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Eco-toxicological bioassay of atmospheric fine particulate matter ($PM_{2.5}$) with *Photobacterium Phosphoreum* T_3

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ABSTRACT

A bioluminescent bacterium, *Photobacterium phosphoreum* T_3 (PPT_3), was used as a bio-indicator for the atmospheric fine particulate matter ($PM_{2.5}$) to determine the eco-toxicity of $PM_{2.5}$. The $PM_{2.5}$ contains toxic chemicals, which reduce light output. The $PM_{2.5}$ samples were collected in the period from March 2014 to January 2015 in Nanjing and analyzed for the chemical composition versus their eco-toxicity. The eco-toxicological responses of each toxicant were detected in $PM_{2.5}$ samples with PPT_3. The dose-response curves obtained were verified using the Weibull fitting function. According to the measured EC_{50} values (EC_{50}, the concentration of a toxicant that inhibits 50% of the bioluminescence), the toxicity sequence was: B[*a*]P > hexa-PCB > tetra-PCB > tri-PCB > Pb^{2+} > DEHP > Cu^{2+} > DBP > BDE_{209} > Zn^{2+} > DMP > DEP, where B[*a*]P is benzo(a)pyrene, PCB is polychlorinated biphenyl, DEHP is diethylhexyl phthalate, DBP is dibutyl phthalate, BDE_{209} is decabromodiphenyl ether, DMP is dimethyl phthalate, and DEP is diethyl phthalate. All the $PM_{2.5}$ samples analyzed proved to be weak toxic for PPT_3. The toxicity of $PM_{2.5}$ was assessed by the dose-addition of organic species and heavy metallic elements existing in $PM_{2.5}$ with PPT_3. The bioluminescence test showed that the metals and organics detected in $PM_{2.5}$ promoted $PM_{2.5}$ toxicity. The total detectable organics (denoted by ΣOrs) exhibited slightly higher toxicity than the total metals (denoted by ΣMs). In contrast, the sum of water-soluble ions (denoted by ΣIons) was beneficial to PPT_3. The $PM_{2.5}$ toxicity increased as the $PM_{2.5}$ trapped more organics or metallic elements from the industrial or densely populated urban areas, where the $PM_{2.5}$ had a high inhibition rate of bioluminescence for PPT_3 in contrast to the residential $PM_{2.5}$ samples, where the minimum inhibition rate was observed. The toxicity of $PM_{2.5}$ samples varied with the mass concentrations, chemical constituents, and sampling locations. The chemicals in $PM_{2.5}$, especially organic species and metallic elements, affected its eco-toxicity. These data provided good understanding of the atmospheric $PM_{2.5}$ pollution in the large portion of the East China.

1. Introduction

Atmospheric fine particulate matter, having a diameter of between 0.1 and 2.5 μm, is designated as $PM_{2.5}$. Due to its small particle size, $PM_{2.5}$ has a life-time of days to weeks and can transport over thousands of kilometers and causes long-term wide-range air pollution. Likewise, owing to its ability to penetrate into the alveolar gas exchange region of human lungs, $PM_{2.5}$ is referred to as respirable particles (Kelly and Fussell, 2012). In 1997, U.S. Environmental Protection Agency promulgated $PM_{2.5}$ standard. This standard is set at 35 μg m^{-3} over a 24-h period and 12 μg m^{-3} for annual average in U. S. The $PM_{2.5}$ standards of China are 35 and 75 μg m^{-3} for annual and daily average, respectively (SEPAC, 2012).

Chinese researchers conducted $PM_{2.5}$ measurements in the early 2000s, much earlier than the Chinese $PM_{2.5}$ standard promulgated in 2012 (Lu et al., 2008). Several studies on the anthropogenic sources of $PM_{2.5}$, including industrial sources, coal combustion, motor vehicles, and secondary aerosols, have already been made in urban areas of China. The emission sources of $PM_{2.5}$ are very similar to what found in the U.S. in the 1980s (Pui et al., 2014).

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$PM_{2.5}$ emitted by anthropogenic sources is the important air pollutant responsible for the adverse health effects because of its respirable and toxic chemical constituents. These fine particles are deposited throughout human respiratory tract, causing lung disease, asthma and premature death. Kelly and Fussell (2012) indicated that $PM_{2.5}$ pollution is responsible per annum, for approximately 0.8 million premature deaths and 6.4 million years of life lost. Riley et al. (2003), Franklin et al. (2008) and Ostro et al. (2008) found that $PM_{2.5}$ composition including organic components, heavy metal elements and organic/elemental carbon (OC/EC) associated with mortality. Previous studies also reported that $PM_{2.5}$ concentration alone cannot sufficiently explain its adverse ecological effects. Many chemical compounds existing in $PM_{2.5}$ are toxic to the biosphere. For example, Ma et al. (2014) observed the probabilistic cancer risk of diethylhexyl phthalate (DEHP) in $PM_{2.5}$. Now, increasing $PM_{2.5}$ contamination is of special concern because of its potential ecological risks.

The bioluminescence test is an available technique for the measurement of environmental contamination and for the toxicity assessment of complex sample matrices in view of its sensitive, reproducible, economic, rapid, easy handling and execution (Fernández et al., 1995). Bioluminescent bacteria grow rapidly, and it is possible to measure toxic effects on several generations over a relatively short period of time (Wang et al., 2014a). They are light-emitting microorganisms which are present in marine, freshwater, and terrestrial environments (Hassan and Oh, 2010). In the presence of chemical toxicants, the bacteria lose their bioluminescence very rapidly, which provides a direct and rapid assessment of the effect of chemical toxicants on microbial metabolism (Hassan and Oh, 2010). Nowadays, the bioluminescence test is mainly used to assess the potential eco-toxicological effects of natural surface water, industrial and domestic effluents, contaminated soils, stream sediments and nanoparticles. Toxicity bioassays for testing soils, sediments and nanoparticles are carried out in water or organic solvent extracts of these solid samples (Fernández et al., 1995; Zhou et al., 2010; Wang et al., 2014a, 2014b). So far, very limited research has been conducted on atmospheric particulate matter (PM), although it is a significant air pollutant.

Despite the lack of available information in the eco-toxicity evaluation of atmospheric PM using PPT_3, there are several other eco-toxicity results already being provided in the published literatures, as reported by Serat et al.(1969), Wang et al. (1998), Vouitsis et al. (2009) and Kessler et al. (2012). In fact, bioluminescent bacteria were used as the bioindicators in the eco-toxicological tests of atmospheric particulates as early as 1969 (Serat et al., 1969). For example, Wang et al. (1998) used different trophic level organisms, *Dunaliella tertiolecta* (a dinoflagellate green alga), *Brassica chinensis* (Chinese white cabbage), *Lolium perenne* (rye grass) and *Photobacterium phosphoreum* (luminescent bacterium), to investigate the correlations between chemical data and the eco-toxicity results of urban dusts collected from Hong Kong and London. In the study of Vouitsis et al. (2009), luminescent bacteria (*Vibrio fischeri*) were used as the toxic receptor to present a thorough physicochemical characterization of PM emitted from light-duty vehicles. Results from this study demonstrated that the rate of bioluminescence produced correlated to the eco-toxicity of the samples. Recently, Kessler et al. (2012) developed a PM cytoxicity bioassay, based on microbial bioreporters, providing a comprehensive view of the cytotoxic effects of atmospheric PM. Nevertheless, to the best of our knowledge, there are no luminescent toxicity bioassays for the chemical analyses of $PM_{2.5}$ and giving information on the whole eco-toxicological impact of the atmospheric pollutant. Then, how does the bioluminescent bacteria test provide overall assessment of the potential eco-toxicity of $PM_{2.5}$? How do the chemical species existing in $PM_{2.5}$ aerosols influence the eco-toxicity of $PM_{2.5}$? Whether bioluminescent bacteria test can be used to study atmospheric particulate matter? So far information is limited on the eco-toxicological bioassay of atmospheric $PM_{2.5}$ to *Photobacterium phosphoreum* T_3 (PPT_3) (Ferreira-Baptista and Miguel, 2005; Kessler et al., 2012). These problems are yet unknown and must be addressed scientifically and rapidly.

PPT_3 is used widely in bioassay to characterize chemical toxicity in the environment (Ge et al., 2014). In this study, the eco-toxicity of $PM_{2.5}$ was assessed by bioluminescence test, in which PPT_3 was chosen as the test bacteria. The objectives of this study are: (1) to investigate the $PM_{2.5}$ distribution in Nanjing, and to determine the water-soluble ions, metals, and organics in $PM_{2.5}$; (2) to measure the effective concentrations (EC_{50}) of individual toxicants detected in $PM_{2.5}$ leading to a 50% decrease in the emission of light for PPT_3; (3) to examine the association between the dose of chemical toxicants and eco-toxicological response for $PM_{2.5}$, and to provide an quantitative assessment of the toxicity of $PM_{2.5}$ ($IR_{addition}$, the total inhibition rate of a mixture).

2. Materials and methods

2.1. Sample collection

$PM_{2.5}$ samples were collected from March 2014 to January 2015 at 3 diverse sites within Nanjing (Fig. 1). One site is located in the urban center area (denoted by S1) with scattered high-rise buildings, residential houses, schools, hospitals, heavily traffic, and several commercial shops nearby. The central part of Nanjing is mainly used for commercial and residential purposes. This site is representative of the ambient air quality in the urban area. Another site (denoted by S2), which lies in Jiangning District, the southern suburbs of Nanjing, is a new residential area. No local industries there contribute to air pollution and no significant anthropogenic sources in the immediate surroundings but several bus and train lines run through. The site S2 is considered to represent the regional background. The third, Luhe's intensive chemical industrial area (denoted by S3), is located at approximately 13 km north of the S1 site. This area is characterized by a combination of traffic, urban living, industry and croplands sources, which is close to National Road and has many industrial enterprises such as thermal plants, petrochemical companies,

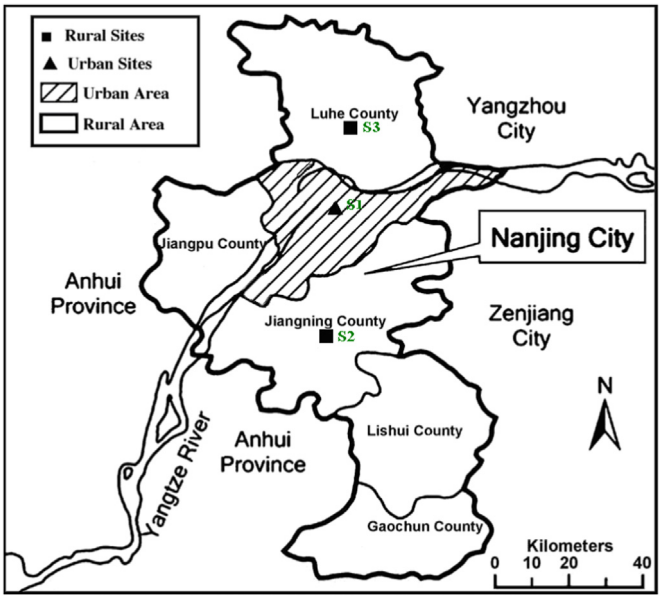

Fig. 1. Locations of S_1–S_3 sampling sites.

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