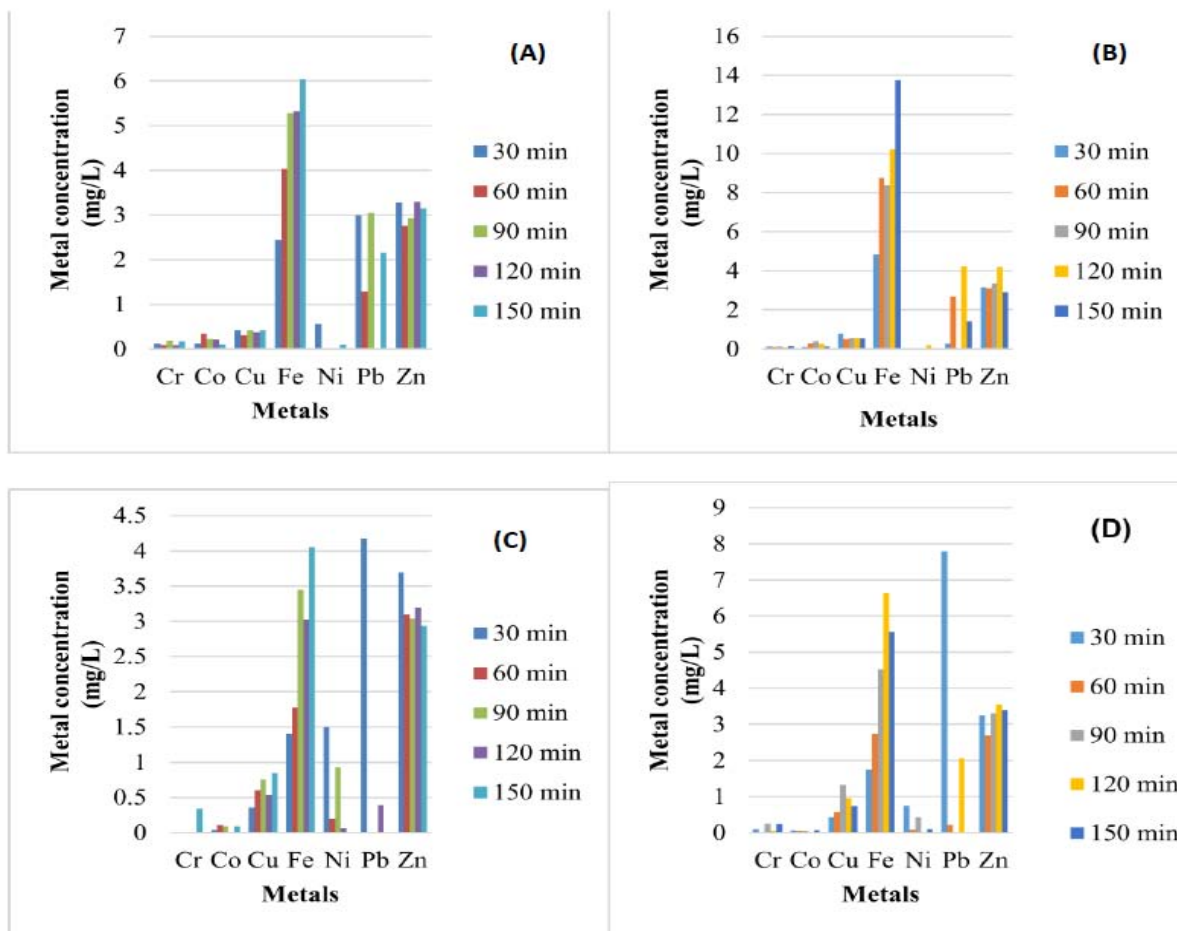


Figure 3: Amount of metal (mg/L) leached from the geophagic clays of Pheramindi: (A) Market sample S/L of 1/100; (B) Market sample S/L of 2/100; (C) Source sample S/L of 1/100; (D) Source sample S/L of 2/100.

When compared to the adequate daily intake of trace elements in the human body, the amounts of elements released from all the geophagic clays samples were relatively high (Figure 1a -Figure3d). The Pb content that was leached from the Pheramindi samples was significantly higher (Figure 3a) than 20 $\mu\text{g/L}$ set as the standard limit for body function. The Cu content leached from all geophagic clays samples was in the range of 0.15 mg/L-0.85 mg/L, less than the standard limit of 1000 $\mu\text{g/L}$ required for body function (Figure 1a - Figure3b). The amount of Zn leached from all the geophagic clays was below 3.81 mg/L which is within the standard limit required for body function, set as 5.0 mg/L.

The average concentration of leached Ni was in the range of 0.06 - 1.5 mg/L, but only 0.025 - 0.03 mg/kg /day is required for body functions. The average Co content in these geophagic clays was in the range of 0.001 - 0.60 mg/L, while the body requirement for this element is 0.002 - 0.1 mg/kg daily. Lead (Pb), Ni, Co and Fe were released in concentrations exceeding the value required for the proper functioning of the human body. The mobility of the metals was mostly dependent on their initial concentration in the clay as well as the mineralogical composition of the clay.

Micro-organisms and potential health risks

Micro-organisms are found everywhere in our body, soil, water and plants. Bacteria can be either beneficial or harmful to people depending on their classification. Soil is an example of an extreme environment that contains large numbers of varied and specific bacteria. In this study, the bacterial community structure and species in the metagenomics of six samples were analysed. The results (Table 4) only show the selected microorganisms with potential effects to the geophagic individuals. The microorganisms that were found on the geophagic clays were introduced in the source and markets by either run-off water, human activities or occurred naturally. Most of the geophagists consume the clay without any pretreatment for disinfection, therefore exposing themselves to pathogenic microorganisms. In our recent study, it was demonstrated that the load of microorganisms in the clay can be significantly reduced by simply exposing the clay to sun light [28]. The risk of infection can at least be minimized through the use of sustainable pretreatment method such as solar disinfection.

Table 4: Microorganisms occurring in geophagic clays from Ikageng, Phelandavha and Pheramindi samples and the potential health risks

Sampling area	Source		Market	
	Microorganisms	Potential disease	Microorganisms	Potential disease
Ikageng	<i>Pseudomonas aeruginosa</i>	Urinary tract infection, Pneumoni, Septic shock, Gastrointestinal infection, Skin and soft tissue infection	<i>Mycobacterium sp</i>	Tuberculosis
	<i>Spiroplasma sp</i>	Creutzfeldt-Jakob (CJD)	<i>Tsukamurella sp</i>	Pneumonia
	<i>Pseudonocardia yunnanensis</i>	Brain and Renal inflammatory disease, Chronic kidney disease	<i>Pseudonocardia yunnanensis</i>	Brain and renal inflammatory disease, chronic kidney disease
	<i>Propionibacterium acnes</i>	Acens, Chronic blepharitis, Endophthamitis	<i>Klebsiella oxytoca</i>	Colitis and sepsis
			<i>Pseudomonas aeruginosa</i>	Urinary tract infection, Pneumonia, Septic shock, Gastrointestinal infection and Skin and soft tissue infection.
Phelandavha	<i>Neisseria meningitidis</i>	Sepsin, Meningitis, Meningococcal disease	<i>Shigella sonnei</i>	Diarrhea, Dysentery
	<i>Synechococcus sp</i>	Cyanotoxins	<i>Escherichia coli</i>	Gastroenteritis, urinary infection, neonatal meningitis
	<i>Streptococcus pneumoniae</i>	Pneumonia	<i>Pseudomonas sp</i>	Nosocomial infection, Urinary tract infection, Pneumonia, Septic shock, Gastrointestinal infection and Skin and soft tissue infection.
	<i>Propionibacterium acnes</i>	Acens, Chronic blepharitis, Endophthamitis	<i>Propionibacterium acnes</i>	Acens, Chronic blepharitis, Endophthamitis
	<i>Pseudomonas aeruginosa</i>	Urinary tract infection, Pneumoni, Septic shock, Gastrointestinal infection, Skin and soft tissue infection	<i>Spiroplasma sp</i>	Creutzfeldt-Jakob (CJD)
	<i>Alpha proteobacterium</i>	Bacteraemia	<i>Klebsiella oxytoca</i>	Colitis and sepsis
	<i>Spiroplasma sp</i>	Creutzfeldt-Jakob (CJD)	<i>Mycobacterium avium</i>	Lungs infection
Pheramindi	<i>Streptococcus mitis</i>	Endocarditis	<i>Peptoniphilus sp</i>	Bloodstream infection
	<i>Streptococcus agalactiae</i>	Neonatal sepsis, Postpartum infection	<i>Streptococcus salivarius</i>	Endocarditis
	<i>Propionibacterium acnes</i>	Acens, Chronic blepharitis, Endophthamitis	<i>Citrobacter amalonaticus</i>	Neonatal meningitis, Gastroenteritis
	<i>Spiroplasma sp</i>	Creutzfeldt-Jakob (CJD)	<i>Lactobacillus jensenii</i>	Urinary tract infection, Pneumonia, Septic shock, Gastrointestinal infection and Skin and soft tissue infection.
			<i>Leptotrichia sp</i>	Endocarditis, Oral diseases
			<i>Streptococcus mitis</i>	Endocarditis
			<i>Propionibacterium acnes</i>	Acens, Chronic blepharitis, Endophthamitis
			<i>Pseudomonas sp</i>	Nosocomial infection, Urinary tract infection, Pneumonia, Septic shock, Gastrointestinal infection and Skin and soft tissue infection.

Susceptibility of Microorganisms

Excessive concentration of heavy metals can be toxic to all organisms. When the soil is ingested and enter into the stomach which has the pH of 2, there is possible reaction with clay minerals and micronutrients contained in soil ingested [23, 29]. The acidic stomach cause a decrease in the pH of ingested clay (Table 3). The bacterial activity decreased over specified time (Figure 4, 5). Figure 4 shows that the number of bacteria in the clay decreases with the increased released of metals into solution; however it is likely that the decrease of the number of bacteria is a result of the combined inhibitory effect of metal and low pH. It has

been reported that the physiochemical parameters such as pH, and metal ion concentration are correlated with microbial community composition [30]. The low pH in stomach increases the solubility of metals and other cations in the gastrointestinal tract [31; 32]. The concentration of metal was low at the initial time (25.3 mg at 30 min) and at the end of exposure the concentration of metals was 34.39 mg (Figure 4). The bacterial activity decreased over specified time ranged from 71.67 CFU/ml -38.33 CFU/ml (Figure 4, 5). Microorganisms that inhabit in metal-contaminated environment are able to develop many various types of resistance mechanisms in order to survive heavy metals, as results not all the bacteria were inhibited [31, 33].

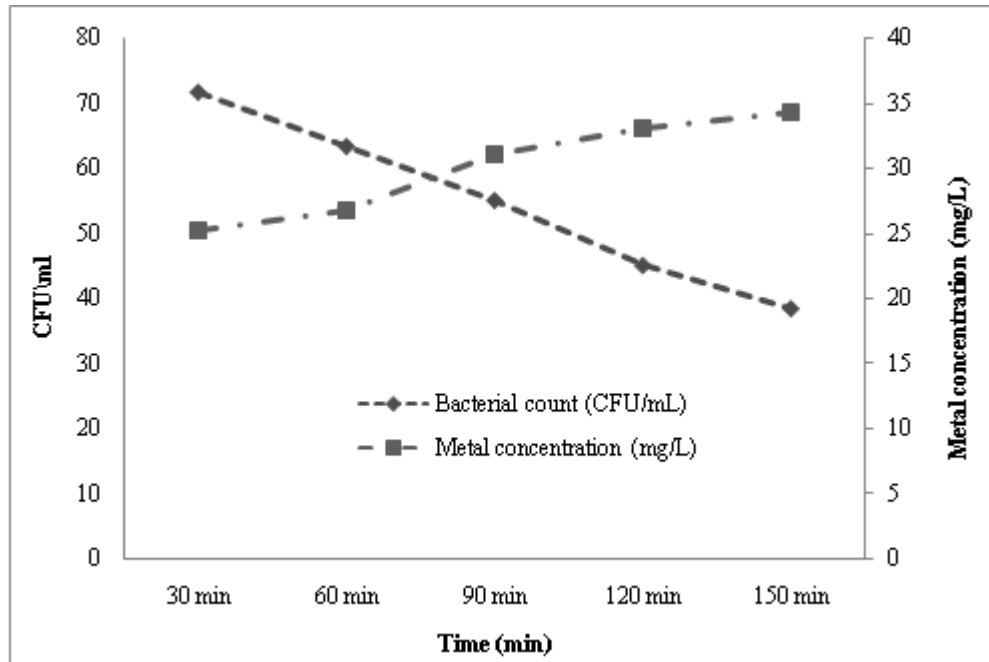


Figure 4: The metals concentration released and the microorganism that survived

Study has reported that heavy metals (Pb, Cd, Hg, Ag, and Cr) are toxic at low concentration and have no known beneficial effects on bacterial cells [33]. Clay particles can also bind to metals cations and microbes and reduce the toxicity of certain metals towards microorganisms; metals such as Zn enter the crystal lattice and become unavailable to organism [34]. Clay minerals such as kaolinite and montmorillonite protect certain bacteria from inhibitory effect of metals [34]. The acidity of the environment increases solubility of metals and decreases the concentration of microorganisms (Table 3, Figure 5). The identification of microorganisms through sequencing of 16S rDNA showed that the more resistant species (still alive after two and half hours) included *Bacillus subtilis*, *Paenibacillus*,

Bacillus cereus, *Bacillus thuringiensis*, *Bacillus humiand* *Arthrobacterarilaitensis* which are all Gram-positive bacteria. Renal failure can be caused by *Paenibacillus sp.* The microbial concentration decreased significantly within the first 30 min while only a slight decrease was observed between 30 and 150 min (Figure 5). The acidity directly affects both metal release and bacterial activity. The low availability of organic carbon is extremely challenging and hostile environment affect the growth of microorganisms. Bacteria needs organic carbon to survive, this can be converted to energy and biomolecules required for cell structure [32].

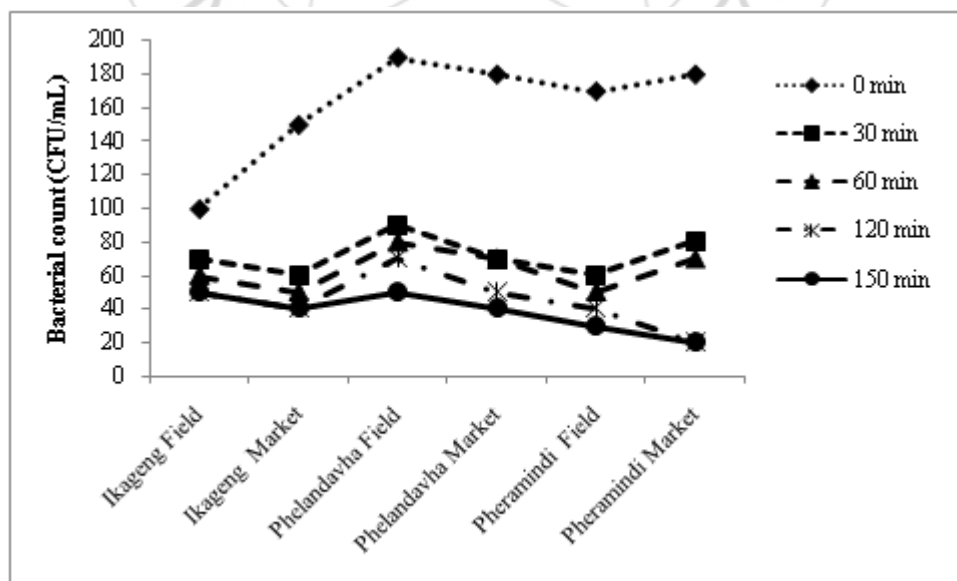


Figure 5: The behavior of microorganism in the geophagic clay subjected to a simulated gastric fluid

4. Conclusion

The aim of this study was to determine the susceptibility of microorganisms to conditions similar to the gastrointestinal tract during ingestion of clays. Our findings show that low pH promotes the release of metals from the clays and the combination of acidity as well as increased concentration of metals contributes to the considerable reduction of the load of microorganisms in the clay. However, it was also found that microorganisms are not totally inhibited under such conditions; Gram-positive bacteria were found to resist after longer exposure and will therefore be likely to reach the intestine of geophagists alive. It therefore ensues that despite the adverse conditions in the estomach some microorganisms are likely to persist in the digestion system and cause disease. It is advisable for consumers to consider the pretreatment of clay prior to ingestion; affordable and sustainable technique such solar disinfection was found to be effective for the treatment of clay [28].

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