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Marine aerosol as a possible source for endotoxins in coastal areas

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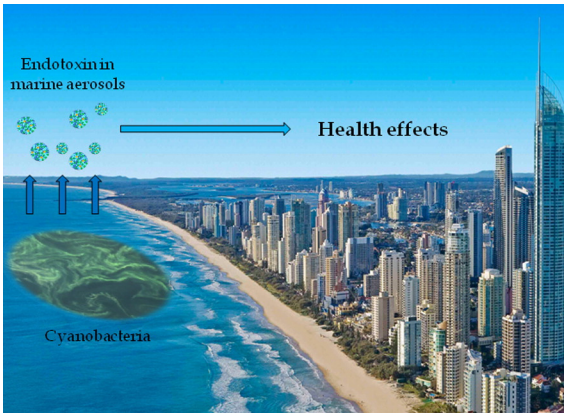
HIGHLIGHTS

- 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

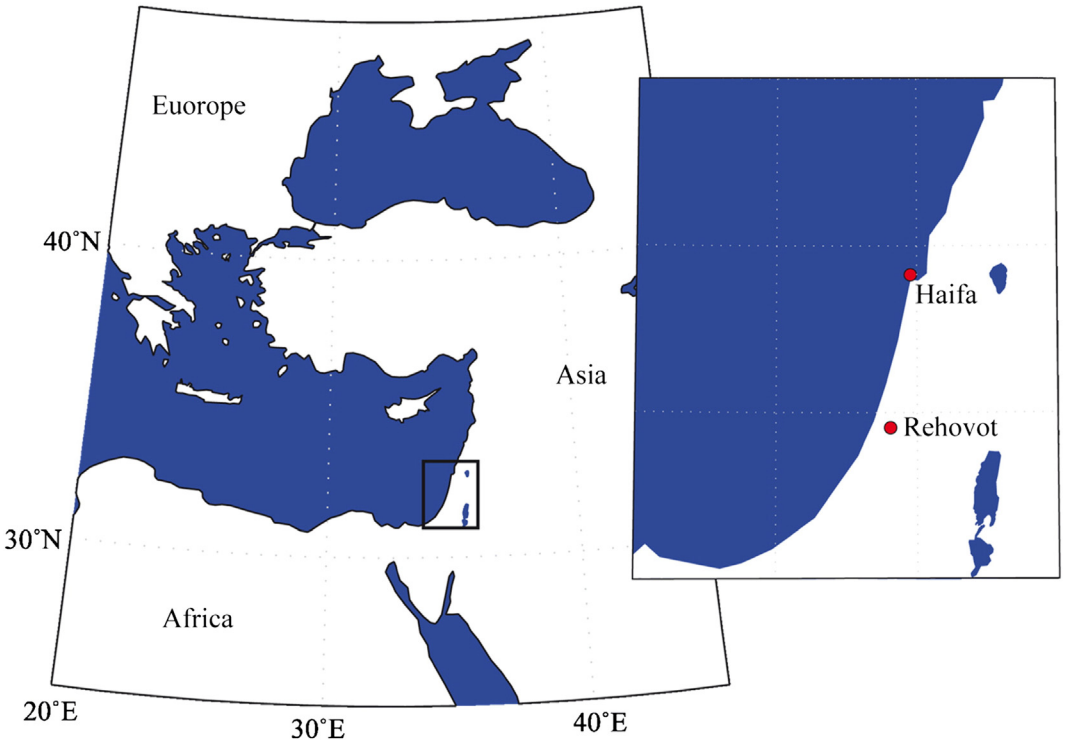

Fig. 1. A map presenting the on-shore sampling site (Haifa), as well as the coastal-inland site (Rehovot).

Inc.) with the Taqman method described previously (Greisen et al., 1994; Nadkarni et al., 2002; Nübel et al., 1997; Rinta-Kanto et al., 2005; Urbach et al., 1992), see Table S1. Calibration curves were derived using standard DNA extracted from *E. coli* K12 (for total and gram-negative bacteria), and Synecocystis 6803 (for cyanobacteria). Triplicates of 10 μl reaction mixtures consisting of 5 μl Gene expression Taq man Master Mix (Applied Biosystems), 1 μl extracted DNA, 0.5 μl of each primer (10 μM), 0.5 μl probe, and 3 μl nuclease free water (NFW). The thermal cycling conditions consisted of an initial 10 min denaturation and enzyme activation at 95 °C, followed by 45 cycles of 15 s denaturation at 95 °C, and 60 s annealing and extension at 60 °C. The MDL was 216.9, 21.6 and 3.5 genome/m^3 for total bacteria, gram-negative bacteria and cyanobacteria, respectively. Bacterial concentration of cyanobacteria and gram-negative bacteria are reported in bacterial copy number per air volume. Total bacteria concentration is represented by 16S copy number (Klappenbach et al., 2001; Větrovský and Baldrian, 2013) and not by genome concentration, as the ribosomal genes are multi-copy genes (Nadkarni et al., 2002). Thus the gene copy-number does not reflect the number of the bacterial copies.

2.5. Back trajectory analysis and satellite data

Back trajectories were calculated using the HYSPLIT Trajectory model (Draxler and Rolph, 2014; Rolph, 2014) for the two sampling locations. By following a parcel of air backward in time, the trajectories provide an estimate to the central path of the air mass before arriving to the sampling location at a given time. Three 24 h trajectories were back-calculated for each 72 h sampling, starting at 50 m above sea level.

Surface chl-a concentrations were derived from the Moderate Resolution Imaging Spectro-radiometer (MODIS) aboard the Aqua satellite. The dataset is comprised of 4 km Level 3 images obtained from the ocean color data distribution site (http://oceandata.sci.gsfc.nasa.gov/). Time series were extracted by averaging all available data over the eastern Mediterranean pelagic waters (23–35E/32.5–36 N), with every time step representing averaged data from 4 consecutive days. Regional winter, spring, summer and autumn Chl-a maps were extracted by averaging data from 15 consecutive images, centered at February 2, April 15, July 15 and October 15, respectively.

2.6. Statistical analysis

Statistical analyses were performed using Origin 9.1 software (OriginLab Corporation, USA). The different parameters (endotoxins, chl-a, total bacteria, gram-negative bacteria, cyanobacteria, cyano/Na and PM 10) were tested for correlation using Spearman correlation test. Correlation was considered significant when $P_{value} < 0.05$. Differences between the two sampling sites for the different parameters were tested using Fisher exact test. The two set were considered significantly different when $P_{value} < 0.05$.

3. Results and discussion

To investigate whether marine aerosols are a possible source for endotoxins, we compared between filters sampled in two close, although different locations: Haifa, located directly on the shoreline of the Mediterranean Sea (on-shore site), and Rehovot, located further inland about 11 km from the coast (Coastal-inland site). The first observed difference between the two locations is demonstrated by the ionic composition of the sampled aerosols (Figure S1), showing higher amounts of sea salt at the on-shore samples. In addition, the aging state in specific samples, indicated by the extensive Cl^-- depletion as well is higher in the costal station. It is established that chemical processes involving secondary aerosol precursors (SO_2, H_2SO_4, NH_3 and HNO_3) and anthropogenic pollutants (SO_2, and NOx) (Moeller, 1990; von Glasow, 2008), may lead to Cl^- depletion, mostly by acid displacement of the more labile acid (HCl). Episodes of high pollution (occasionally detected in the on-shore sampling location (Eitan et al., 2010)), could explain the different depletion levels observed. Nevertheless, we did not identify any correlation between the aging levels of the aerosol and endotoxin or bacterial content. In addition, at the coastal-inland site all samples show low levels of sea salt, therefore the Na^+ to Cl^- ratio is sensitive to noise, therefore less representative.

Sampled filters from the on-shore site and the coastal-inland site that have similar or close sampling dates were compared. We focused on 25 samples from the on-shore station, and 20 from the coastal-inland station (see Table S2). The values of endotoxins (A) total bacteria (B), gram-negative bacteria (C) cyanobacteria (D), and the corresponding PM10 (E) for both on-shore and coastal-inland sites are shown in Fig. 2. The shaded bars (Roman numbers) represent specific events where bacterial concentrations from filters sampled at the on-shore site were higher than the seasonal average at all levels (i.e. total bacteria, gram-negative bacteria and cyanobacteria). These specific events are indicated in Table S2 in bold, and summarized in Table S3. The cyanobacteria concentrations were further analyzed and scaled to the Na^+ content (see Table S4) detected on the filter (cyano/Na), yielding the cyanobacteria concentration per sea-salt aerosol mass (μg). This calculation was performed assuming that cyanobacteria concentrations are reflected by the marine-originated fraction of aerosols, at least in the on-shore site.

We observed in the fourth event at the on-shore site (27/05/2010) high PM 10 and total bacteria levels, as well as high Al concentrations (Table S5), however no significant increase in endotoxins was detected. It is suggested that higher wind speeds associated with low pressure system, led to increased sea spray and the consequent primary aerosol emission, and the cyanobacteria and endotoxins content in the aerosols. For an elaborate discussion, see SI (P. 14).

Seasonally averaged values and range of endotoxins and the bacterial concentrations are summarized in Table 1. The highest endotoxin, cyanobacteria and cyano/Na averaged levels at the on-shore samples were detected in the winter (2.34 ± 0.09 EU/m^3, 483.97 ± 257.45 genome/m^3 and 33103.18 ± 11507.63 genome/μg respectively). Both total bacteria content as well as PM10 highest averaged values were observed during winter (5744.29 ± 1118.2 16S gene/m^3 and 230.4 ± 60.3 μg/m^3 respectively), while gram-negative bacteria highest averaged values were observed in the spring (2116.34 ± 624.09 genome/m^3). At the coastal-inland samples, the highest endotoxins and cyanobacteria averaged levels were observed during spring (0.98 ± 0.36 EU/m^3 and 381.7 ± 46.15 genome/m^3), while cyano/Na, total bacteria and PM 10 highest averaged levels were observed in the winter (6006.5 ± 1738.0 genome/μg, 42045.4 ± 12166.1 16S gene/m^3 and 263.6 ± 17.3 μg/m^3 respectively). Gram-negative highest average values were obtained during autumn (3237.2 ± 531.95 genome/m^3).

We found significant correlations between endotoxin values and cyanobacteria, cyano/Na and total bacteria in the on-shore station (Haifa, r = 0.59, 0.48 and 0.48 respectively, Table S6). In the coastal-inland site (Rehovot), endotoxin significantly correlated with cyanobacteria and total bacteria (r = 0.49 and 0.68 respectively), however, the correlation to cyano/Na was weak and insignificant. It is noted that cyano/Na highly correlated with PM 10 in both the on-shore and the coastal-inland sites for the entire dataset (r = 0.71 and 0.73 respectively). The reasons for the high correlations between PM10 and cyano/Na are different for the two sampling sites. In the on-shore sampling site an inverse correlation between dust and marine aerosols is observed, as was previously shown in this region (Furman, 2003). Therefore, when PM 10 increases, Na^+ levels decrease, and hence the cyano/Na value increase. We suggest that it is probably not due to a direct link between cyanobacteria and high PM10. This also explains the fact that cyanobacteria absolute values did not show high correlation with PM10 values in this sampling location.

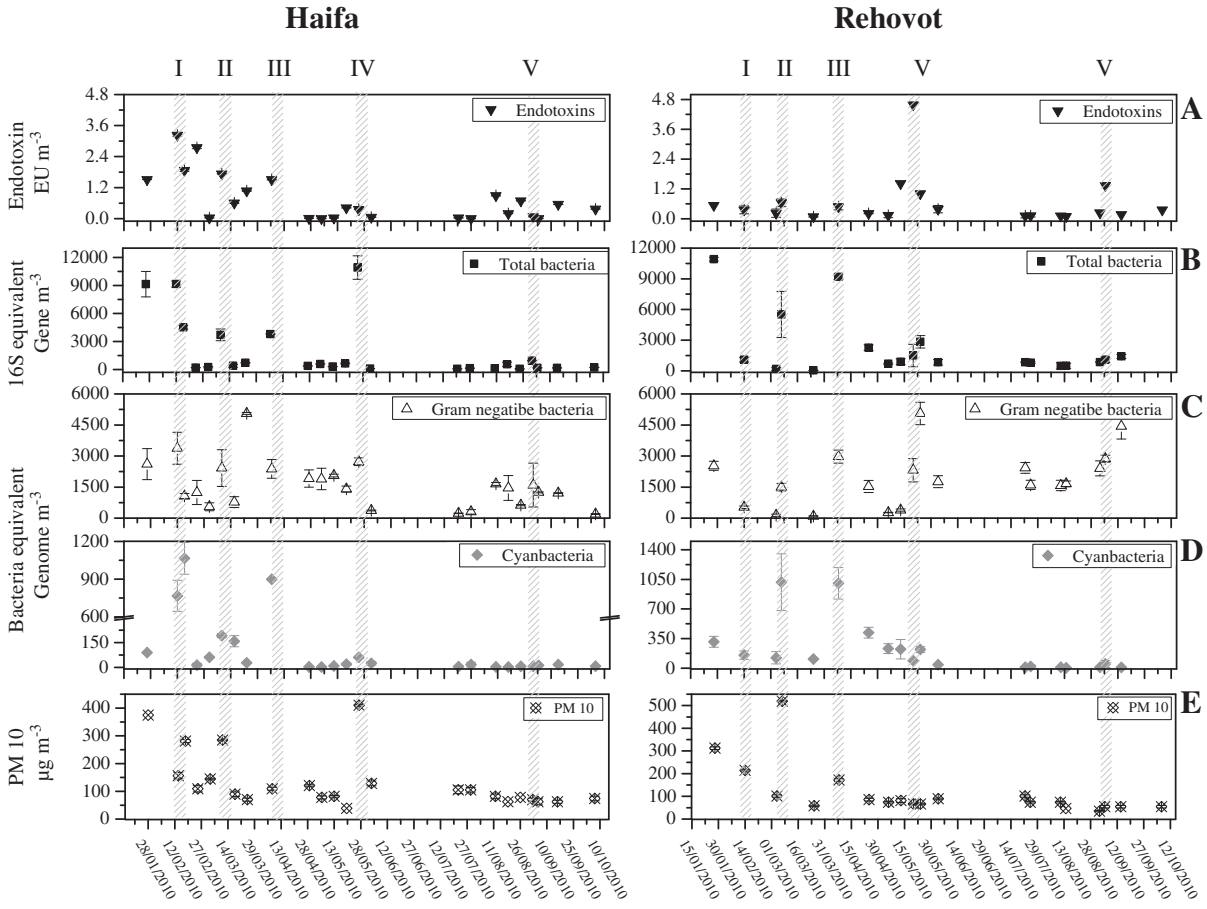

Fig. 2. Annual distribution of endotoxins (A), total bacteria (B), gram-negative bacteria (C), cyanobacteria (D) and PM 10 (E) at both Haifa (on-shore site) and Rehovot (coastal-inland site). Shade bars (I–V) represent the dates where consistent increase was observed in all genomic levels.

Table 1
Seasonally averaged values (range) of endotoxins, total, gram-negative and cyanobacteria, cyano/Na, and PM 10 detected on the filters.

	Endotoxin (EU/m^3)	Total bacteria (16S gene/m^3)	G. negative (genome/m^3)	Cyanobacteria (genome/m^3)	Cyano/Na (genome/μg)	PM 10 (μg/m^3)
Haifa						
Winter	2.34 (1.51–3.23)	5744.29 (153.3–9157.2)	2072.42 (1065.9–3375.4)	483.97 (13.9–1065.5)	33103.18 (234.5–100403.8)	230.39 (109.34–374.88)
Spring	0.57 (0.00–1.73)	1927.2 (259.5–10922.3)	2116.34 (552.0–5056.5)	143.22 (2.2–898.7)	4171.92 (83.5–18866.4)	142.7 (39.05–410.02)
Summer	0.31 (0.00–0.89)	198.8 (73.8–568.3)	773.9 (234.8–1650.8)	9.69 (2.1–24.6)	298.5 (64.2–946.4)	93.7 (62.48–128.86)
Autumn	0.25 (0.00–0.55)	246.3 (147.6–623.1)	947.6 (198.2–1596.7)	8.44 (6.1–17.2)	113.6 (73.6–168.7)	67.4 (62.33–74.19)
Entire year	0.75 (0.00–3.23)	1856.8 (73.8–10922.3)	1597.9 (198.2–5056.5)	144.5 (2.1–1065.5)	7349.1 (73.6–100403.8)	132.6 (62.33–410.02)
Rehovot						
Winter	0.45 (0.36–0.54)	6006.5 (1090.6–10922.3)	1526.2 (526.6–2525.8)	233.0 (153.6–312.5)	388863.8 (65294.1–712433.4)	263.6 (214.77–312.40)
Spring	0.98 (0.08–4.59)	2569.6 (54.2–9192.8)	1584.6 (90.6–5056.5)	381.7 (88.5–1016.1)	52470.1 (2697.0–230205.5)	136.2 (58.57–519.36)
Summer	0.16 (0.08–0.38)	690.9 (488.4–843.3)	1805.6 (1577.4–2428.8)	17.2 (4.2–41.1)	734.3 (196.3–1467.3)	77.3 (46.86–101.53)
Autumn	0.52 (0.16–1.34)	836.6 (849.6–1413)	3237.2 (2403.5–4435.5)	23.7 (9.2–50.5)	619.7 (227.9–1368.6)	49.8 (35.14–54.67)
Entire Year	0.63 (0.08–4.59)	2097 (54.2–10922.3)	1897.5 (90.6–5056.5)	213.6 (4.2–1016.1)	66078.4 (196.3–712433.4)	116.9 (35.14–519.36)

In the coastal-inland sampling site, PM 10 also correlated with cyano/Na as well as to cyanobacteria for the entire data set. However, unlike the on-shore site, here the high correlation between cyano/Na and PM 10 is maintained for the high cyanobacteria episodes. This might be due to the additional decrease in Na^+ and Cl^- concentration (as indicated in Table S4) when aerosols are transported inland. Moreover, the coastal-inland station, in addition to its eastern position, is located south of the Haifa site, increasing the frequency of dust storms episodes during the spring and fall seasons, where cyanobacterial blooms were observed. The combination of these two factors is arguably the cause for the high correlation.

When narrowing down the correlation analysis to the specific events (upper shaded values, Table S6), in the on-shore station, a significant high correlation between endotoxins and cyano/Na is observed ($r = 0.9$), while the correlation between cyano/Na to PM 10 is reduced dramatically, with no statistical significance. This strengthens our suggestion that there is no direct correlation in the on-shore site between PM 10 and cyanobacteria. In the coastal-inland station, a non-significant correlation was found between endotoxins and each of the other parameters analyzed from the filters.

To further support the link between endotoxins and cyanobacteria, we used satellite-derived chl-a data to trace possible endotoxin sources, with the assumption that in the Mediterranean Sea, chl-a is often indicative of cyanobacteria (Rahav et al., 2013; Yogev et al., 2011). In Fig. 3 we present MODIS satellite images of the eastern Mediterranean Sea at the different seasons for the on-shore and coastal-inland samples (Haifa and Rehovot respectively). A significant bloom is observed during winter (Fig. 3A), while the lowest chl-a levels were detected in the summer (Fig. 3D).

Back trajectories for the events with elevated genomic levels were added for the relevant seasons in Fig. 3. Each line-color in the graph represents 3 consecutive sampling days for the same filter. In the analysis it is shown that for each sample, at least one out of the three trajectories was transported over the bloom area for both sampling sites. At the summer period, no event was detected; therefore, no back trajectory lines are shown. The lack of events is supported by the low chl-a level observed.

In order to quantitatively understand the correlation of endotoxins with chl-a concentrations, we averaged chl-a values over a total of 8 days to overcome cloud interferences. Fig. 4 shows the endotoxin levels versus chl-a concentrations derived from MODIS for each filter sampled at both on-shore and coastal-inland sites. It can be seen that the correlations in the on-shore site are higher and more significant for the high genomic level events ($r = 0.95$). In the coastal-inland site, on the other hand, the correlation for the events is insignificant.

Similarly to the satellite observations in Fig. 3, the seasonal average ranking of chl-a (Table 2) reveal highest levels during winter (0.15 ± 0.01 mg/m^3), decreasing during spring (0.08 ± 0.03 mg/m^3) and summer (0.04 ± 0.01 mg/m^3), and increasing again in the autumn (0.05 ± 0.00 mg/m^3). A similar trend was previously reported in the continental shelf-slope of the Mediterranean Sea (Herut et al., 2000).

In both sampling sites, back trajectory analysis shows that the air mass samples had similar histories. Nevertheless, there is a significant inconsistency between ranking of seasonally averaged values of endotoxins, cyanobacteria and chl-a. The rapid decrease in endotoxin levels after crossing to the inland could be linked to the cyanobacteria levels in the sea, far away from the shoreline. In a report by Herut et al. (2011) a North/South gradient of cyanobacteria biomass was observed presenting an increasing trend when going up in latitude of the sampling location. Therefore, it can be expected that Rehovot, located at lower latitude, will encounter lower cyanobacteria biomass. Another possible sink of the endotoxins coming from the sea could be either deposition (wet or dry) or degradation of the toxins, possibly through gas phase or heterogeneous chemical reactions with anthropogenic pollution in the heavily polluted coastal plain. A previous study on the effect of airborne endotoxin on asthma in children in the presence of nicotine and NO_2 showed that higher NO_2 levels lead to a reduction in endotoxins activity, and the consequence decrease in the induced health effect (Matsui et al., 2013). When endotoxin is transported over an urban environment, a similar reduction in potency may occur.

The highest endotoxins levels at the coastal-inland site were observed during the spring (20–23 and 24–27/05/2010), and are most probably associated with other types of gram-negative bacteria, as they are not associated with cyanobacteria or chl-a high levels. Other sources for the high endotoxin levels could be from agricultural harvesting, which is common in this area (Castellan et al., 1987; Spaan et al., 2006). It was previously reported by Viet et al. (2001) that high endotoxin levels were detected during wheat harvesting season in Colorado, USA, with levels exceeding up to 744.4 EU/m^3. Mineral dust can also

Table 2
Seasonally averaged values and range of Chl–a* analyzed from MODIS satellite images.

	Chlorophyll-a	
	Average	Range
Haifa		
Winter	0.15 ± 0.01	(0.14–0.16)
Spring	0.08 ± 0.03	(0.05–0.12)
Summer	0.04 ± 0.01	(0.03–0.05)
Autumn	0.05 ± 0.00	(0.04–0.06)
Whole year	0.08 ± 0.04	(0.03–0.16)
Rehovot		
Winter	0.15 ± 0.00	(0.15–0.16)
Spring	0.07 ± 0.01	(0.05–0.12)
Summer	0.04 ± 0.00	(0.04–0.05)
Autumn	0.05 ± 0.00	(0.04–0.06)
Whole Year	0.07 ± 0.02	(0.04–0.16)

* Eight days averaged around the sampling dates.

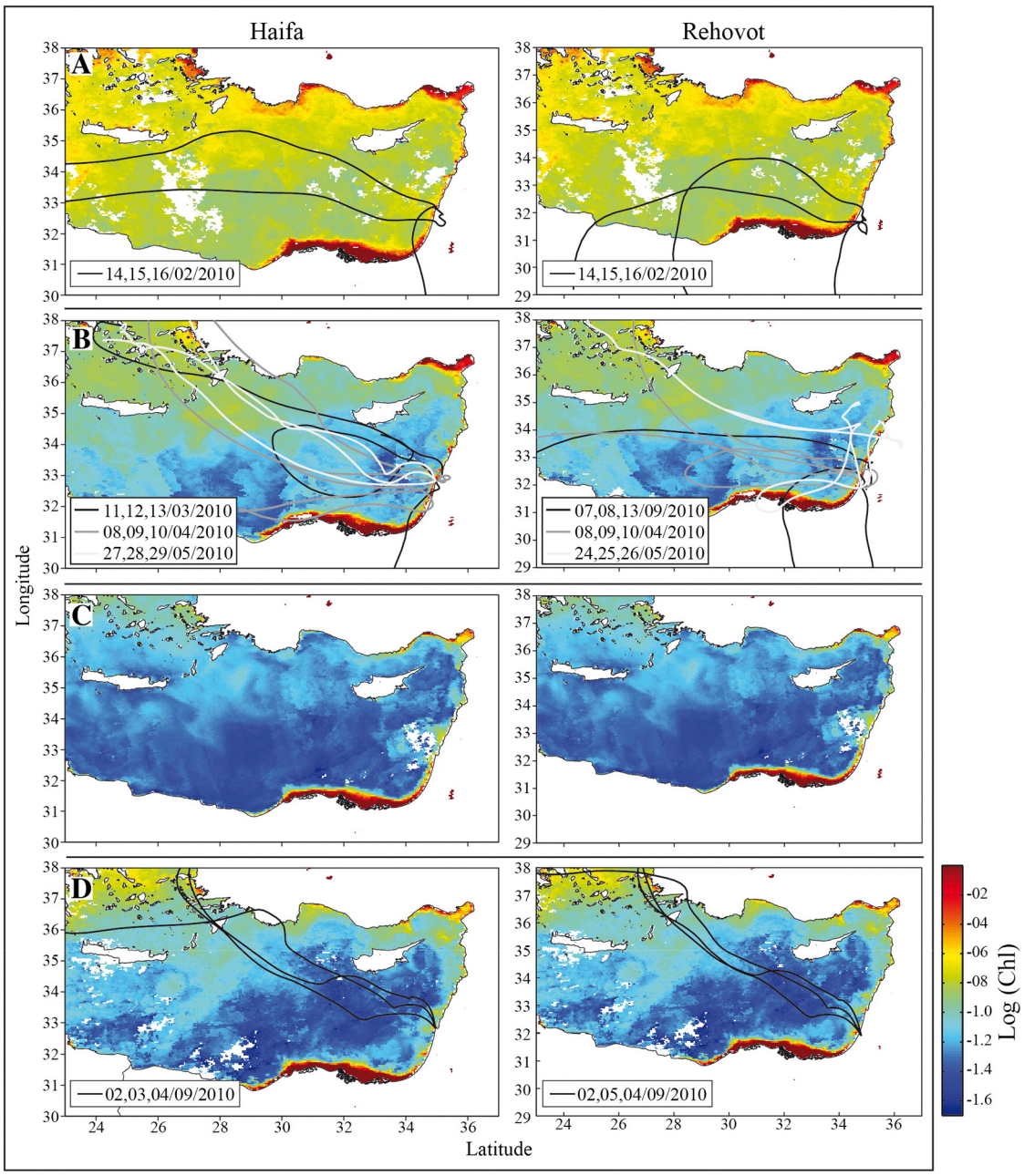

Fig. 3. Satellite image of chl-a averaged concentrations (log (mg/m^3) during winter, spring, summer and autumn (A, B, C, D) 2010 in the East Mediterranean Sea for the on-shore site (Haifa, left hand side) and the coastal-inland site (Rehovot, right hand side). Back trajectories analyses for 3 consecutive days of the events are illustrated for each sampling date at the related season. The trajectories provide an estimate to the central path of the air mass before arriving to the sampling location at a given time.

contribute to endotoxin content, as it was previously reported to carry bioaerosols ((Griffin, 2007) and reference therein).

The Dutch Expert Committee on Occupational Safety and Health (DECOS) suggested that the levels of endotoxins that can induce adverse health effects are above 90 EU/m^3 (Mulder et al., 2010). The endotoxin levels measured in this study are substantially lower. However, they are comparable with previously reported levels (Chen and Hildemann, 2009; Cheng et al., 2012; Heinrich et al., 2003; Morgenstern et al., 2005; Mueller-Anneling et al., 2004). In a study published by Mueller-Anneling et al. (2004), they discuss the gap between their results and the higher levels associated with occupational thresholds for adverse health effects. While ambient endotoxin concentrations were below the "no-effect" levels, the endotoxin concentration in the PM were comparable with the levels of endotoxins measured indoors, which were associated with health effect (Thorne et al., 2005). This discrepancy may be due to different parameters that can influence the extraction efficiency of endotoxins from sampled filters, as was reported previously (Thorne et al., 2003). The duration of sampling and sampling rates were not considered so far, and could also contribute to the significant differences in the reposted levels of endotoxin. In addition, the distance from the source (700 km in our case) can also be an important factor that decreases the endotoxin levels. Nevertheless, near shore cyanobacteria blooms, frequently occur (Howarth, 2008; Qin et al., 2010), can lead to substantially higher levels of endotoxins at shoreline areas compared to the levels detected in this study.

Specifically, the Mediterranean Sea is a source for cyanobacteria that can drift towards the shoreline, with average concentrations of 2.5×10^4 cells/mL (Flombaum et al., 2013). Other coastal regions could also be

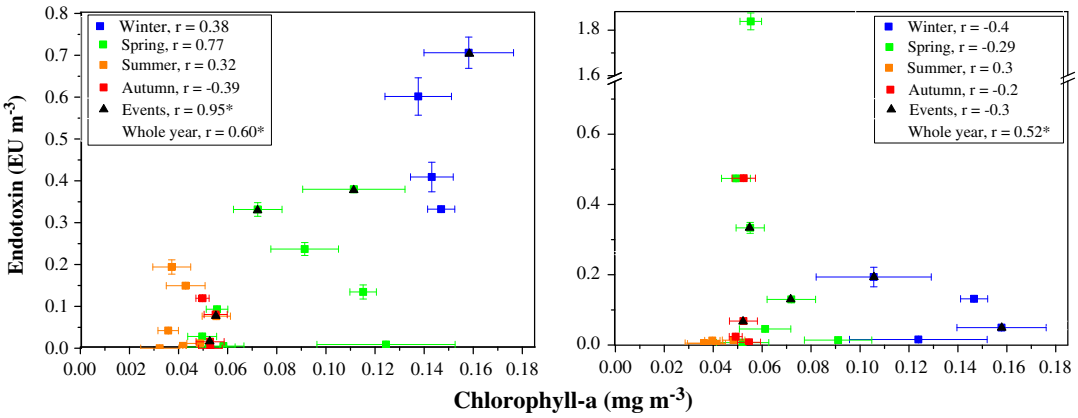

Fig. 4. Endotoxin levels against chl-a concentration in the eastern Mediterranean sea for the different filter samples in both on-shore (Haifa) and coastal-inland (Rehovot) sites. *Statistical significance.

exposed to high cyanobacteria levels (e.g. Central America, the west side of North America, Saudi Arabia, the islands between north Australia and India and China, etc.), up to an annual average of 5×10^4 cells/mL (Flombaum et al., 2013). Higher fluxes of endotoxins can thus be expected in such locations possibly leading to more pronounced health outcomes.

It was previously hypothesized that global climatic changes are expected to induce increase in cyanobacterial biomass at eutrophic waters (Jeppesen et al., 2007; Mooij et al., 2005; Paerl and Huisman, 2009). This was also documented and quantified (Wagner and Adrian, 2009). In addition, it has been shown that changes in turbulence in marine environments, driven, for instance by climate change, may shift dramatically the species composition of phytoplankton communities (Huisman et al., 2004). Hence it may consequently lead to increased endotoxin content in marine aerosols, and as shown in our study, may lead to an increase in endotoxin levels at shoreline areas. In addition, as population constantly increases in coastal areas, there are increasing odds that sensitized individuals will be subjected to these allergenic agents of marine origin.

4. Conclusions

In the on-shore station, all events of high endotoxins levels, as well as the seasonally averaged values, were accompanied by elevated genome concentrations of cyanobacteria per sea salt aerosols and MODIS-derived chl-a levels, with high correlation. Due to its olygotrophic characteristics, (Bosc et al., 2004; Krom et al., 2005), high chl-a levels in the Mediterranean Sea indicate high cyanobacteria content (Rahav et al., 2013). Therefore, we suggest that marine blooms of cyanobacteria are the probable source for the high concentration of cyanobacterial genomes observed in our filters, and the high cyanobacteria is the source of the endotoxin detected on the filters sampled at the on-shore station. Thus we propose that marine sources of endotoxins can affect health in shoreline locations at times of oceanic blooms.

5. Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2014.08.054.

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