ECOTOXICOLOGICAL ANALYSIS OF AEROSOL SAMPLES

THESES OF THE PHD DISSERTATION

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Introduction and Objectives

The adverse health effects of aerosols usually occur in the polluted air and plume of air of busy cities and industrial areas. The aerosol particles smaller than 10 micrometers and 2.5 micrometers (PM\(_{10}\) and PM\(_{2.5}\)) are defined as the most harmful air pollutant to human health by the European Union 2008/50/CE no. Guidelines for Ambient Air Quality. One of the reasons is that this inhalable fraction of aerosols can be the cause of a number of respiratory diseases and lesion. The main source of the fraction of urban suspended particulate matter in the size range of PM\(_{2.5}\) is traffic, wood firing and agitated, settled dust (Schauer et al. 1996). The aerosol particles originating from traffic increasingly affect urban air as a result of the increasing levels of urbanization experienced in Europe and around the world; the main source of the PM\(_{10}\) and PM\(_{2.5}\) fraction of these particles are primarily the exhaust emission of diesel vehicles as well as the ultra-fine grained powder generated from the abrasion of brake pads and tyres.

The human toxicological effect of aerosols is already well known (Kappos, 2010), but our knowledge of the ecotoxicological effects of aerosols, including the aerosols emitted by diesel-powered vehicles is rather limited. To carry out these investigations is difficult due to the small sample size of aerosol samples. *Vibrio fischeri* bioluminescence inhibition assay is most commonly used as an ecotoxicological test. The basic version of the *V. fischeri* bioluminescence inhibition test (ISO 11348-2009) is applied for aqueous phase as *V. fischeri* is a marine bacterium. A number of systems exist, which are based on this version, such as ToxAlert (Merck), Microtox (AZUR Environmental), LUMIStox (Hach-Lange) or BioTox (ABOATOX). The test can be carried out by preparing extracts in case of aerosol samples. The toxicity studies using aerosol extracts, however, do not reflect the real environmental exposure route. With the use of organic solvents we can mobilize such ingredients which are generally biologically inaccessible (Harkey and Young, 2000), so the aerosol toxicity overestimate, so the aerosol toxicity can be overestimated. In order to obtain a more realistic exposure route for the assessment of the ecotoxicity of solid samples, we must provide a direct contact between the particles and test organisms, since the toxic effect mainly depends on the particle-bound compounds.

A new protocol has been developed for testing solid and/or colour patterns (direct contact test), which was standardized in 2010 (ISO 21338: 2010: Water quality - Kinetic determination of the inhibitory effects of sediment, other solids and coloured samples on the light emission of
Vibrio fischeri kinetic luminescent bacteria test /). The Ascent Luminometer (Flash System) of the Finnish Aboatox Co. uses this protocol.

My research aims:

1. Our research group has developed such a sample preparation protocol (Kováts et al., 2011) using the kinetic version of the test based on Vibrio fischeri bioluminescence inhibition test (the so-called Flash Test), which allows the use of a direct contact test. My primary goal was to certify that this enhanced test protocol (1) is adequately sensitive, (2) represents a real route of exposure, and (3) eliminates false toxicity arising from the possible turbidity of the sample.

2. For this purpose I compared the sensitivity and reliability of various test systems. I examined our aerosol samples (solid phase) intentionally with the Ascent Luminometer (Flash System) distributed by Aboatox Co. In parallel to this I carried out comparative measurements on aerosol extracts using the ToxAlert® 100 system.

3. As a further research goal I used Artemia salina mortality tests in order to determine the ecotoxicity of aerosol samples, as well as in vivo enzymatic examinations on A. salina brine shrimp to determine the ecotoxicity of aerosols.
Theses

1. I used the direct contact test developed by our research team for the ecotoxicity analysis of solid phase aerosol samples. The test is based on the ISO 21338:2010: Water quality - Kinetic determination of the inhibitory effects of sediment, other solids and coloured samples on the light emission of *Vibrio fischeri* /kinetic luminescent bacteria test/, developed for the analysis of solid and/or coloured samples. I found that the protocol is suitable for testing a variety of aerosol samples, and thus it is adaptable for the ecotoxicity analysis of different environmental aerosol samples.

2. I compared the literature ecotoxicological data measured using PAH samples (Eom et al., 2007) with the ecotoxicological data measured using the Ascent Luminometer (Flash system) I used. According to the results there is no significant difference in the results gained from the two methods, so the Flash System proved to be sensitive. The two methods were statistically evaluated as well, in which medium positive correlation ($r = 0.57, p = 0.15$) was found between the two methods using *V. Fischeri*.

3. I compared the Ascent Luminometer (Flash System) I used on aerosol samples with the ToxAlert luminometer, which has long been using a standard (ISO 11348-3: 2009) in our country. Based on the results, I concluded that the Flash system is capable of eliminating the false toxicity arising from the colour or the turbidity of the sample.

4. The ecotoxicity values of aerosol samples from the emissions of diesel powered passenger vehicles and buses, measured in a Flash system, were compared with the genotoxicity results of the samples measured with SOS Chromotest. In the case of passenger cars there was a correlation observed between the ecotoxicity and genotoxicity results. In the case of buses a very close positive, significant correlation was found between the ecotoxicological and genotoxicological results ($r = 1, p <0.001$).

5. The ecotoxicity results measured using the Flash system were compared to the results of the mortality test carried out on the *Artemia salina* small-sized cyst, and also with the results of the enzymatic examinations carried out on the *A. salina* small-sized cyst. We found a strong
positive, significant correlation between the results measured with a Flash system and mortality test results \((r = 0.842, p = 0.004)\). The results obtained by enzyme activity assays showed a similar trend with the results of mortality tests as well as *V. fischeri* bacterial tests.

6. The protocol using the Flash system works with an extremely small amount of sample, thus allowing fast ecotoxicological analysis of individual motor vehicle emissions.
Publications used in the thesis


Publications not used in the thesis


Presentations


Kovács, A., Kováts, N., Ács, A., Ferincz, Á, Beatrix Turóczy: Ecotoxicological analysis of aerosol samples – methodological approach, VII. Kárpát-Medencei Környezettudományi Konferencia, Kolozsvár, Románia


Kováts, N., Ács, A., Kovács A., Kárpati, Á.: Alternatív Daphnia teszt szennyvíz toxicitásának becslésére; LII Hidrobiológus Napok, Tihany