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Toxicity assessment of atmospheric particulate matter in the Mediterranean and Black Seas open waters

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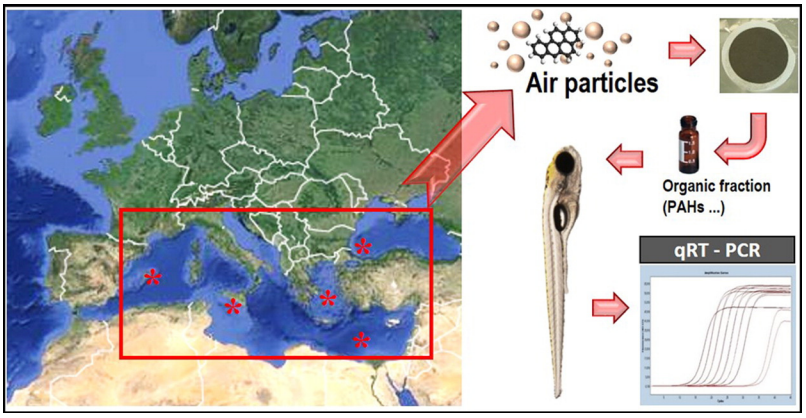
HIGHLIGHTS

- PM organic constituents from the Mediterranean basin showed dioxin-like activity.
- Dioxin-like activity correlated with the samples' content in PAHs.
- Induction of some air pollution-related genes also correlated to PAH content.
- Pancreatic gene markers showed a distinct geographical pattern of activation.
- Airborne PM can increase the toxic burden of aquatic organisms.

GRAPHICAL ABSTRACT



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ABSTRACT

Atmospheric deposition of particulate matter (PM) is recognized as a relevant input vector for toxic compounds, such as polycyclic aromatic hydrocarbons (PAHs), into the marine environment. In this work we aimed to analyse the biological activity and potential adverse effects of PM constituents to aquatic organisms. Organic extracts of atmospheric PM samples from different sub-basins of the Mediterranean and Black Seas were screened using different toxicological tests. A yeast-based assay (AhR-RYA) revealed that dioxin-like activity correlated with the concentration of total PAHs in the PM samples, as well as with their predicted toxic equivalent values (TEQs). Although the zebrafish embryotoxicity test (the ZET assay) showed no major phenotypical adverse effects, up-regulation of mRNA expression of *cyp1a*, *fos* and development-related genes (previously described as related to PM toxicity) was observed in exposed embryos when compared to controls. Results showed that mRNA patterns of the studied genes followed a similar geographic distribution to both PAH content and dioxin-like activity of the corresponding extracts. The analysis also showed a distinct geographical pattern of activation of pancreatic markers previously related to airborne pollution, probably indicating a different subset of uncharacterized particle-bound toxicants. We propose the combination of the bioassays tested in the present study to be applied to future research with autochthonous species to assess exposure and potential toxic effects of ambient PM. The

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present study emphasizes the need for more in-depth studies into the toxic burden of atmospheric PM on aquatic ecosystems, in order to improve future regulatory guidelines.

1. Introduction

Ambient particulate matter (PM) is considered as one of the most harmful air pollutants to human health by different regulatory agencies (EEA, 2012; WHO, 2004). One of the major contributors to its toxic effects are organic compounds, such as polycyclic aromatic hydrocarbons (PAHs), and their derivatives of photochemical oxidation (Cavanagh et al., 2009; Kanakidou et al., 2005; Mesquita et al., 2014b).

PAHs are formed through the incomplete combustion of organic matter (e.g. biomass burning, fossil fuel-burning, motor vehicle exhaust, waste incineration, home heating) and are considered priority pollutants due to their mutagenic, carcinogenic and teratogenic properties (EC, 2000; IARC, 1998; Zhang and Tao, 2009). Once emitted into the atmosphere, PAHs tend to bind to the soot carbon of air particles due to their semi-volatile character (Dachs and Eisenreich, 2000; Lohmann and Lammel, 2004). When coated onto ambient PM, PAHs have relatively long half-lives, persisting in the environment and travelling long distances before they deposit. Particle-bound PAHs can be removed from the atmosphere through dry deposition (i.e. gravitational settling) or wet deposition (scavenging by rain, snow, fog).

Researchers have highlighted the importance of atmospheric dry deposition as a main vector for the entrance of the most hydrophobic PAHs into coastal aquatic systems and open oceans (Arzayus et al., 2001; Castro-Jiménez et al., 2012; Franz et al., 1998; González-Gaya et al., 2014; Jurado et al., 2004; Kroflič et al., 2015). In parallel, an increasing number of studies have been directed to the assessment of the potential toxic burden of airborne particle-bound PAHs to aquatic biota (Mesquita et al., 2014a,b; Sheesley et al., 2004, 2005). Indeed, an effort has been made to broaden the scope of atmospheric aerosol toxicology to begin to include effects on aquatic organisms (Mesquita et al., 2014a,b; Sheesley et al., 2004, 2005). However, regulatory directives do not contemplate the effects of airborne pollution to aquatic organisms. The latest air quality report of the European Environmental Agency (EEA) addresses the need to protect the environment from the adverse effects of particulate air pollution, but no critical level, target/limit value or long-term objective is defined for PM (neither for airborne PAHs), in order to protect terrestrial or aquatic organisms (EEA, 2014).

The Mediterranean and Black Seas are semi-enclosed basins surrounded by highly populated countries, with corresponding high levels of atmospheric PM. As a consequence, air masses laden with anthropogenic PM move across the Mediterranean and Black Seas, partially depositing into water. In particular, atmospheric deposition has been referred to as a significant non-point source of PAHs in remote (i.e. non-coastal) areas of the Mediterranean and Black Seas (Castro-Jiménez et al., 2012; Grimalt et al., 1988; Lipiatou et al., 1997; Parinos et al., 2013; Tsapakis et al., 2003, 2006) and in Mediterranean Sea sediments (Lipiatou et al., 1997; Tsapakis et al., 2003).

The organic constituents of atmospheric PM from urban, semi-rural and rural environments have been shown as biologically active and capable of inducing adverse effects on aquatic species, with strong correlations with the PAH content of samples (Mesquita et al., 2014b, 2015; Olivares et al., 2011, 2013; Sheesley et al., 2004, 2005). However, no information is available on the biological activity and effects of PM organic constituents from the atmosphere of open marine environments, such as the Mediterranean and Black Seas. The concentrations of PM organic constituents on such environments, distant from emission sources, are expected to be lower than over continents, however the mixture of chemical compounds bounded to PM should be biologically active, possibly contributing to the toxic burden of marine biota.

A fundamental aspect determining the toxic effects of organic compounds, such as PAHs, is their ability to bind and activate the aryl hydrocarbon receptor (AhR), a key regulator of detoxification cascades (Hankinson, 1995; Nebert et al., 1993). This biological activity is commonly known as dioxin-like activity. This activity can be monitored by the AhR-recombinant yeast assay (AhR-RYA), in which the AhR is challenged with extracts of PM samples to determine their toxic potential (Mesquita et al., 2014b; Olivares et al., 2011).

The zebrafish (*Danio rerio*) embryo is a widely used vertebrate model in toxicology (Westerfield, 2000). The zebrafish embryotoxicity (ZET) test provides a unique opportunity to analyse survival, morphological alterations, and specific gene expression changes that bring insight on the toxic mechanism of action of PM constituents (Mesquita et al., 2014b; Olivares et al., 2011). The use of transcriptomic tools on zebrafish embryos has proven successful to study and early anticipate potential adverse outcomes of environmental pollutants (Mesquita et al., 2015; Piña and Barata, 2011; Raldúa et al., 2012; Scholz et al., 2008).

In a previous work, we used microarray technology to identify genes of interest to be used as potential markers for the biological effects of PM organic constituents (Mesquita et al., 2015). These genes included the cytochrome P450 1a (*cyp1a*), a classical AhR-responsive gene, and other genes related to xenobiotic response, proliferation and inflammation, and pancreatic exocrine function (Mesquita et al., 2015). In the present study we tested the organic extracts of atmospheric PM samples from the Mediterranean and Black Seas, using the AhR-RYA and the ZET assays. The expression of those genes of interest was determined on exposed embryos, by quantitative reverse transcription-polymerase chain reaction (qRT-PCR). The results were correlated with the PAH composition of PM samples.

The working hypothesis is that the chemical mixture of organic compounds bound to PM from the marine area under study, should elicit measurable biological effects, possibly contributing to the toxic burden of aquatic organisms. To the best of our knowledge, this is the first study to analyse the biological activity and toxic potential of atmospheric PM organic constituents in open waters from different sub-basins of the Mediterranean and Black Seas.

2. Material and methods

2.1. Study area and sampling

The Mediterranean and Black Seas are semi-enclosed environments, with areas of about 2.5×10^6 km^2 and 4.2×10^5 km^2, respectively. The Mediterranean Sea can be divided in Western and Eastern basins, and subdivided in different sub-basins. In the present work, we analysed 29 samples from Western Mediterranean Sea (W Med), Ionian Sea, Aegean Sea, South-East Mediterranean Sea (SE Med), and Black Sea (Fig. 1). Air samples were collected on board the R/V Garcia del Cid during two sampling cruises made on June 2006 and May 2007 (Fig. S1). Both campaigns started and finished in Barcelona (Spain), with Istanbul (Turkey) and Alexandria (Egypt) as intermediate stops (Fig. 1). All further details regarding the sampling procedures and strategy can be found in Castro-Jiménez et al. (2012). Briefly, air samples were collected using a high-volume air sampler (MCV, Barcelona, Spain), located at the upper deck of the boat close to the bow. Samples were collected while cruising (transects), with the exception of samples P6, P7, P13, P27, P28 and P29, which were collected at stations (Fig. 1, Table S1). Samples were generally collected within twelve hours (average volume of