COVER SHEET

Investigation of linearity of the UVAPS fluorescent signals

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The fluorescence emitted by aerosol particles is registered by the UVAPS in one of 64 channels, depending on the intensity of the signal. The UVAPS does not provide the nominal values for the fluorescence intensity of the particles in arbitrary units such as relative fluorescence units (rfu), as is typically reported by the spectrophotometers. Instead, the intensity of the signals is reported on a scale from 1 to 64, corresponding to the channel numbers.

Accordingly to the Beer-Lambert law, the fluorescence intensity should be directly proportional to the amount of fluorophores in the aerosol particles, in the absence of quenching. In order to facilitate interpretation of the UVAPS data in regard to the composition of airborne biological particles, a linearity of the fluorescent signals of the spectrometer was investigated.

As the amount of intrinsic fluorophores in bacteria may vary depending on viability status of the cells (Agranovski et al., 2003), the linearity was initially investigated for the non-microbial aerosols (NADH, NADPH, or riboflavin), with preset concentrations of fluorescent material in aerosol particles. The succeeding tests were performed with bacterial aerosols containing carefully washed *Bacillus subtilis* or *Micrococcus luteus* vegetative cells.

The fluorescence intensity was found to be linearly proportional to particle volume for all tested aerosols (Figs. 1-2). The results for the NADH aerosols generated from the 0.1 mg/ml, 0.05 mg/ml, and 0.025 mg/ml solutions are presented in Fig.1. The slope of the lines decreased by the same factor as the dilution (within the limits of the experimental error) demonstrating the linearity of the signals. Similar results, in terms of linearity, were obtained for the NADPH and riboflavin aerosols.

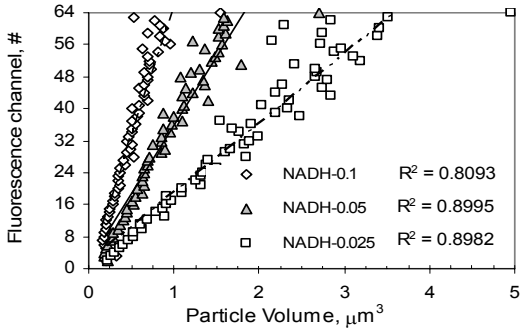

Fig.1. Fluorescent signals from the NADH aerosols. Each data point represents a mean value of the five replicate measurements.

Comparative analysis of the data for the bacteria in various metabolic states showed that fluorescence intensity of the particles containing cells harvested during the stationary growth phase was higher than the fluorescence intensity of the log-phase cells. For example, inspection of the data for the *B. subtilis* aerosols (Fig. 2) showed that the stationary-phase particles of the same size as the log-phase particles typically produced stronger fluorescence, corresponding to higher channel numbers. In addition, while the fluorescent signals for the log-phase aerosols were recorded only in the first 15 channels, the stationary-phase aerosols were detected in up to the 33rd channel. Similar tendencies were also observed for the *M. luteus* aerosols, generated with the cells harvested at different growth phases. In addition, the heat-treated (stressed) *M. luteus* cells produced less fluorescence than the untreated ("healthy") cells. Although both aerosols produced signals in the first 34 channels, the comparison of the dependence of the fluorescence signals for the particles of the same size showed that the amount of fluorophores in the heat-stressed bacteria was smaller ($P < 0.1$) than in the healthy bacteria. Namely, intensity of aerosol particles of the same size containing healthy *M. luteus* cells that were untreated by heat was higher than for the particles containing the heat-stressed cells.

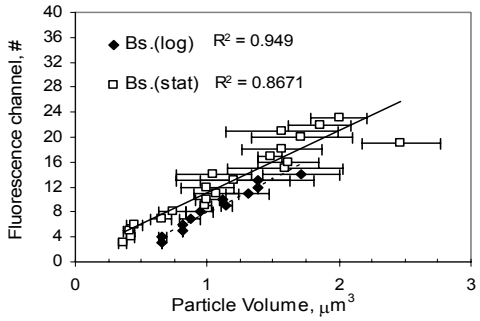

Fig.2. Fluorescent signals from *B. subtilis* aerosols.

Due to apparent linearity of the signals, it was concluded that the UVAPS can be used to estimate the number of individual bacterial cells in aerosol particles and thus to provide information on the concentration of airborne microorganisms, in addition to the number of microbe carrying particles.

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