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A toxicological and genotoxicological indexing study of ambient aerosols ($PM_{2.5-10}$) using in vitro bioassays

Saida Aammi [a, *], Ferhat Karaca [b], Mustafa Petek [c]

[a] Istanbul University, Biotechnology Department, Beyazit, 34452, Fatih, Istanbul, Turkey
[b] Nazarbayev University, Civil Engineering Department, 010000, Astana, Kazakhstan
[c] Fatih University, Department of Genetic and Biomedical Engineering, 34500, Buyukcekmece, Istanbul, Turkey

HIGHLIGHTS

- PM extracts collected from the Istanbul megacity showed toxicity and genotoxicity activities.
- We found significant seasonal differences in the toxicity and genotoxicity activities.
- The source of PM directly influences the toxicity and genotoxicity activities.

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ABSTRACT

This study evaluates the toxicity and genotoxicity levels of atmospheric particulate matter (PM) samples collected at several locations of a megacity (Istanbul, Turkey) with different urban and industrial characteristics. The ambient air samples, in the form of a coarse fraction of inhalable particulates, $PM_{2.5-10}$, were collected on Teflon filters using a passive sampling method on a monthly basis during a one-year period. Later, they were extracted into both the lipophilic and hydrophilic phases using dimethyl sulfoxide (DMSO) and ultra-pure water, respectively. The obtained aqueous extracts were tested for acute toxicity and genotoxicity using the photo-luminescent bacterium *Vibrio fischeri* Microtox® and SOS Chromotest® assays, respectively. Statistically significant differences greater than background levels were obtained in both measurements, indicating the presence of toxic substances absorbed on particulate matter. The $PM_{2.5-10}$ extracts identified significant seasonal and locational differences in the toxicity and genotoxicity levels. Local anthropogenic activities and factors were associated with the quantified higher levels. Finally, a qualitative inner comparison study of regional toxicity and genotoxicity indexes was suggested to provide a clearer picture of the pollution and risk levels (or occurrences) in the Istanbul urban area. In this indexing study, the threshold levels for the urban background and episodic occurrences of the toxicity and genotoxicity levels in $PM_{2.5-10}$ samples were identified to be 1.11 TU *(Toxicity Unit)* and 8.73 TU and 0.72 IF *(Induction Factor)* and 1.38 IF, respectively.

1. Introduction

Particulate matter (PM) is a mixture of many subclasses of pollutants, each of which contain a different chemical species (Lippmann et al., 2003; Harrison and Yin, 2000). PM is considered to be the most significant environmental problem in many densely populated urban and industrials areas (Roig et al., 2013). Many toxic and harmful substances, which are responsible for adverse health effects, can be carried in the respiratory system in the form of PM (Osornio-Vargas et al., 2003; Claxton et al., 2008). According to their physical and chemical properties, PM can persist in the atmosphere for different time periods (lifetime) in their original, or often more toxic, transformed forms. The inhalable suspended particles can be subdivided into different size fractions according to their aerodynamic diameters: coarse ($PM_{2.5-10}$), fine ($PM_{2.5}$) or ultrafine particles ($PM_{0.1}$) (Mantecca et al., 2009). The fine particles are considered to be highly dangerous due to their carcinogenic characteristics since they can easily and effectively penetrate deeper in the respiratory system, into the alveolar lung regions where blood exchange occurs. However, coarse particles are also

* Corresponding author.
E-mail addresses: aammisaida@yahoo.fr, aammisaida@gmail.com (S. Aammi).

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associated with certain health risks for particular groups, e.g., asthmatics, elderly people and children (Chang et al., 2007; Yeatts et al., 2007; Ault et al., 2012), and are most frequently associated with acute health impacts such as allergy, toxic effect coughing, etc. (Bari et al., 2014; Alberg et al., 2014).

In addition to the variety of health impacts of the atmospheric aerosols, the atmospheric cleaning capabilities of atmospheric air are also limited, so it is necessary to take measures to protect against excessive air pollution. Commonly, the first step in an "air quality evaluation study" starts with sample collection if onsite measurement and identification is not possible, followed by identification of the physical and chemical parameters of the air. However, this "monitoring" or "sample and analyze" process alone does not give enough information to assess the health impacts of a wide variety of environmental pollutants.

Therefore, apart from analytical methods, it is necessary to employ a consequence analysis (e.g., comparison studies, dose-response assessments or bio-indication tests) for effective risk assessment of the potential health impacts of pollutants (Beer, 2006; Krzyzanowski, 1997; Landa et al., 1997; Mesquita et al., 2016; Izhar et al., 2016; Morales et al., 2015).

Toxicity refers to the degree to which a substance can damage a whole organism as well as the effect of the substance on a substructure of the organism, such as a cell or an organ. Genotoxicity refers to the ability of a chemical or physical agent to react with the genetic material of an organism, causing reversible or irreversible changes in its structure and altcbberations to the information carried by DNA in cells (Quillardet and Hofnung, 1985). Urban atmospheric particles, as a reservoir of toxic compounds and genotoxins, are typically dangerous for human health due to their physicochemical compositions and their ability to enter the respiratory system. From a risk assessment perspective, the toxicity and genotoxicity levels of atmospheric particles can be considered the direct consequences of air pollution exposure. Thus, biomonitoring in the form of toxicity and genotoxicity testing can be considered an essential supplement to risk assessment studies in atmospheric pollution research. As a result, both acute toxicity and genotoxicity tests have gradually been applied to assess the impacts of pollutants on the environment (e.g., water, soil, food, etc.) during the last decade; however, only a limited number of studies exist on atmospheric particles (Mesquita et al., 2016; Ozaki et al., 2012; Sheesley et al, 2004, 2005; Schauer et al., 2001; Adamson et al., 1999; Filep et al., 2015; Traversi et al., 2015; Novak et al., 2014; Pereira et al., 2013; Piekarska et al., 2011). The use of bacterial in vitro assays such as Microtox® and SOS Chromotest® have been applied as the traditional methods for toxicological evaluation of environment mixtures (Sheesley et al, 2004, 2005; Schauer et al., 2001; Adamson et al., 1999; Filep et al., 2015; Traversi et al., 2015). There are only a few reports in the literature on the testing of particulate extracts using the SOS Chromotest assay that show a high correlation to *Salmonella typhimurium* (Škarek et al., 2007a; Gilli et al., 2007; Claxton et al., 2004; Buschini et al., 2001; Škarek et al., 2007b). The genotoxicity activity of environmental mixtures is often determined with *Salmonella typhimurium* (Ames test). However, the application of the SOS Chromotest assay for the detection of genotoxicity in air pollution has many advantages, such as the testing of many environmental samples in a short time (24 h), the use of fewer laboratory consumables, and the identification of all changes in cellular DNA (which is not identified using the Ames test). All chemical compounds that can induce DNA damage and lead to mutation are considered to be genotoxic.

The main goal of the present study is to assess the toxicity and genotoxicity levels of atmospheric PM samples collected in several locations of a megacity (Istanbul, Turkey) with different urban, land use and anthropogenic impact characteristics. This study evaluates the seasonal and spatial differences of the measured parameters and provides regional indexes on the airborne PM toxicity and genotoxicity levels.

2. Materials and methods

2.1. Sampling sites, collection and extraction

For the purpose of this study, samples of airborne particulate matter PM were collected from three districts, namely the Esenyurt, Beylikduzu, and Buyukcekmece districts in Istanbul, Turkey. These areas are characterized by the fastest population, urbanization, and industry growth rates during the last decade in the megacity context. They are located in the northwestern side of Istanbul, forming an identifiable zone.

Ten sampling sites were installed in this area using grid base (1 km × 1 km) locations. Two additional sampling sites were employed in this study to provide a systematic comparison and reference for the pollution toxicity and genotoxicity levels. One of these reference sites was the "clean air site", and the other was the "polluted air site".

The clean air site was used to identify the background atmospheric PM toxicity and genotoxicity levels. This site was located on the eastern coastline of the Turkish Black Sea border in the Cilingoz National Park, apart from human settlements and in the upwind direction of the megacity, approximately 70 km away from the study area.

The polluted air site was located in Dilovasi, which was identified as one of the most polluted (anthropogenic) local air zones in Turkey in earlier national studies (Ozturk et al., 2015; Hamzaoglu et al., 2011; Karaca, 2012). Dilovasi is approximately 85 km away from the sampling area and is heavily polluted by its local industry. The locations of the study area, sampling sites and reference sites are given in Fig. 1. The characteristics of the sampling sites including potential local PM emission sources are summarized in Table 1. Approximately 30-day sampling campaigns were performed during a one year period, and a minimum of nine samples per site were collected from March 2014 to February 2015.

Coarse size PM samples ($PM_{2.5-10}$) were collected directly on two

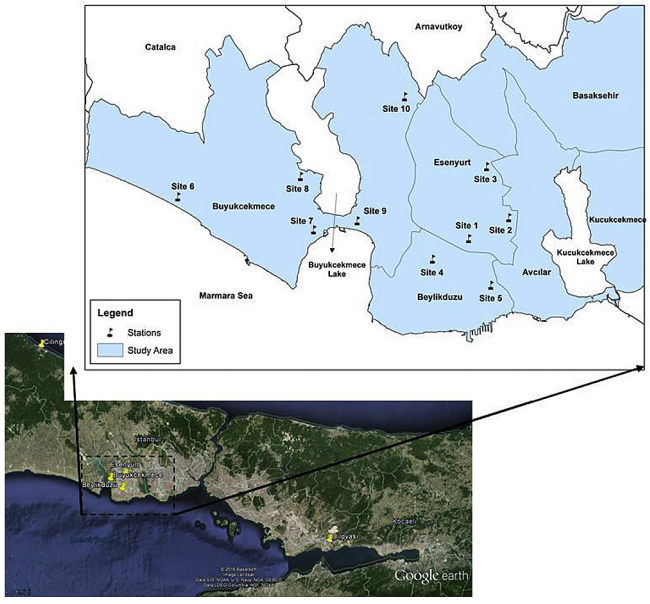

Fig. 1. Sampling sites in the study area.