Screening of cytotoxicity, oxidant generating capacity and inflammatory potential of two selected coal mine dusts as a contribution to the European ROCD* project

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Highlights

- Two lignite coal mine dust samples (size fractions $PM_{2.5}$ and PM_{10}) from a coal mine in Velenje (Slovenia) induced ROS production, cytotoxic effects and release of the cytokine IL-8 in A549 human lung cells in a concentration-dependent manner.
- The observed toxic effects of these two coal mine dusts in human lung cells A549 appear to correlate with their hydroxyl-radical-generating and superoxide-generating capacities.
- The results of this study show that using the oxidant generating capacity of coal mine dusts as a metric allows toxicological grouping of coal mine dust samples and prediction of human health effects.

Introduction

Despite international efforts to limit worker exposure, coal mine dusts continue to impact the health of thousands of miners across Europe. Modern, practicable assessment tools and devices are urgently needed to protect workers, particularly from the fine fraction ($PM_{2.5}$; Fig. 2 shows a typical SEM image) of coal mine dust, which is increasingly implicated in human disease. To predict dust toxicity of different coal mine dusts and mining scenarios, a set of toxicological assays are necessary to identify a successful improvement of risk management targeting mitigation measures. Various studies indicate that the surface area and the potential to form reactive oxidants (Fig. 1) are highly promising metrics to predict the toxic potency of fine and ultrafine dusts.
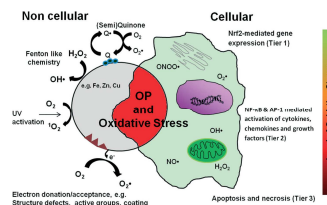

Fig. 1: Non cellular oxidant production by PM (left), oxidants formed upon cellular interaction of lung cells with PM (right) and levels of oxidative stress (after Helleck et al. 2012)
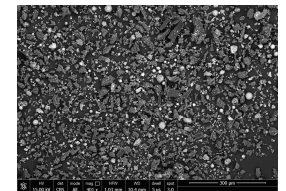

Fig. 2: SEM image of the $PM_{2.5}$ fraction of an lignite coal mine dust from Velenje (Slovenia)

Materials and Methods

In the frame of the European ROCD project, two lignite coal mine dusts with different fractions ($PM_{2.5}$ and PM_{10}) obtained from a coal mine in Velenje (Slovenia) were investigated for their non-cellular radical generation capacity ($DMPO/H_2O_2$ for $OH\cdot$ and CPH for $O_2\cdot$) using Electron Spin Resonance Technique (ESR) and their oxidant generating capacity (DCF-assay), cytotoxic activity (WST-1 assay) and inflammatory potential (IL-8; ELISA) in the human alveolar epithelial cell line A549 and the human monocytic cell line THP-1. Furthermore, to relate the observed effects to the hydroxyl-radical ($OH\cdot$)-generating and superoxide anion ($O_2\cdot$)-generating activities of these samples. The approach is based on the aligned ESR technique with 5.5-dimethyl-1-pyrroline-N-oxide (DMPO) as spin trap and hydrogen peroxide as substrate, and is specifically sensitive to Fenton-type reaction mediated generation of hydroxyl radicals.

Results and Conclusion

The results show that the two lignite coal mine dust samples investigated produce ROS (Fig. 5), induce cytotoxic effects (Fig. 6) in A549 and THP-1 cells, and release cytokine IL-8 (Fig. 7) in A549 cells in a concentration-dependent manner, with a similar potency to the two reference substances quartz mineral dust (QMD) and coal fly ash (CFA). Moreover, the toxic effects of coal mine dusts observed in human lung cells A549 appear to correlate with the hydroxyl-radical-generating and superoxide anion-generating capacity of a coal mine dust sample (Fig. 3 and Fig. 4). The two studied coal mine samples and two reference substances with known constituents reveal the intrinsic hydroxyl-radical-generation method to be a sensitive tool for prediction of adverse health effects of particulate matter.

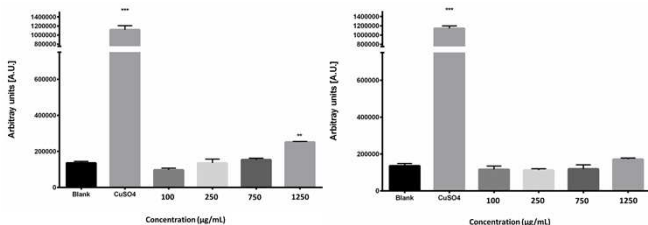### Non-cellular OH and O_2 radical generation capacity using ESR

Fig. 3 : Concentration dependency of $OH\cdot$ generation induced by $PM_{2.5}$ (left) and PM_{10} (right) of an lignite coal mine dust from Velenje (Slovenia) measured by ESR. Error bars ± SD. p≤0.05*; p≤0.01**, p≤0.01***; N=3
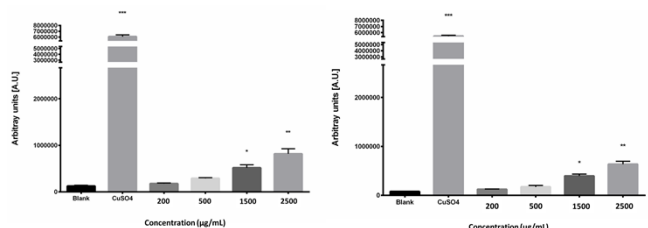

Fig. 4: Concentration dependency of $O_2\cdot$ generation induced by $PM_{2.5}$ (left) and PM_{10} (right) of an lignite coal mine dust from Velenje (Slovenia) measured by ESR. Error bars ± SD. p≤0.05*; p≤0.01**, p≤0.01***; N=3

Cellular oxidant generating capacity, cytotoxicity and inflammation in human lung cells (A549) and in human monocytic cells (THP-1)
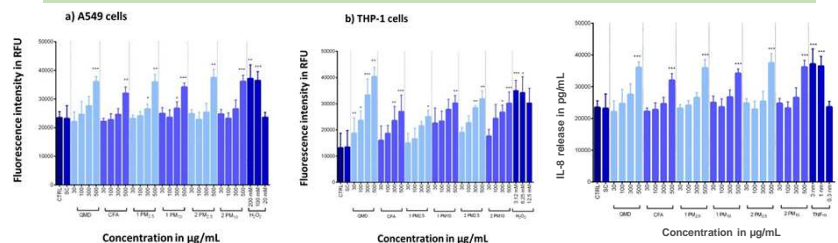

Fig. 5: Oxidative stress in A549 cells (left) and THP-1 cells (right) exposed to $PM_{2.5}$ and PM_{10} of the two lignite coal mine dusts 1 and 2 for 1 h using DCF-DA-assay. Error bars ± SD. p≤0.05*; p≤0.01**, p≤0.01***; N=3
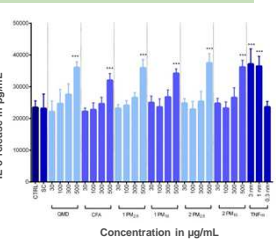

Fig. 7: Release of IL-8 in A549 cells exposed to $PM_{2.5}$ and PM_{10} of the two lignite coal mine dusts 1 and 2 for 24 h. Error bars ± SD. p≤0.05*; p≤0.01**, p≤0.01***; N=3
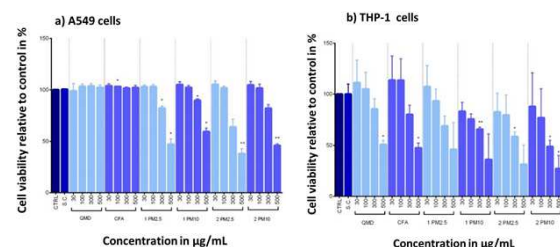

Fig. 6: Cellular viability of A549 cells (left) and THP-1 cells (right) exposed to $PM_{2.5}$ and PM_{10} of the two lignite coal mine dusts 1 and 2 for 24 h using WST-1 assay. Error bars ± SD. p≤0.05*; p≤0.01**, p≤0.01***; N=3