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Marine aerosol as a possible source for endotoxins in coastal areas

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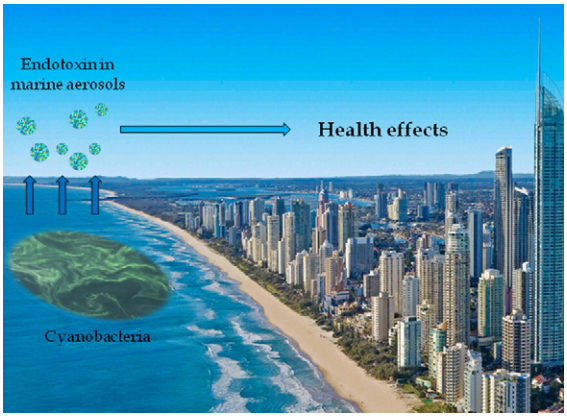
HIGHLIGHTS

- Comparison of endotoxin content in sampled marine aerosols in two sites: on-shore and coastal-inland.
- Endotoxin annual distribution as well as bacterial genome content is analyzed.
- Cyanobacteria are suggested as a source for endotoxins at coastal areas.
- Satellite images and back trajectory analyses provide supporting evidence.

GRAPHICAL ABSTRACT



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ABSTRACT

Marine aerosols, that are very common in the highly populated coastal cities and communities, may contain biological constituents. Some of this biological fraction of marine aerosols, such as cyanobacteria and plankton debris, may influence human health by inflammation and allergic reactions when inhaled. In this study we identify and compare sources for endotoxins sampled on filters in an on-shore and more-inland site. Filter analysis included endotoxin content, total bacteria, gram-negative bacteria and cyanobacteria genome concentrations as well as ion content in order to identify possible sources for the endotoxins. Satellite images of chlorophyll-a levels and back trajectory analysis were used to further study the cyanobacteria blooms in the sea, close to the trajectory of the sampled air. The highest endotoxin concentrations found in the shoreline site were during winter (3.23 ± 0.17 EU/m^3), together with the highest cyanobacteria genome (1065.5 genome/m^3). The elevated endotoxin concentrations were significantly correlated with cyanobacterial levels scaled to the presence of marine aerosol ($r = 0.90$), as well as to chlorophyll-a ($r = 0.96$). Filters sampled further inland showed lower and non-significant correlation between endotoxin and cyanobacteria ($r = 0.70$, P value $= 0.19$), suggesting decrease in marine-originated endotoxin, with possible contributions from other sources of gram-negative non-cyanobacteria. We conclude that marine cyanobacteria may be a dominant contributor to elevated endotoxin levels in coastal areas.

Abbreviations: Cl-, Chloride; chl-a, Chlorophyll-a; cyano/Na, Cyanobacteria concentrations/Na^+ content; HCl, Hydrochloric acid; LAL, Limulus amebocyte lysate; LPS, Lipopolysaccharids; MDL, Method detection limit; MODIS, Moderate Resolution Imaging Spectro-radiometer (MODIS); NFW, Nuclease free water; LRW, Pyrogen-free water; qPCR, Quantitative PCR; Na+, Sodium; CSE, Standard endotoxin; DECOS, The Dutch Expert Committee on Occupational Safety and Health.

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1. Introduction

Marine aerosols are a significant portion of the global aerosol load. They are composed of inorganic sea-salt ions and organic material that includes carbohydrates, lipids, microorganisms, marine viruses and algae (Fitzgerald, 1991; Rinaldi et al., 2010; Spracklen et al., 2008; Vignati et al., 2010). The biological components are primarily emitted into the atmosphere through a bubble bursting mechanism, usually as a part of a mixed aerosol composed of other organic and inorganic compounds (Aller et al., 2005; Després et al., 2012; O'Dowd et al., 2004). It was suggested that during microbial or algal blooms, the biological fraction in marine aerosols increases, with detectable enhancement of the organics in the aerosolized matter compared to the sea-surface microlayer (O'Dowd et al., 2004). This view has been recently challenged (Quinn et al., 2014). However, while the total organic content may not change significantly, its composition could be affected by the biological content in the water.

Some marine microorganisms and algae contain a variety of exotoxins that are secreted from the organism, and can damage different mammalian tissues (Alexander and Rietschel, 2001; Gentien and Arzul, 1990). These materials can be aerosolized and transported to the coast by the winds (Pierce, 1986). Other toxic compounds such as endotoxins, inherent compounds of the organism (mostly lipopolysaccharids, LPS, in gram-negative bacteria), may be exposed when cells are damaged and disintegrate (Galanos and Freudenberg, 1993). The LPS endotoxin is the most abundant component in the gram-negative cell wall, and can stimulate acute inflammatory response towards pathogens (Galanos and Freudenberg, 1993; Ngkelo et al., 2012; Sweet and Hume, 1996). Previous investigations on exotoxins emitted during the Florida Red Tide, *karenia brevis*, reported human exposure levels to brevetoxin, which is secreted from the organisms during the bloom (Pierce et al., 2003). Cyanobacteria which are common in bloom events in fresh (Oliver and Ganf, 2002) and sea water (Paerl, 2002) can also be an important marine source for endotoxin. High endotoxin levels are commonly reported at agricultural areas (Castellan et al., 1987; Spaan et al., 2006), in relation to high PM10 (Heinrich et al., 2003; Mueller-Anneling et al., 2004) after floods (Solomon et al., 2006) and indoors (Gehring et al., 2002; Gereda et al., 2000). However, despite the vast abundance of cyanobacteria, records of aerosol-borne endotoxin levels in coastal locations have not been reported. This may have significant implications as the population at coastal areas is constantly increasing globally (Wilson and Fischetti, 2010).

The eastern Mediterranean Sea, a semi-enclosed olygotrophic sea with low amounts of nutrients, is a favorable environment for cyanobacteria growth compared to algae and larger phytoplankton (Rahav et al., 2013; Yogev et al., 2011). Relatively high concentrations of cyanobacteria were reported in both pelagic and coastal waters of the eastern Mediterranean, suggesting that it may be an important source of endotoxins in coastal Mediterranean cities (Efrati et al., 2013).

In this study we examined the possible source for endotoxins extracted from aerosols collected on filters sampled directly on the eastern Mediterranean Sea shore, compared to aerosols sampled further inland. Both sampling sites were in an urban location. We correlated the measured endotoxin levels to the presence of gram-negative cyanobacteria, gram-negative bacteria and total bacterial DNA content. While detecting endotoxins on filter-samples cannot provide information about their sources, coupling their measured values with genomic analysis of biological species or chemical tracers in the aerosol may be a useful tool for better characterization of their sources. Sea salt aerosol sodium (Na^+) and chloride (Cl) are useful markers for the determination of the contribution of marine aerosols to the collected mass on the filter. While the Na^+ concentration in the aerosol is quite stable, chloride can react with sulfuric or nitric acid and form labile hydrochloric acid (HCl) leading to its depletion in aged sea salt aerosol (Finlayson-Pitts and Pitts, 2000; Moeller, 1990). Correlating meteorological conditions, aerosol composition and chlorophyll-a (chl-a)-related satellite data with genomic and endotoxin levels may thus enable to differentiate between terrestrial and marine sources for endotoxins in coastal locations.

2. Methods

2.1. Aerosol sampling

Ambient air was sampled on the rooftop at two locations (see Fig. 1): the National Institute of Oceanography, located directly on the Mediterranean Sea shore, in Haifa bay (32.8249 N, 34.9553E, on-shore site), and at the Weizmann institute of Science, located in the city of Rehovot (31.9075 N, 34.8092E, located about 11.5 km from the shore, costal-inland site). Thermally pretreated (450 °C) 20.3 × 25.4 cm^2 quartz filters (Whattman) were stored at −20 °C until sampling, using high volume sampling (HVS3000, Ecotech) at atmospheric pressure, with a 10 micrometer cutoff diameter head, for a period of 72 hr, with flow rates kept on 67.8 m^3 hr^{-1}. After sampling, the filters were wrapped with aluminum foil and stored at −20 °C until the end of the sampling campaign, and then archived at −80 °C until analyses were carried out to avoid degradation of the organic and biological material. To check for sampler contaminations, blank samples were taken, in which filters were placed in the sampler cascade for 1 minute, without operating the air-pump.

2.2. Gravimetric analysis

Filter cuts (1×1 cm^2) were weighted using a microbalance scale (BP-121S, Sartorius) before and after sampling. Before weighting, each filter cut were placed in Petri dish and equilibrated at constant room temperature (23 °C) and relative humidity (60%) for 24 h.

2.3. Endotoxin analysis

Endotoxins were extracted from 1 × 1 cm^2 subsampled filters, shaken in 1 mL pyrogen-free water (LRW, Associate of Cape Cod, Inc.) for 60 min at room temperature. The samples were than centrifuged at 2000 RPM for 10 min, as previously described in Thorne et al. (2003). The endotoxin concentration in the collected particles was determined using the Limulus Amebocyte Lysate (LAL) commercial kit (Cape Cod Inc.), reported in endotoxin unit (one unit equivalent approximately to 100 pg of E. coli lipopolysaccharide, EU) per air volume. For each assay, standard curves were generated over the concentration range 0.187–50 EU/mL using a standard endotoxin (CSE, Escherichia coli O113:H10; Associate of Cape Cod, Inc.). As it is not clear if endotoxins on sampled filters could interact with other sampled compounds, leading to reduction in extraction efficiency (Mueller-Anneling et al., 2004), we spiked standard dilutions on sampled-filter cuts and used the same extraction method as for the filter samples. The reaction included 50 μl of endotoxin standard, sample-extracted endotoxins or blanks, in a pyrogen-free microtiter plates (TC MicroWell 96 F SI w/lid 167008, Nunc) and 50 μL of LAL reagent (Pyrotell LAL, Associate of Cape Cod, Inc.) in triplicates. The Method detection limit (MDL) and precisions were 0.001 EU m^{-3} and ±8.4%, respectively. The plate was placed in a microplate reader (Synergy™ HT Multi-Mode Microplate Reader, BioTek), agitated to mix the lysate and sample, and the assay was carried at 37 °C for 1.5 h. Absorption measurements at 405 nm were taken every 5 min.

2.4. Genomic analyses

Extraction of DNA was performed directly from 1 × 1 cm^2 subsampled filters, using the PowerSoil DNA Isolation Kit (MO BIO Laboratories Inc.), as previously described (Hospodsky et al., 2010; Lang-Yona et al., 2012). The concentrations of total, gram-negative and cyanobacteria in the sampled aerosols were determined using quantitative PCR (qPCR) instrument (StepOnePlus Real-Time PCR, Applied Biosystems