The bioreactivity of 'red clays' from basaltic terrains.

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For over 50 years an epidemiological correlation has been recognised between specific human health issues and basaltic terrains with 'red clays'. This correlation has been seen worldwide, including counties along the African Great Rift, Central America, India and others. The health effects are due to the internalisation of the clay minerals into the body's circulatory systems, resulting in skin discolouration and 'mossy' textures, and disruption of the balance of interstitial fluids resulting in oedema in lower limbs. These health issues are invariably seen in the poorer communities in these countries, and millions of people are affected worldwide. The basaltic terrains of concern are also usually characterised by high elevations, relatively warm average temperatures, and significant amounts of rainfall. We have collected 'red clays' from a number of such locations and compared their bioreactivity with characterised 'non-red clays'; and a consistent result is the enhanced bioreactivity of red clays with high iron content. All the results in this presentation relate to 'red clay' samples collected from the Atlantic island of Madeira, which was our most bioreactive clay. These Madeira clays are dominated by kaolinite and resemble spheroidal halloysite under SEM. Once collected the clays were not subjected to any pre-treatment other than size sorting by suspension in double-distilled water, which allowed the separation of clays grains of 2 microns or less. These extremely small grains are the ones capable of translocation around the human body via the circulatory system.

The plasmid scission assay uses the bacterio-phage ΦX174, which is susceptible to damage by Reactive Oxygen Species (ROS) and metals. The lack of a cell or its components makes it suitable to study the effect of ROS directly on DNA. The general principle of the assay is that free radicals on the surface of the mineral grains cause damage to the super coiled DNA, leading to single strand breaks, double strand breaks, or complete DNA fragmentation. The relative proportion of damage can be measured and quantified by gel electrophoresis. The bacterio-phage was exposed to a range of Madeira clay concentrations in aqueous suspension from 50 to 1000 ug/ml. A clear dose response is seen with the highest doses causing 35% DNA damage.

Vibrio fischeri is a bioluminescent marine bacteria. Toxicity tests using *Vibrio fischeri* have been used extensively to monitor environmental contamination of soils, with the reduction of bioluminescence being indicative of toxicity. Incubation of *Vibrio fischeri* with Madeira clay resulted in a significant drop of 70% in viability within the first minutes, almost mirroring the response to the positive control.

The haemolysis assay monitors the lysis of human red blood cells (erythrocytes) that are susceptible to damage by ROS and metals. Blood samples were collected from three donors and were centrifuged to separate the erythrocytes, which were then exposed to a range of concentrations of Madeira clay. The degree of lysis is determined by the optical density of samples. There was a clear dose response with 1000 ug/ml causing between 1% and 2% erythrocyte lysis.

The effect of clays on oxidative stress in immune cells was determined by measurement of intracellular H_2O_2 production by THP-1, cultured monocyte cells. Cells incubated for 1 hour with 0.5 mg/ml of Madeira clay produced more H_2O_2 (MFI=262) than cells incubated with control Kaolin (MFI=189). Incubation of THP-1 cells with Madeira clay for 24 hours increased secretion of all cytokines.

The bioreactivity of 'red clays' from basaltic terrains is clearly demonstrated. The 'red clays' have the potential of cause damage to DNA, reduce cell viability and cause haemolysis. In addition 'red clays' have immunological effects which include increased oxidative burst and production of a range of inflammatory cytokines in immune cells.