Bio-reactive Clay Minerals and Anthrax Decontamination: a Novel Antimicrobial Solution

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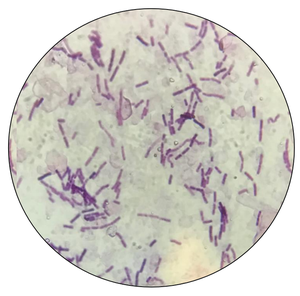
Anthrax

Bacillus anthracis, causative agent of Anthrax, is endemic to large areas of the planet.
Bacillus spp. produce hardy endospores resistant to desiccation; pH change; extreme temperatures; and high pressure. All, whilst remaining highly infectious when returned to favourable germination conditions[1].

Germination of the spores in a host results in contraction of inhalational, gastrointestinal, or cutaneous Anthrax (by inhalation, ingestion, or skin lesion contact with spores, respectively).

Decontamination of contaminated sites is very difficult, usually resulting in the use of large quantities of toxic chemicals.



Gram stain of *Bacillus anthracis* Sterne.
Sterne strain anthrax was imaged using a Leica DM750 Light microscope at 100x magnification.

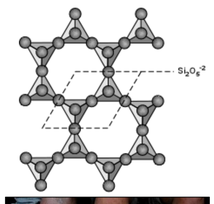
Bio-reactive Clay

The use of clay minerals for medical treatment is a common facet of much non-western medicine. Geophagy and topical use are the most common; topical use on the face is widespread even in western countries.

It has been demonstrated that some clay minerals possess antimicrobial properties. One of these is "*French Green Clay*" from the Republic of Côte d'Ivoire; it has successfully been used to treat Buruli ulcer (cutaneous *Mycobacterium ulcerans* infections)[2].

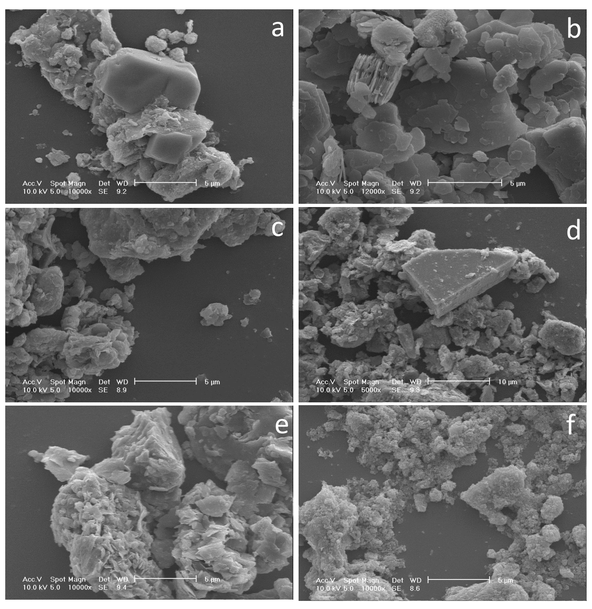
It is thought that the Iron and Aluminium ions in these clays provide antimicrobial properties, specifically those found in specific 2:1 phyllosilicate formations[3].

Clays from Basaltic terrains contain high levels of bio-available Iron. Exposure is the primary cause of Podoconiosis (non filamentous elephantiasis).



Bio-reactive clay minerals possess specific chemical phyllosilicate structures (Figure **a)** capable of housing ions with partially empty electron shells. These can result in inflammation (non filamentous elephantiasis) (Figure **b**).

Geochemical Results

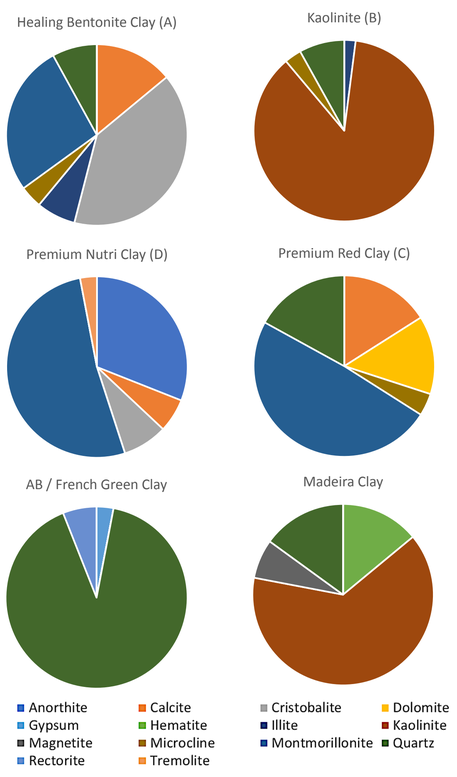


SEM showed wide variations in the fabric and texture of the morphologies of 6 different clay minerals. Images presented are representative of ~20 images per sample. (**a**, 'Healing Bentonite Clay'; **b,** Kaolinite; **c**, 'Premium Red Clay'; **d**, 'Premium Nutri Clay'; **e**, French Green Clay; and **f**, Madeira clay) sourced from various locations – some from companies that claimed biological activity.

X-ray diffraction studies have determined the mineral composition of each of the candidate clays. All show vastly differing compositions. Several of the samples contained different minerals to those purported by the seller.

French Green Clay was composed of approximately 91% quartz mineral, and therefore has a completely different composition to the other samples.

Bulk analysis was carried out on the powdered samples. Scans were run using the Philips PW1710 Automated Powder Diffractometer using Cu Kα radiation at 35kV and 40mA, between 2 and 70 °2θ at a scan speed of 0.04 °2θ/s.



From the scans, phases were identified using Philips PC-Identify software and from the peak areas, semi quantitative analysis was performed and a percentage of each phase present calculated.

Methodology

Suspension Production
- Suspensions were created using 0.15g/ml of clay suspended in phosphate buffer solution (PBS). The suspensions were sterilised by autoclaving at 121˚C for 15 mins.

Leachate Production
- Leachates were produced using 0.05g/ml clay. Suspensions were: sonicated for 15 mins (60Hz, RT); shaken for 24 hours (200rpm, 37˚C); and centrifuged at 5000 x g for 1 hour. The supernatant was then removed and sterilised by autoclaving.

Incubation with vegetative bacteria
- Clay suspensions were incubated 1:1 with 10^5 *B. anthracis* Sterne bacteria and 10^6 *B. cereus* 6A1 bacteria. Samples were shaken for 20 hours (200rpm, 27˚C)

Incubation with spores
- Clay leachates were incubated 1:1 with 10^6 *B. anthracis* Sterne spores. Samples were shaken for 20-24 hours (200rpm, 27˚C). Samples were washed 10x in 1ml of sterilised distilled water. Centrifugation at 20000 x g was used to separate the supernatant

Miles & Misra
- Samples were serial diluted to 10^{-7} and 20µl drops were pipetted onto Tryptone Soya Agar plates.
- Plates were incubated at 27˚C overnight and the mean number of colonies were counted to determine CFU/ml

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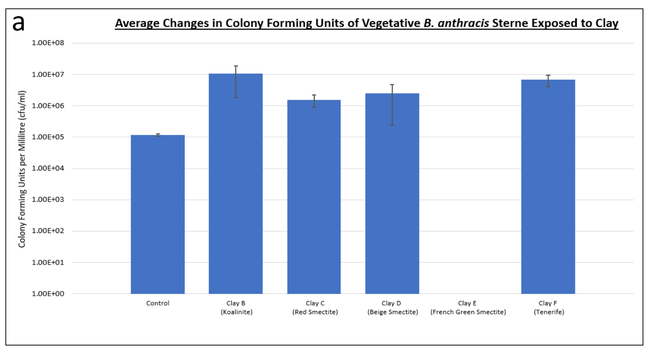
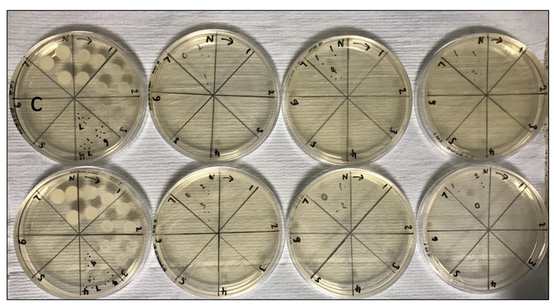
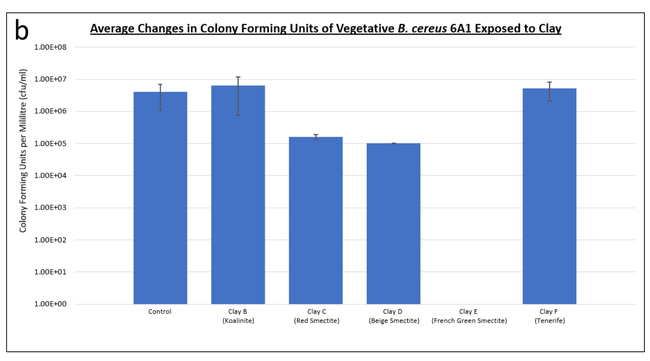
Microbiological Results

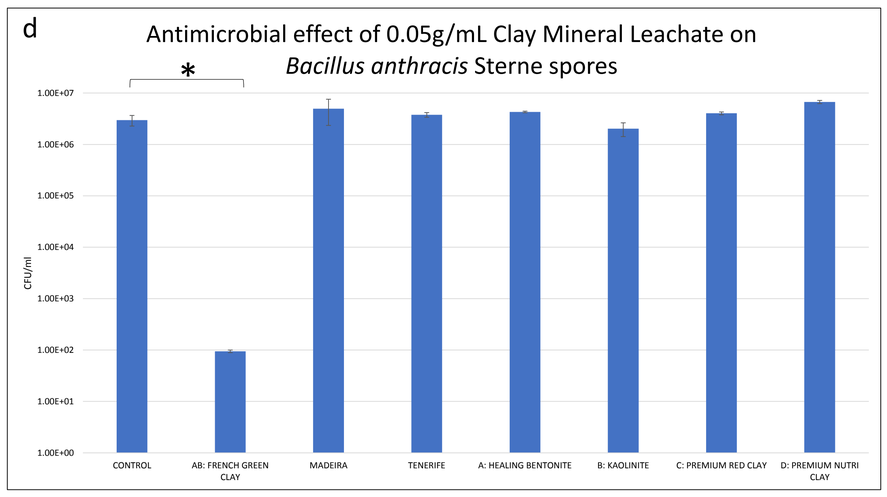
The French Green Smectite showed an antimicrobial effect against both vegetative *B. anthracis* Sterne (Figure **a**) and *B. cereus* 6A1 (figure **b**) cells, reducing CFU/ml by 100%. Other test clays had little effect on CFU/ml after repeated exposure of *B. anthracis* Sterne (n=3) and *B. cereus* 6A1 (n=2) to clay suspensions.

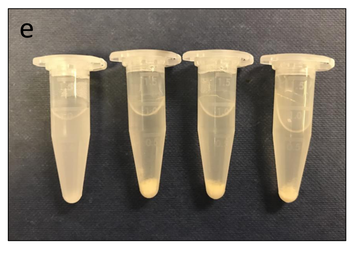






French Green Clay clay showed a $Log_{10}5$ reduction in total viable spores (Figure **c** and **d**). Other tested clay minerals showed no statistically significant difference in total viable spore numbers. * Indicative of where median Colony Forming Units per millilitre differed significantly – Control = $3.72x10^6$; French Green Clay = $1.00x10^2$ (Wilcoxon Mann-Whitney *U* – 12, $n_1 = 4$, $n_2 = 3$, $p = <0.05$. A noticeable difference in samples is evident at a pre-washing and final wash stage when compared to the control (as shown for AB/French Green in **e** and **f**, respectively).





Future Directions

1) Toxicological investigation of the candidate clays, *in-vitro*, will reveal whether they are toxic to mammals. If toxic, they would not function as a replacement for current best practice for decontamination.
2) Environmental-SEM or Transmission EM of spores in suspension with clay will determine whether the mechanism of action is a result of clay morphology; i.e. whether the clay minerals attach to the bacterial spores.
3) Confirm theories regarding Fe^{2+} activity in the presence of Al^{3+}: no ROS damage activity observed without membrane protein misfolding by Al^{3+}.

References

(1) Inglesby TV, Henderson DA, Bartlett JG, et al. Anthrax as a Biological Weapon: Medical and Public Health Management. JAMA. 1999;281(18):1735-1745. **(2)** Williams L.B. and Haydel S.E. *Int Geol Rev.* 2010;52(7/8):745-770. **(3)** Milenkovic J. *et al. Environ Sci Pollut Res Int.* 2017;24(25):20273-20281.

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