

Toxicity of fine and coarse atmospheric particles using *Vibrio fischeri*

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Abstract

In the present study, the toxicity of fine (<2.5μm) and coarse (2.5-10μm) particulate matter was determined using a rapid and cost-effective bioluminescence assay, the Microtox bioassay. Samples were collected in the city of Kozani which is a heavy industrialized area in the north-western part of Greece and characterized by complex topography. Near Kozani, lignite power stations (PS) operate with a total installed generating capacity of more than 4.7 GW. These PS contribute to about 57% of the total electrical energy produced in Greece. The lignite used by these power stations is mined in the nearby open-pit mines. Dust emissions seem to be the most serious problem in the area, as the measured ambient concentrations of suspended particles are at high levels and exceed local and international standards.

The organic contents from filters were extracted in two stages. Firstly by reflux and then by using ultrasound bath. Then concentration extracts were further concentrated to 1 mL and the half part, after addition of internal standard, was analyzed by gas chromatography - mass spectrometry technique for the determination of the 16 polycyclic aromatic hydrocarbons (PAHs) listed by the U.S. EPA as priority pollutants. The other part of extract was exchanged with 0.5 ml of DMSO and then diluted to freshwater. Following the samples were analyzed for their ecotoxicological properties with *Vibrio fischeri*. Five consecutive dilutions of the sample were tested in order to determine the EC50 values (the % of sample concentration that causes 50 % effect on the test organism) as a result of different size of particulates. The results showed correlation between toxicity of airborne particles and PAHs content.

Keywords: suspended particles, toxicity, bioassays

1. INTRODUCTION

Suspended particulates are an important health concern in urban areas, especially with respect to a number of chronic respiratory diseases. Particulate pollution is of paramount importance in areas with open-pit mines because of its human health related effects [1,2].

Knowledge of the distribution of airborne particulate matter (PM) into size fraction has become an increasing area of focus when examining the effects of particulate pollution [3]. Particle size distribution is important for human exposure and risk assessment, as well as for understanding the mechanisms of atmospheric processes. Particles with the size less than 10 μm (PM_{10}) have long been implicated in causing adverse health effects and increased mortality whereas fine ($\text{PM}_{2.5}$) and ultrafine particles impose even higher risk [4-6]. In addition, much in vitro work with human and rat cell lines has further expanded the collective knowledge of the biological response mechanisms to PM [7-9]. However, due to the complex nature of biological testing, few of these studies have combined detailed chemical analysis with biological characterization, focusing instead on bulk or physical characterization (elemental and organic carbon, inorganic ions, particle sizing, etc.) or a limited set of detailed analytes such as PAHs. Over the past years PAHs have been found to be ubiquitous constituents of urban airborne particles and have become a major health concern mainly due to their well-known carcinogenic and mutagenic properties [10,11].

The Microtox system approved by the U.S. Environmental Protection Agency (U.S. EPA) and the American Society for Testing and Materials (ASTM) [12,13] is used widely for assessing both acute toxicity and genotoxicity of various contaminants, including the testing of industrial effluents, municipal wastewater, pesticides, inorganic and organic chemicals, soils, sediments, and air pollutants [14-17]. This bioassay uses freeze-dried bioluminescent bacteria (*Vibrio fischeri* NRRL B-11177) as the test organism and assesses acute toxicity by measuring the reduction in light emission of bioluminescent bacteria. The Microtox bioassay can be used to test liquids or solids. However, to date, only a few studies exist evaluating the toxicity testing of air samples using Microtox. This is because the Microtox system requires all samples to be in aqueous solution for testing purposes. Thus, for compounds with limited solubility in water, Microtox is not suitable without some modification.

In the Kozani - Ptolemais area lignite power stations produce the most percentage of the electrical energy produced in Greece. The lignite is mined in the nearby open-pit coal-mines and transported to the power stations. Considerable amounts of pollutants are emitted from the PS stacks and mining activities [3]. The air quality control around the area is monitored by a measurement stations network, which has been installed by the Greek Public Power Corporation. Specific atmospheric pollution measurements are also carried out by the Laboratory of Atmospheric Pollution and Environmental Physics of T.E.I of West Macedonia [18].

However the ecotoxicological effects of the particulate matter, has not been investigated, thus the current study is a preliminary effort to correlate physicochemical properties of particulate matter to biological impact.

2. MATERIALS AND METHODS

2.1 Sample collection

Size-segregated particulate samples fine and coarse particles were collected in the urban area of Kozani in Greece, for a period of one year (December 2005 to October 2006). The samples were collected by dichotomous sampler with a PM_{10} inlet to collect particles in the PM_{10} size range for gravimetric analysis. The samples were collected by passing air through a 37 mm teflon PTFE filter. The technique for

monitoring the size distribution of airborne aerosols with a dichotomous sampler is inertial impaction of particles into a void (virtual surface). The airflow for coarse particle sampling was 1.7LPM (liter per min.), while that for fine particle sampling was 15LPM, and the total airflow was 16.7LPM.

Before sampling, the filters have been equilibrated to constant temperature and relative humidity conditions and weighed. After sampling, the filters have been again equilibrated to the constant temperature and humidity conditions and again weighed. The concentration is calculated by dividing the weight of the particulate captured on the filter by the volume of air (at ambient conditions) that passed through the sampler. The flow rate is required to be maintained within 5% of 16.67 L P M with a coefficient of variation of less than 2%.

2.2 Treatment of samples

PAHs were recovered from filter according to procedures previously optimized [19]. Briefly PAHs are extracted quickly and in high portions by the use of supersonic chambers, however, this may lead to partial P A H degradation when are found in high concentration. Thus, the teflo filters were separated from polypropylene ring with care in order to avoid loss of sample and then, they were extracted in a two stage procedure. First, they were purred in 50 ml liners with 5 ml hexane. The samples were placed in magnetic shaker for 30 min while heated below boiling temperature. In the second stage the extract was taken off by the addition of 5 ml hexane - acetone mixture (1:1) and then, the liner was placed in the supersonic chamber for 15 min. During both stages it has been used water freezer for the condensation of the solvent in order to avoid loss of the low molecular weight substances.

After cutting off filters end, the extracts were transferred in conical 10 ml vials and the volume was reduced to 1 ml under ultra pure nitrate gas flow. 0.5 ml of the extract after addition of PAHs internal standard was analyzed by gas chromatography - mass spectrometry technique for the determination of polycyclic aromatic hydrocarbons (PAHs) content.

The other part of extract was exchanged with 0.5 ml of DMSO and then diluted with reconstituted hard freshwater to 150 mL. The reconstituted hard freshwater extracts were also diluted to 150 mL with additional reconstituted hard freshwater before use in the bioassays. A dilution series composed of five different concentrations was run for each sample.

All instrumentation was washed up by acetone and hexane prior use. The samples after centrifugation for the removal of solid endings were isolated from sunbeams and stored in freezer (-20°C) until of their analysis.

2.3 Toxicity Testing with *Vibrio fischeri*

This test was based on the measurement of bioluminescence inhibition of the marine bacteria *Vibrio fischeri* within a short exposure time. The bacteria were in freeze-dried form and activated prior to the use. The salinity of the samples was adjusted with the addition of 2% NaCl. The % bioluminescence inhibition or the EC₅₀ value (the % of sample concentration that causes 50 % effect on the test organism) of a sample was calculated as an end point. The light emitted from the control sample and the samples have been measured using the Microtox model 500 analyzer (Azur Environmental) after 30 min exposure of the bacteria (Microtox Manual, 1998).

3. RESULTS AND DISCUSSION

Table 1 gives the particle bound PAHs concentrations and the acute toxicities of airborne particles at the sampling sites. Mean particulate PAH concentrations (mean \pm standard deviation) for fine samples were 5.54 ± 6.92 ng/m³ and for coarse samples were 1.05 ± 0.81 ng/m³. At warm period (15 April - 15 October) for fine samples concentrations were 4.16 ± 6.92 ng/m³ and for coarse samples were 1.08 ± 0.95 ng/m³. Finally at cold period for fine samples concentrations were 6.57 ± 5.68 ng/m³ and for coarse samples were 1.03 ± 0.71 ng/m³.

These results indicate that the concentrations of airborne particulate PAHs varied seasonally, and generally were decreasing with increasing temperature. The decrease in particulate PAH concentrations during warmer periods may be due to the temperature dependency of vapor pressure which controls particle/gas partitioning [20]. In addition, the mean concentration of fine particulate PAHs was significantly higher than that measured in coarse, by a factor of five.

Table 1 Particulate PAHs Concentrations and Acute Toxicity of Airborne Particles

	N	Fine (ng/m ³)		Coarse (ng/m ³)	
		EC ₅₀	ΣPAH	EC ₅₀	ΣPAH
mean	22	0.03±0.02	5.54±6.20	0.25±0.55	1.05±0.81
warm	11	0.04±0.03	4.16±6.92	0.29±0.65	1.08±0.95
cold	11	0.03±0.02	6.57±5.68	0.21±0.47	1.03±0.71

All data represent mean \pm SD.

EC₅₀ values for one year period was for fine particles 0.03 ± 0.02 ng/m³ and for coarse samples 0.25 ± 0.55 ng/m³ indicating that the finer particles are more toxic to aquatic organisms than coarse, probably due to higher PAHs concentrations and other organic toxicants. At cold period both fine and coarse particles had higher but similar toxicity than during warm period. At cold period EC₅₀ values of fine samples were 0.03 ± 0.02 ng/m³ and for coarse were 0.21 ± 0.47 ng/m³. Similarly at warm period for fine samples EC₅₀ values were 0.04 ± 0.03 ng/m³ and for coarse samples were 0.29 ± 0.65 ng/m³. The results show that the toxicity of airborne particles varied seasonally but not as high as PAHs do.

With reference to investigate the toxicity of PAHs to *Vibrio fischeri* test standard PAHs samples prepared and examined for toxic effects to organisms. The samples had equivalent (for 24h air sampling) total ΣPAH concentrations 22, 33 and 67 ng/m³ with was the higher concentration found for the one year period. Figure 1 shows the EC₅₀ measured for testing samples.

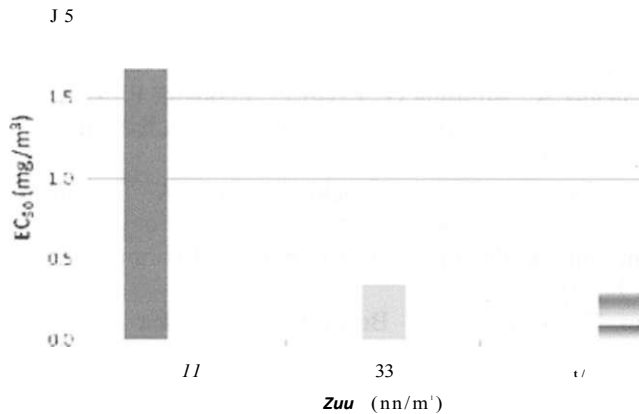


Figure 1. EC50 values of PAHs standard samples

The results have shown that the PAHs standards had an increasing toxicity with the increase of PAHs concentration. However, total toxicity of particles was significantly higher, proving the influence of other substances in the total toxicity.

4. CONCLUSIONS

Ambient samples are able to allow investigation of naturally occurring mixtures to specific organisms, the combination of bioassay techniques and ambient sampling, assimilates atmospheric transformations and thus the findings of the toxicity testing should be taken into account. However it cannot be clearly estimated whether the toxicities are attributed to specific sources.

Summarizing and according to the results obtained in this work, it could be concluded that the atmospheric particles show toxicity and higher toxicity have those with smaller diameter (fine particles). In general fine particulates exhibited increased toxic effects to *Vibrio fischeri*, thus the EC50 values are lower than those of the coarse particulate matter. The calculation of EC50 was performed by measuring the effects of five serial dilutions of each sample, in many cases further dilutions were required in order to determine the effective concentration.

Furthermore the results have shown that there is a direct relationship between the total PAHs content bound on the particulate matter, particle size lower than 10 microns, emitted from the power stations and their corresponding toxicity: the higher their PAHs content, the higher their ecotoxicity. However, total toxicity of particles was significantly higher, proving the influence of other substances in the total toxicity.

References

1. Glantz, S.A., 1993: Heart disease and the environment. *J. Am. Coll. Cardiol.*, 21, 1473-1474.
2. Seaton, A, McNee, W., Donalson, K., Godden, D., 1995: Particulate air pollution and acute health effects. *Lancet*, 345, 176-178.
3. Triantafyllou, A.G., Zoras, S., Evagelopoulos, V.: 2006, 'Particulate matter over a seven year period in urban and rural areas within, proximal and far from mining

- and power station operations in Greece, *Environmental Monitoring and Assessment*, (2006) 122:41-60.
4. Crebelli R., Fuselli S., Meneguz A., Aquilina G., Conti L., Leopardi P., Zijno A., Baris F., Carere A., 1998. In vitro and in vivo mutagenicity studies with airborne particulate extracts, *Mutat. Res.* 204 1988 565-575.
 5. Crebelli R., Fuselli S., Turrio Baldassarri S., Ziemacki G., Carere A., Benigni R., 1995. Genotoxicity of urban air particulate matter: correlations between mutagenicity data, airborne micropollutants, and meteorological parameters, *Int. J. Environ. Health Res.* 5 1995 19-34.
 6. Pagano P., De Zaiacomo T., Scarcella E., Bruni S., Calamosca ML, 1996. Mutagenic activity of total and particle-sized fractions of urban particulate matter, *Environ. Sci. Technol.* . 30 1996 3512-3516
 7. Seagrave J.; Mauderly J. L.; Seilkop S. K., 2003. In vitro relative toxicity screening of combined particulate and semivolatile organic fractions of gasoline and diesel engine emissions. *J. Toxicol. Environ. Health, Part A* 2003, 66 (12), 1113-1132.
 8. Friedman, M. S. 2001. Impact of changes in transportation and commuting behaviors during the 1996 Summer Olympic Games in Atlanta on air quality and childhood asthma. *J. Am. Med. Assoc.* 2001, 285 (7), 897-905.
 9. Zanobetti, A.; Schwartz, J.; Dockery, D. W., 1988. Airborne particles are a risk factor for hospital admissions for heart and lung disease. *Environ. Health Perspect.* 2000, 108 (11), 1071-1077.
 10. W.K. De Raat, 1988. Polycyclic aromatic hydrocarbons and mutagens in ambient air particles, *Toxicol. Environ. Chem.* 16 1988 259-279.
 11. Oanh, N. T. K.; Nghiem, L.; Phyu, Y. L., 2002. Emission of polycyclic aromatic hydrocarbons, toxicity, and mutagenicity from domestic cooking using sawdust briquettes, wood, and kerosene. *Environ. Sci. Technol.* 2002, 36 (5), 833-839.
 12. American Society for Testing and Materials (ASTM), 1995. Standard Test Method for Assessing the Microbial Detoxification of Chemically Contaminated Water and Soil Using a Toxicity Test with a Luminescent Marine Bacterium, in *Annual Book of ASTM Standards* (Philadelphia: ASTM, 1995), D5660-95.
 13. U.S. Environmental Protection Agency (U.S. EPA), 1999. Evaluation of Toxicity and Bioaccumulation of Contaminants in Sediments Samples from Waukegan Harbor, Illinois, EPA-905-R-99-009 (1999).
 14. Kahru A., Tomson K., Pall T, and Kulm I., 1996. Study of Toxicity of Pesticides Using Luminescent Bacteria *Photobacterium Phosphoreum*, *Water Science and Technology* 33 (1996):147-154.
 15. Kleinheinz G. T. and Bagley S. T., 1998. Biofiltration for the Removal and "Detoxification" of a Complex Mixture of Volatile Organic Compounds, *Journal of Industrial Microbiology and Biotechnology* 20 (1998): 101-108.
 16. Ghosh S. K., Patel T. S., Doctor P. B., Kulkarni P. K., Shah S. H, Desai N. M., and Derasari A., 2001. Study on Indoor Air Pollutants: Toxicity Screening of Suspended Particulate Matter, *Bulletin of Environmental Contamination and Toxicology* 67 (2001): 149-154.
 17. Tsiroidis V., Petala M., Samaras P., Hadjispyrou S., Sakellaropoulos G., Kungolos A., 2006. Interactive toxic effects of heavy metals and humic acids on *Vibrio fischeri* *Ecotoxicology and Environmental Safety* 63 (2006) 158-167

18. Triantafyllou A.G., Evagelopoulos V., Zoras S., 2006. Design of a web-based information system for ambient air quality data, *Journal of Environmental Management*, 80(2006) 230-236.
19. Evagelopoulos V., Albanis T.A., Triantafyllou A.G., Zoras S., Asvesta A. and Garas S., 2008. Development of a LVI - GC/MS method for detecting particle PAH bound in PM10 and PM2.5 samples. 3^o Macedonian Environmental Conference, 2008, March 14-15, Thessaloniki, Greece, 52.
20. Odabasi M., Vardar N., Sofuoglu A., Tasdemir Y., and Holsen T. M., 1999. Polycyclic Aromatic Hydrocarbons (PAHs) in Chicago Air, *Science of the Total Environment* 227 (1999):57-67.