

Do ferrous-minerals in Arctic circle coal fly-ash mediate respiratory toxicity in humans?

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Svalbard is a Norwegian Archipelago located in the Arctic Circle that has a long history of coal-mining. The coal is used by the local power-station to provide electricity to the residential settlements, as well as being exported to countries, e.g. Norway and Germany. Bulk coal fly-ash (CFA) produced from the Longyearbyen Power-Station was collected by Dr Lisa Mol in 2014. Here we report the first geo-toxicological investigation of CFA derived from this Arctic region.

The bulk CFA (Figure 1a) from Svalbard was separated into inhalable particles (Figure 1b), as a means to test their oxidative capacity in the lung environment. Dry CFA was resuspended inside a rotating-drum fitted with a PM10-selective inlet head attached to an air-pump. The air-flow rates of 5, 10 and 20 litres per minute produced different sized (i.e. PM 1, 2.5, 10, respectively) particles for geo-toxicological analysis.

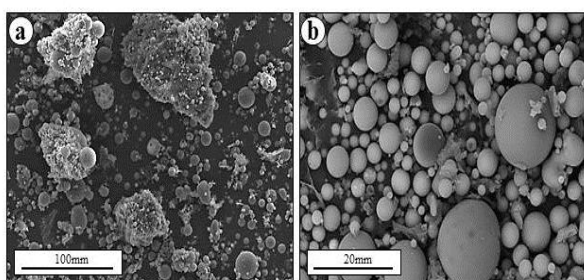


Figure 1. FESEM Svalbard CFA*.

Analysis of CFA physicochemical properties has been on-going (e.g. surface area, size, shape, number, metal chemistry) as they are known drivers of the oxidative capacity of particulate matter (PM). Knowledge of particle size informs on probable deposition sites within the lung, and thereby, estimation of toxic potential. For example, PM <0.1µm will have the greatest toxic effect(s) because nano-sized particles readily enter alveoli in the distal respiratory tract to provoke inflammation. The main cause of PM bioreactivity is believed to be through their induction of oxidative stress by generating reactive oxygen species (ROS).

Ferrous minerals can be found in varying levels in CFA depending on the coal geochemistry, and have been indicated to cause the most damage through inducing ROS via Fenton reactions (Brown *et al.*, 2011). Svalbard CFA was exposed to a neodymium magnet in order to extract magnetic components. All CFA fractions demonstrated a very high-presence of ferrous minerals.

Accordingly, pure magnetite (Fe₂O₄) was selected to be a positive control particle for assessing CFA toxicity.

Preliminary physicochemical characterisation involved field-emission scanning electron microscopy (FE-SEM) and associated energy dispersive X-ray microanalysis (EDX) to determine the inorganic elemental composition of bulk and inhalable-sized CFA fractions. The oxidative capacity of Svalbard CFA was determined by the Plasmid Scission Assay (PSA). PSA employs a ROS-sensitive plasmid that exhibits different DNA morphologies relative to specific levels of 'damage' (i.e. relaxed, linear and fragmented) versus DNA 'un-damaged' (i.e. super-coiled); that are captured via gel electrophoresis.

Svalbard CFA caused DNA damage in a dose-responsive manner; thus inferring toxicity (Figure 2). Preliminary results have implied that a dose as low as 5µg/ml induced minor (i.e. relaxed) DNA damage, with the total amount of damage being increased gradually to approximately 40% as the CFA concentration increased. A TD₄₀ (i.e. Toxic Dose 40%) refers to the dose required to kill 40% of the 'test subject' (e.g. plasmid DNA or lung cells) being challenged by a toxicant.

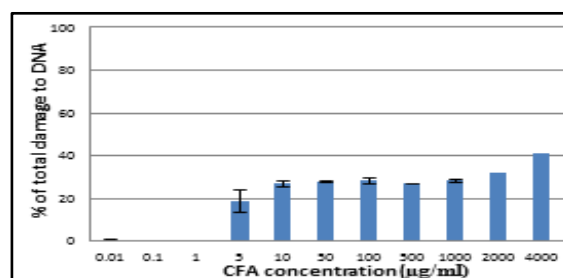


Figure 2. PSA results on Svalbard CFA

Further analysis of this Arctic CFA is required to determine the parameters causing bioreactivity. Conventional toxicology will include cellular and whole organism ROS-assays, including lysis of human red blood cells (haemolyses) and detection of bioluminescence in *Vibrio fischeri*, respectively, to confirm the acellular PSA results. The indirect effects that Svalbard CFA may have on human lungs will require investigation of soluble-components (via leaching in lung fluids) and bio-availability of other metals (using metal-chelators).

*Brown, P., Jones, T. and BéruBé, K. (2011). *Environmental Pollution* 159 (12):3324-3333.