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Microorganisms and heavy metals associated with atmospheric deposition in a congested urban environment of a developing country: Sri Lanka

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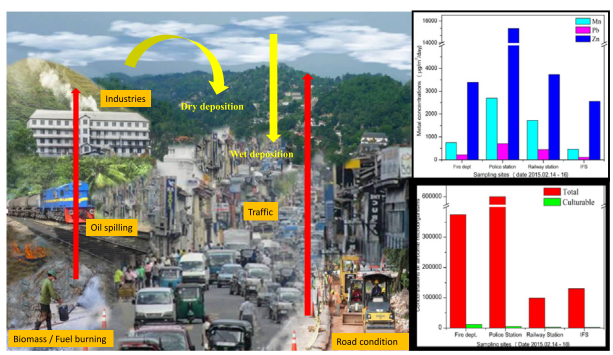
HIGHLIGHTS

- Nine bacterial species were identified, six Gram-negative and three Gram-positive.
- Al and Fe, geogenic in origin and may be re-deposited by vehicular traffic.
- High Cr, Mn, Ni, Cu, Cd and Pb are traffic influenced, galvanized roofs release Zn.
- Bacteria and heavy metals in deposition create human and ecosystem health risks.

GRAPHICAL ABSTRACT



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ABSTRACT

The presence of bacteria and heavy metals in atmospheric deposition were investigated in Kandy, Sri Lanka, which is a typical city in the developing world with significant traffic congestion. Atmospheric deposition samples were analyzed for Al, Cr, Mn, Fe, Ni, Cu, Zn, Cd and Pb which are heavy metals common to urban environments. Al and Fe were found in high concentrations due to the presence of natural sources, but may also be re-suspended by vehicular traffic. Relatively high concentrations of toxic metals such as Cr and Pb in dissolved form were also found. High Zn loads can be attributed to vehicular emissions and the wide use of Zn coated roofing materials. The metal loads in wet deposition showed higher concentrations compared to dry deposition. The metal concentrations among the different sampling sites significantly differ from each other depending on the traffic conditions. Industrial activities are not significant in Kandy City. Consequently, the traffic exerts high influence on heavy metal loadings. As part of the bacterial investigations, nine species of culturable bacteria, namely; *Sphingomonas* sp., *Pseudomonas aeruginosa*, *Pseudomonas monteilii*, *Klebsiella pneumonia*, *Ochrobactrum intermedium*, *Leclercia adecarboxylata*, *Exiguobacterium* sp., *Bacillus pumilus* and *Kocuria kristinae*, which are opportunistic pathogens, were identified. This is the first time *Pseudomonas monteilii* and *Ochrobactrum intermedium* has been reported from a country in Asia. The culturable fraction constituted ~0.01 to 10%. Pigmented bacteria and endospore forming bacteria were copious in the atmospheric depositions due to their

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capability to withstand harsh environmental conditions. The presence of pathogenic bacteria and heavy metals creates potential human and ecosystem health risk.

1. Introduction

Atmospheric deposition is potentially an important part in the biogeochemical cycling of different pollutants such as heavy metals (HMs), bacteria and polycyclic aromatic hydrocarbons (PAHs) (Bari et al., 2014; Liang et al., 2016; Smets et al., 2016). The deposition of atmospheric particulate matter (PM) on ground surfaces can occur via dry and wet deposition processes. Dry deposition (DD) occurs through gravitational settling while wet deposition (WD) occurs through scavenging by precipitation such as rain, snow or fog (Bari et al., 2014; Kara et al., 2014). These particulates along with associated pollutants such as HMs and bacteria are transported by stormwater runoff to receiving waters, posing significant risks to human and ecosystem health (Herngren et al., 2006; Wijesiri et al., 2016). Natural sources such as soil inputs and anthropogenic activities such as traffic, industrial processes, and incineration of sewage sludge and solid waste are among the primary sources of HMs to the atmosphere (Azimi et al., 2003; Tian et al., 2015). Similarly, natural sources such as agriculture, decaying organic matter, (Jeon et al., 2011; McEachran et al., 2015; Smets et al., 2016) and anthropogenic activities such as solid waste disposal and sewage treatment (Fang et al., 2005; Fang et al., 2007; Smets et al., 2016) contribute to the presence of bacteria in the urban atmosphere.

Pathogenic airborne bacteria attached to dust particles can cause detrimental human health impacts such as respiratory diseases, allergies and skin rashes in both, humans and animals (Deng et al., 2016; Kumar et al., 2011; Smets et al., 2016). However, only limited research studies have been conducted on bacteria attached to atmospheric deposited particulate matter (PM) (Bowers et al., 2013; Gao et al., 2015). Heavy metals are not biodegradable and can accumulate in fauna and flora, water bodies, and soils causing adverse impacts on ecosystem health (Duruibe et al., 2007; Soriano et al., 2012). Further, these metallic pollutants are able to travel over long distances by binding to small particles (Azimi et al., 2003). Past research studies have reported elevated concentrations of HMs in deposited atmospheric particles (Gunawardena et al., 2015; Khillare et al., 2004; Samara and Voutsa, 2005).

A major limitation in studies undertaken in relation to HMs and bacteria associated with atmospheric deposition is that they have generally been confined to urban areas in developed countries (for example Barberán et al., 2015; Gao et al., 2016) and, only a limited number of research studies have been conducted in developing countries. Though numerous studies have focused either on HMs or bacteria, the simultaneous investigation into bacteria and HMs in atmospheric deposition in urban environments is also limited (Abdel Hameed and Mounirb, 2016). This limits the ability to develop specific and evidence-based policies and control measures to mitigate the adverse impacts on human and ecosystem health. The primary objective of this study was to undertake a detailed characterisation of common HMs and bacteria present in atmospheric deposition particles in a typical city of a developing country, Sri Lanka. The study outcomes are generic and are expected to contribute to strengthening the environmental management practices across Sri Lanka and can be adopted for similar developing countries.

2. Materials and methods

2.1. Study area and sampling sites

The sampling sites were located in Kandy, which is the second largest city in Sri Lanka. Kandy is a historical city with a high population density of about 6000 persons per km^2. The City has a permanent population of >170,000 people and a daily transient population of around 100,000 people (Wickramasinghe et al., 2011) with characteristics that are typical for a city in a developing country. Kandy has a 26 km^2 land area surrounded by high mountains, facilitating thermal inversions within the city atmosphere. The daily traffic flow is over 100,000 vehicles through the four main entrances to the city center. Due to high vehicular volume and bottlenecks in the road system, the city experiences both inner-city and through traffic congestion. (Wickramasinghe et al., 2011). Incomplete combustion of fuel and construction activities are significant sources of atmospheric pollution in Kandy. Use of firewood for cooking may also contribute to atmospheric pollution. There are no significant industrial activities within the city and nearby areas. Building constructions are continuous, which enhances the emission of dust and other pollutants into the atmosphere in Kandy (Wickramasinghe et al., 2011). The average day time ambient temperature is in the range of 28–32 °C, while the monthly rainfall is in the range of 52–398 mm and the daytime relative humidity is in the range of 63–83%.

Four sampling sites with intensive traffic activities were selected for the collection of atmospheric deposition samples. These sites were designated as Fire Brigade Station (F), Police Station (P), Railway Station (R) and the National Institute of Fundamental Studies (I) (Fig. 1). Sites, F, P and R, are located near 3 or 4-way road intersections with heavy traffic, whereas site I is in a tea plantation with low vehicular and other anthropogenic activities compared to the city centre and it was considered as the control site. Fig. 1 also provides a summary of the main characteristics of the four sampling sites.

2.2. Sample collection

Atmospheric deposition samples were collected based on three consecutive rainfall events. The first rainfall event was after four antecedent dry days while the other two rainfall events were after two and three antecedent dry days. The sampling system used for collecting dry and wet atmospheric deposition samples is illustrated in Fig. S1 in Supplementary Information. The samplers were made using high density polyethylene (HDPE) bottles with polyethylene funnels and connected to a star picket bar and fixed at a height of 1.5 m above ground to minimize contamination from re-suspended particles. The sample collection system was previously described by Gunawardena et al. (2013). Dry deposition is the amount of deposition over a particular antecedent dry period. Bulk deposition (BD) is the dry and wet deposition at the end of a particular rainfall event including the preceding antecedent dry period (Gunawardena et al., 2013). One sampling head was used to collect DD, while the other was used to collect BD. BD and DD samples were collected on the same day, just before and just after a rainfall event. Bulk and dry deposition collectors were installed at the same time after a rainfall event as the atmospheric pollutant loads are minimum at that stage (Gunawardena et al., 2013; Ravindra et al., 2003). Prior to installation, the sampling bottles and funnels were washed with deionized water followed by an acid wash with 1:1 HHO_3 solution as part of the quality assurance measures. At the end of each sample collection, all collection materials were replaced. After collection, the funnels were covered by clean plastic bags and sealed to avoid contamination. Sample bottles were sealed and were transported to the laboratory immediately following standard quality control procedures.

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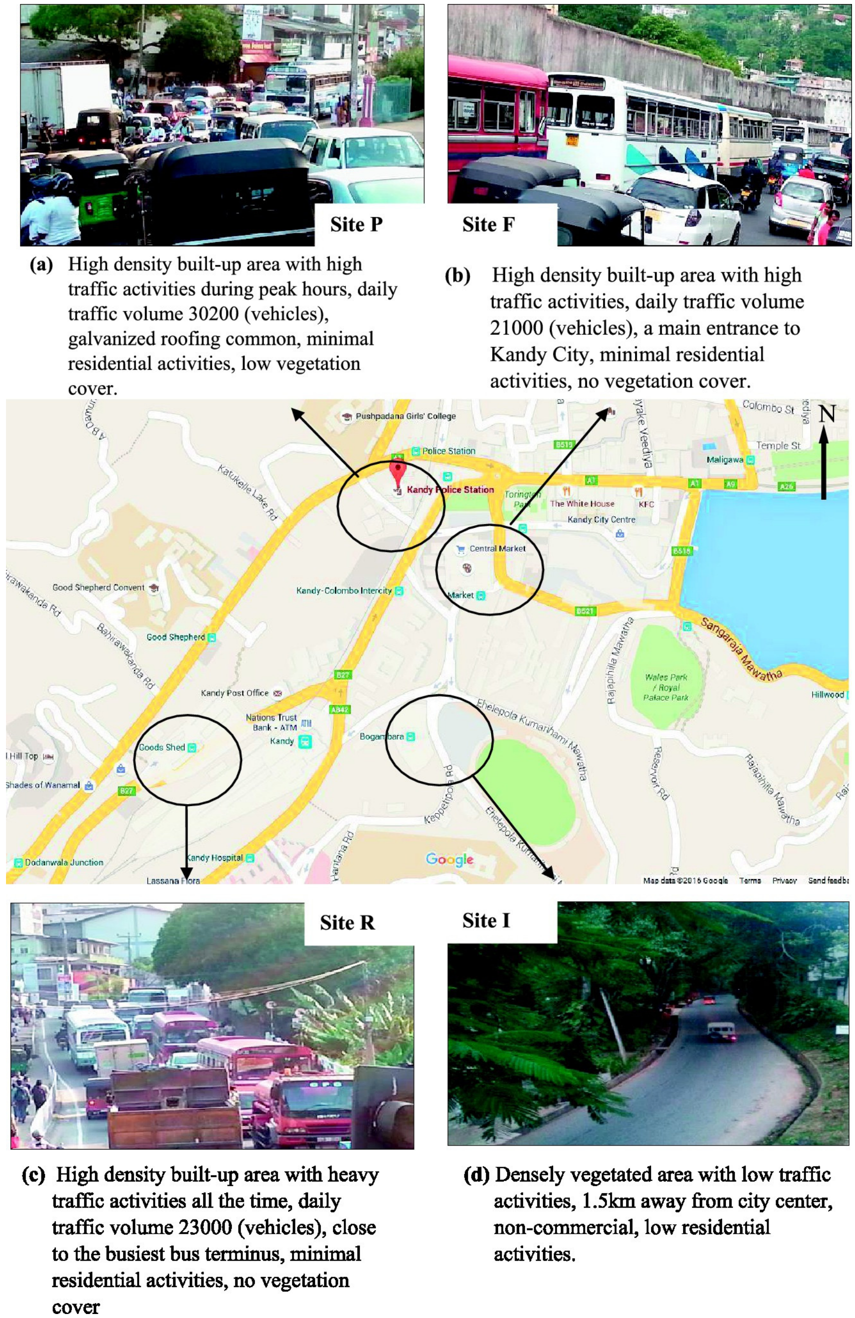

Fig. 1. Map of the study area and locations of the four sampling sites. Police Station (P), Fire Brigade (F), Railway Station (R), National Institute of Fundamental Studies (I).

2.3. Laboratory analysis

The HMs investigated in this study were Al, Cr, Mn, Fe, Ni, Cu, Zn, Cd, and Pb, as these metals are commonly present in the urban environment. Since Al is a crustal element, it was selected as an identifier of geogenic metals (Ziyath et al., 2016). After samples were brought to the laboratory, the funnels and bottles were washed with autoclaved deionized water in order to transfer samples to polyethylene bottles (200 mL for DD samples and 50 mL for BD samples). The samples were stored at 4 °C temperature until laboratory analysis was carried out. The HM concentration was determined according to the US EPA method 200.8 (USEPA, 1994a,b) using an Agilent 8800 Triple Quadrupole Inductively Coupled Plasma Mass Spectrometer (ICP-MS). All the quality assurance and quality control (QA/QC) samples were prepared and tested as specified in US EPA Method 200.8 (USEPA, 1994a,b). The method recommends testing of calibration blanks, laboratory reagent blanks, field reagent blanks, calibration standards, and internal standards as part of the quality assurance measures, which were adopted in the laboratory testing undertaken. The ICP Quality Control Standard #3 (100 μg/mL in 5% HNO_3, AccuStandard®) was used to prepare five calibration standards at concentrations of 0, 1, 10, 100, 1000 and 3000 ppb for the nine HMs.

Calibration curves were set up and ensured that the residual mean square (R^2) was greater than or equal to 0.98. Consequently, certified reference material (CRM) recovery was compared against values given in standard certificate. CRM recovery was found to be within 85–115%, which was considered acceptable as described in US EPA method 200.8. Multi-element standard solution V for ICP-MS prepared by TraceCERT® was used as the CRM. Calibration blank was a volume of deionized water acidified with the same acid matrix as the calibration standards. Internal standards were prepared according to the US EPA method 200.8 (USEPA, 1994a,b). Multi-element solution containing Indium (In), Rhodium (Rh) and Rhenium (Re) at 100 μg/L in HNO_3 were prepared in the laboratory and used as the internal standard. The diluted internal standard was spiked into the samples at 5 μg/L prior to the ICP-MS analysis to ensure all the samples are above the method detection limits as described in US EPA method 200.8 (USEPA, 1994a,b).

The deposited bacterial amounts were determined using two methods. The colony counts for the culturable bacteria was measured using standard spread plate technique (Pant et al., 2016). A 100 μL solution from the 1 mL sample with suspended PM which was taken from wet deposition (WD) and DD (eluted to autoclaved de-ionized water) was spread on Luria-Bertani (LB) agar plate and incubated for 1 to 2 days at 25 °C (Shaffer and Lighthart, 1997). The concentrations of culturable atmospheric bacteria were calculated as colony forming units (CFU/mL). Bacterial cultures were identified by sub culturing the isolated bacterial colonies in LB agar plate or LB broth at 25 °C for 24 to 48 h. Pure cultures were separated according to their Gram staining characteristics and morphology. Solutions with suspended PM were used for total bacterial abundance measurements via epiflourescence microscope (CK × 41 epiflourescence microscope). A 100 μL of sample was stained with 0.2 μg/mL of 2-(4-amidinophenyl)-1H-indole-6-carboxamidine (DAPI) solution to count both, viable and non-viable bacteria cells at a sample to DAPI ratio of 10:1. Further dilution was undertaken if the cell count exceeded >100 cells per field. Bacterial abundance was expressed as cells per mL, taking into account the dilution (Bowers et al., 2009).

DNA was extracted from the cultured isolates using the modified Cetyltrimethylammonium bromide (CTAB) method (Somerville et al., 2005), while polymerase chain reaction (PCR) was carried out for extracted DNA using 16S rDNA primers. Bacterial 16S rDNA was amplified using real-time PCR Instrument System (Rotor GeneQ) and conventional PCR machine (Techne, TC-3000) using universal primers 16sA1 (5′ AGR GTT TGA TCM TGG CTC AG 3′) and 16sB1 (5′ GGY TAC CTT GTT ACG ACT T 3′) (Chen et al., 2009). The reaction was carried out in a 25 μL mixture containing 375 ng of bacterial DNA, 0.1 mM of each dNTP, 0.4 μM of each forward and reverse primer, 1× Taq buffer (Promega), 1.5 mM $MgCl_2$ (Promega), 1 unit of Taq DNA polymerase (Promega). The thermocycle program was set at 94 °C for 2 min for initial denaturation, followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 50 °C for 1 min, and elongation at 70 °C for 2 min, and a final extension at 70 °C for 20 min (Chen et al., 2009). The amplified DNA was visualized by gel documentation system (SYNGENE) after gel electrophoresis in 1.5% agarose and ethidium bromide staining. A 1 kbp DNA marker was used to identify a correct fragment of 1.5 kbp. Amplified DNA fragments were purified using gel extraction kit (Promega) and were commercially sequenced by Macrogen Inc., South Korea, using ABI 3730XL sequencers.

2.4. Data analysis

The metal data matrix was analyzed for normality using the Quantile-Quantile (Q-Q) probability distribution plots and the Shapiro-Wilk test (Ogunkunle et al., 2016). Kruskal-Wallis one-way analysis of variance followed by Tukey's honest significance difference was used to investigate the significance of the metal load variations and for bacterial analysis (Hinton, 2004). The above analyses were conducted using Matlab (MathWorks, 2013). Preference ranking organization method for enrichment evaluation (PROMETHEE) was used to rank the study locations according to the degree of pollution, while the Graphical analysis for interactive assistance (GAIA) method was used to investigate the relationships between the study sites and the metal deposition pattern. PROMETHEE and GAIA were performed using the Visual PROMETHEE software (Brans and Mareschal, 2005). Further details regarding PROMETHEE and GAIA can be found in Kokot and Ayoko (2004).

3. Results and discussion

3.1. Metal load in deposited samples

The data matrix consisted of the loads of nine metal species tested using the dry and wet deposited samples collected from the four different sampling locations. The normality test was conducted separately for dry and wet deposition samples for each metal species. Generally, the frequency distributions cannot be approximated to normal distribution according to the Q-Q plots (Fig. S2 in Supplementary Information). This conclusion was further confirmed by the Shapiro-Wilk test results (Table S1 in Supplementary Information). Hence, non-parametric statistical techniques were employed for the data analysis.

Descriptive statistics relating to the metal loads in dry and wet samples are presented in Table 1. Relatively high standard deviations for metal loads suggest that the metal composition in the deposited atmospheric particles varies significantly among the study sites. This may be attributed to the variation in the intensity of anthropogenic activities in the vicinity of the study locations. In comparison to DD, WD loads are slightly higher (Table 1). However, it has been reported that DD generally contributes significantly to the total metal deposition loads (Morselli et al., 2003; Pan and Wang, 2015; Powell et al., 2015). This difference could be attributed to geographic and climatic factors including the number of antecedent dry days, rainfall frequency and intensity, wind speed, and terrain.

Kruskal-Wallis one-way analysis of variance test showed that there is a statistically significant difference in the metal loads in both, dry [$H(8) = 86$, $p < 0.05$] and wet [$H(8) = 48$, $p < 0.05$] deposition. The post-hoc test was conducted using Tukey's honest significance test and the results are presented in Fig. 2. According to Fig. 2, Al and Fe have the highest load in both, dry and wet depositions. Among the metal loads, Al and Fe are the major components in geogenic materials (Ziyath et al., 2016). As such, these metals could have been contributed by disturbed soil and suspended by anthropogenic activities such as traffic and eventually deposited via dry and wet deposition. The other metal species investigated in this study are commonly related to traffic activities and are present in relatively low quantity (Table 1). Interestingly, Zn was found to be present in relatively higher quantity than the other metals in both, dry and wet deposition in the study area. Vehicle emissions are one of the important sources of Zn in atmospheric deposition (Duan and Tan, 2013). Direct emissions from exhaust, re-entrainment by traffic of dust enriched with Zn, lubrication oil additives, emissions from tyre and brake wear and corrosion of galvanized automobile components can also be major sources of Zn deposition in the study area. Additionally, the wide use of Zn coated roofing materials in the study area may also be attributed to the Zn loads in Kandy City.

Heavy metals in DD are depicted in Fig. 3a and b. Considering the study period, comparatively low HM concentrations in DD loads have been reported for February 11th to 12th, which was Wednesday to Thursday. The highest average metal concentrations in DD have been reported for the period 14th to 16th February which was Saturday to

Table 1
Descriptive statistics of metal loads in dry and wet deposition samples (mg/m^2/day).

Metal	Dry deposition (DD)			Wet deposition (WD)		
	Mean	SD[1]	Range	Mean	SD	Range
Al	46	62	6–230	54	65	0–204
Cr	0.3	0.3	0.0–1.0	0.3	0.2	0.0–0.8
Mn	1.0	0.9	0.2–2.7	1.2	1.5	0.0–4.6
Fe	66	65	9–206	94	121	4–368
Ni	0.2	0.2	0.0–0.6	0.2	0.1	0.0–0.4
Cu	0.3	0.2	0.1–0.8	0.5	0.5	0.1–1.4
Zn	4.4	4.3	0.6–15.3	8.4	12.3	0.5–37.3
Cd	0.04	0.04	0.00–0.15	0.06	0.06	0.00–0.20
Pb	0.3	0.2	0.0–0.7	0.4	0.5	0.0–1.2

Note: [1]SD – Standard Deviation.

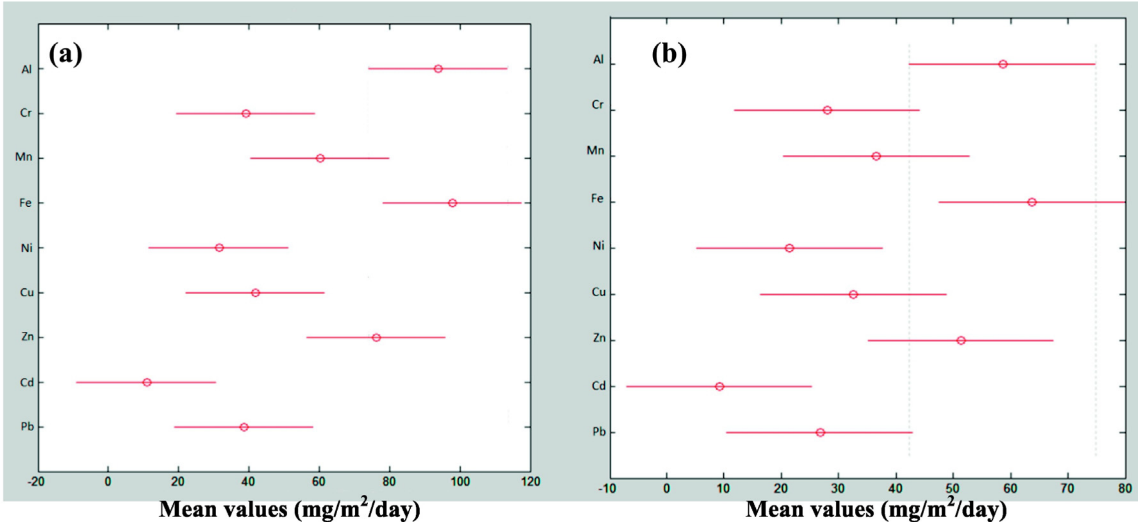

Fig. 2. Post-hoc Tukey's honest significant test results for: (a) Dry deposition and (b) Wet deposition.

Monday (Fig. 3a and b). Fridays to Mondays are when the highest traffic volumes are experienced in the Kandy City. Due to the presence of sites of historical and religious importance, tourists and devotees numbering over 300,000 and >106,000 vehicles visit the City during weekends, which is more than during weekdays (Meetiyagoda, 2016). As Kandy City does not have significant industrial activities which could result in the emission of HM loads, it can be concluded that traffic exerts the primary influence on HM loadings.

3.2. Ranking of study sites and sampling episodes

PROMETHEE was used to rank the sampling episodes according to the level of metal pollution to investigate whether there are unique trends in terms of deposition characteristics in the context of the study. The data matrix used for PROMETHEE analysis consisted of nine variables (metal loads) and 20 objects. The data matrix is provided in Table S2 in Supplementary Information. The objects were the deposition samples collected from the four study sites over three dry and two wet sampling episodes. The three modelling parameters, i.e. ranking sense, weighting and specific preference function required for each variable in PROMETHEE analysis were defined as follows: (a) maximum was chosen as the ranking sense to rank the sampling episodes from the highest to the lowest pollution level; (b) 1 was chosen as weighting to ensure that all variables have equal significance; and (c) linear preference function was chosen since it facilitates the definition of an indifference threshold (Behzadian et al., 2010).

PROMETHEE produces net ranking out flow values (φ) by comparing the objects pairwise against the criteria (Espinasse et al., 1997). The φ values are used to rank the objects in the ascending or descending order according to the ranking sense chosen. The PROMETHEE results suggests that the level of pollution for sampling episodes is ordered as follows: FW1 (0.88) > PD3 (0.66) > RD3 (0.15) ≈ RD1 (0.14) > RW1 (−0.02) > FD1 (−0.04) > IW2 (−0.06) ≈ PW1 (−0.06) ≈ PW2 (−0.06) > RW2 (−0.08) > PD1 (−0.10) > IW1 (−0.12) ≈ FD3 (−0.13) ≈ ID3 (−0.14) > ID1 (−0.15) > FD2 (−0.16) ≈ ID2 (−0.17) ≈ FW2 (−0.17) ≈ PD2 (−0.18) ≈ RD2 (−0.19), where the φ values are given in brackets, the first letter represents the study location (F – Fire Brigade Station, P – Police Station, R -Railway Station and I - National Institute of Fundamental Studies), the second letter specifies whether dry (D) or wet (W) deposition, while the numerals indicate the

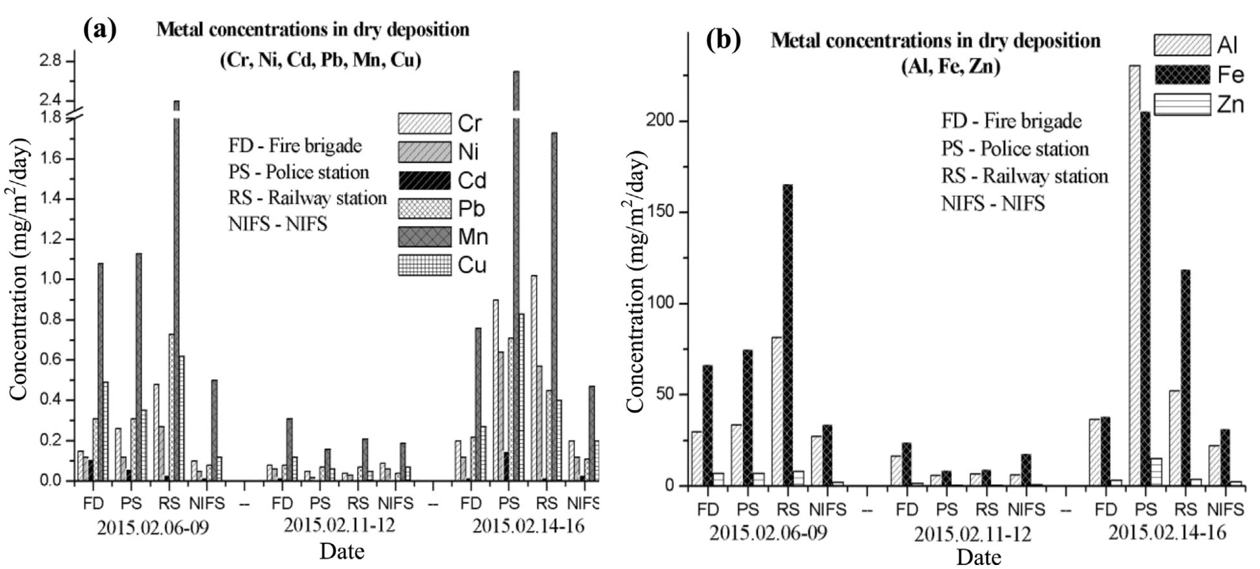

Fig. 3. (a) Metal concentrations of Cr, Ni, Cd, Pb, Mn and Cu in dry deposition in Kandy City during February 2015; (b) Metal concentrations of Al, Fe and Zn in dry deposition in Kandy City during February 2015.

different sampling episodes. The ranking of sampling episodes does not follow any specific pattern based on either the sampling or the deposition type, illustrating the complexity of the pollution sources in Kandy City, which are influenced by traffic congestion and climatic conditions.

GAIA analyses were conducted separately for each study location to understand the deposition characteristics in order to identify potential strategies to mitigate metal pollution. The data matrices consisted of nine metal loads (variables) in both, dry and wet deposition samples (objects) from each study site. GAIA plots were developed based on the decomposition of the φ values calculated using the PROMETHEE algorithm (Ni et al., 2002). For GAIA analysis, the same PROMETHEE modelling parameters discussed above were used. For the interpretation of GAIA plots, rules outlined by Espinasse et al. (1997) were used. For example, the influence of a variable on objects is high if the vector corresponding to the variable is relatively longer.

In general, vectors related to geogenic metals such as Al and Fe are negligible in size as evident from Fig. 4, suggesting that their loads do not exert a significant influence at the study sites. This also means that Al and Fe loads did not significantly vary during different sampling periods, indicating that consistent natural processes have contributed these metals to the atmosphere and consequently to deposition. Anthropogenic activities would have limited influence on the deposition of Al and Fe. Zn also showed a similar pattern except at site 'P', further confirming the predominant contribution by Zn coated roofs since the site is in the vicinity of high density built-up area compared to the other sampling sites. Vectors related to the rest of the metals are generally longer suggesting that the loads varied during the sampling episodes (Fig. 4). Hence, anthropogenic activities, in particular traffic activities, could have been the predominant source of these metals. Based on the above findings, it can be concluded that mitigation strategies such as easing congestion, and improving fuel and vehicle quality can reduce the pollution of Mn, Pb, Cu, Ni, Cd and Cr significantly, while Zn pollution may be mitigated by improving fuel and vehicle quality as well as reducing or phasing out the use of Zn coated roofs.

The metal concentrations in BD obtained from the present study were compared with the values reported in past studies (Table 2). It can be noted that most of the urban sites in developed regions have reported high concentrations of Zn, compared to other HMs, which corroborates with this study. Other than Al and Fe which have crustal origin, Zn concentrations were higher than the other metals. Apart from Zn; Mn, Cu and Pb are among the anthropogenic metals reported from the urban sites in developed countries. The present study reports similar results.

3.3. Bacterial analyses

3.3.1. Spatial variation of atmospheric deposition bound bacteria

Total bacterial concentrations in the deposited particles ranged from 2.40×10^4 to 1.15×10^6 cells/mL (Table 3) with the mean concentration of 5.87×10^5 cells/mL. There was no significant difference in total bacteria at the four different sites according to Kruskal-Wallis test followed by Tukey's hsd (MathWorks, 2013) (Fig. S3 in Supplementary Information). Culturable bacterial survival in deposited PM ranged from 1.00×10^1 to 2.27×10^4 CFU/mL (Table 3) with a mean concentration of 1.14×10^4 CFU/mL. Similar to the total bacterial count, there was no significant difference in culturable bacteria in atmospheric deposition at the four sites ($P > 0.05$) (Fig. S4 in Supplementary Information). These findings on culturable bacteria, corroborate with the data from the other countries. Although the method of sampling was different, culturable bacteria in a study in Beijing, China ranged from 4.8×10^2 to 2.4×10^4 CFU/m^3 (Fang et al., 2008). A study conducted in a grass field in Oregon USA, which was confined to vegetation, had culturable

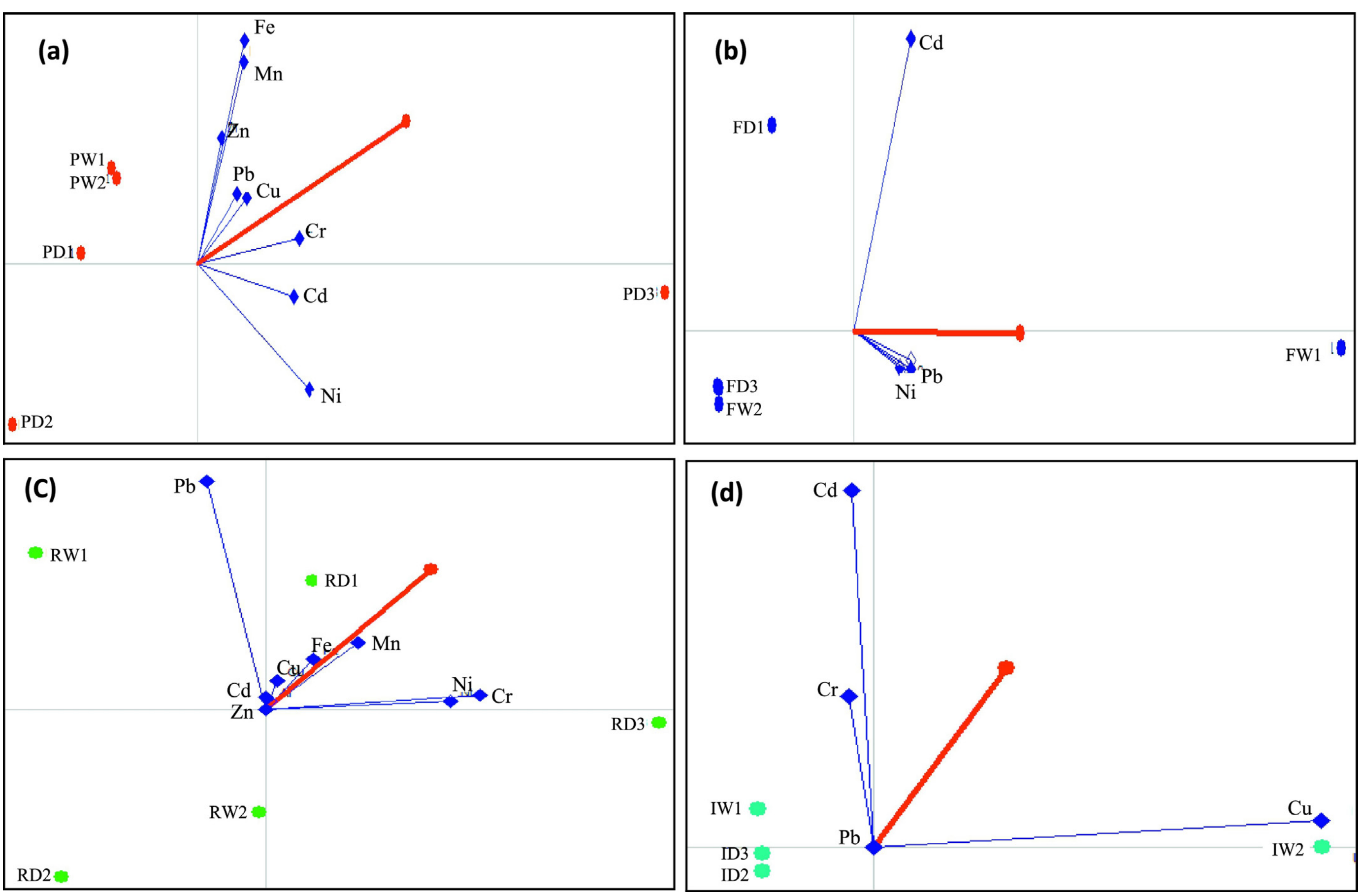

Fig. 4. GAIA biplot for (a) Police Station - P, (b) Fire Brigade Station - F, (c) Railway Station - R and, (d) National Institute of Fundamental Studies - I.

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Table 2
Comparison of present study results with studies from developed countries on heavy metal concentrations in bulk deposition.

Country	Cd	Cu	Pb	Zn	Cr	Mn	Ni	Unit	period	Reference
Kandy, Sri Lanka	0.1	0.8	0.6	12	0.5	1.9	0.3	mg/m^2/d	2015	This study
Santander, Spain	0.1	11.8	4.5	183	5.2	153	1.5	μg/m^2/d	2009–2013	Fernández-Olmo et al. (2015)
Paris, France	0.66	16.4	11.5	82.2			1.7	μg/m^2/d	2001–2002	Motelay-Massei et al. (2005)
London, UK	0.11		11.5	48.9			1.8	μg/m^2/d	2004	Brown et al. (2006)
Brisbane, Australia	0.32	5.5	5.9	46.0	1.8	16.0	1.0	μg/m^2/d	2007–2008	Huston et al. (2012)
Galicia, Spain	0.00065	0.05	1.0	1.41				kg/ha	2010–2011	Ares et al. (2015)

bacterial concentrations of 3.22×10^1 to 1.3685×10^3 CFU/m^3 (Lighthart and Shaffer, 1995). Studies in Poland (Bugajny et al., 2005) and in Spain (Soto et al., 2009) also demonstrated similar data, 3.0×10^3 and 5.0×10^3 CFU/m^3, respectively.

The culturable bacteria at the four sampling sites accounted for 0.01 to 10% of the total bacterial cells which was similar to previous studies (Lighthart, 1997). It also confirmed that all the bacteria attached to PM could not be cultured. Culturable bacteria are the organisms that can be cultured in a laboratory supplied growth media under given laboratory conditions. None of the synthetic culture media is appropriate to supply total nutrients needed for the bacteria in a mixed population. Some of the bacteria are unable to culture under laboratory conditions and may require a long time to appear in the growth media and require special growth media to produce visual colonies (e.g. Appearance of *Mycobacteria* spp. in Lowenstain-Jensen medium and in Middlebrook 7H11 require >4 weeks) (de Azevedo Issa et al., 2016; Palange et al., 2016). The ability to get bacteria into culture gradually decreases with aerosolisation and with increased HM concentrations (Kaushik and Balasubramanian, 2012). Moreover, atmospheric deposition is not a nutrient rich growth substrate for bacteria. Therefore, the limitations in nutrients create starvation conditions for bacteria, causing physiological and morphological adaptations. Due to these adaptations, bacteria lose the ability for rapid colonization in nutrient rich environments such as in laboratory supplied growth media (Lievens et al., 2015). Thus, culture based methods are not adequate for obtaining an in-depth understanding of the quantity of bacteria in an environmental sample. Hence, it is important to enumerate the total bacteria present in deposition with culture independent techniques in order to obtain a detailed understanding of the quantity of bacteria. For example, 4′,6-diamidino-2-phenylindole (DAPI) is a DNA binding stain which can be used for direct enumeration of total bacteria (Culturable, non-culturable viable or dead) in a sample.

Interestingly, Gram negative rods were the highest culturable bacteria observed at all four sites (Table S3 in Supplementary Information) in the collected sample set, whereas, in other past studies Gram positive bacteria have been found to be the most abundant in the urban atmosphere (Deng et al., 2016). The reason for the presence of more Gram negative bacteria could be due to their survival capabilities under harsh conditions and the ability to survive even under droplet evaporating process during dry and wet deposition (Xie et al., 2006). Most of the Gram negative bacteria are able to produce toxins affecting human health (Deng et al., 2016) and are responsible for 30% of hospital acquired infections mostly associated with ventilator-associated pneumonia (Peleg and Hooper, 2010). Hence, the presence of high load of Gram negative bacteria demonstrates a potential public health risk.

3.3.2. Identification of bacteria associated with atmospheric deposition

Nine bacterial species belonging to eight genera were isolated, cultured and identified. There were six species (five genera) of Gram-negative bacteria and three species (three genera) of Gram-positive bacteria which were culturable. *Sphingomonas* sp. (KT985361, KT985371), *Pseudomonas aeruginosa* (KT985363), *Pseudomonas monteilii* (KT985367), *Klebsiella pneumonia* (KT985366), *Ochrobactrum intermedium* (KT985368) and *Leclercia adecarboxylata* (KT985369), *Exiguobacterium* sp. (KT985362), *Bacillus pumilus* (KT985365, KT985370) and *Kocuria kristinae* (KT985360, KT985364) were identified.

Six out of the nine species were found to be Gram-negative bacteria in contrast to previous studies (Amato et al., 2007; Smith et al., 2012). Bacterial species such as *Exiguobacterium* sp., *Pseudomonas* sp., *Ochrobactrum intermedium* are soil inhabitant and capable of surviving in low nutrient moisture levels, and high radiation levels. They can survive even under evaporation process of atmospheric droplets (Xie et al., 2006). Vegetation and soil are the main sources for the bacteria in the atmosphere and to a lesser extent, anthropogenic factors also contribute to the numbers (Smets et al., 2016). Several identified bacteria such as *Sphingomonas* sp., *Exiguobacterium* sp. were yellow in colour. These pigmented bacteria are abundant in atmospheric depositions due to their adaptation for survival in harsh conditions.

Gram-positive bacteria of the genus *Bacillus* were quite common in the samples as the endospores of this genus are highly resistant to ultraviolet radiation and desiccation (Polymenakou, 2012; Rao et al., 2016; Soni et al., 2016). *Pseudomonas* sp., is also a pathogenic bacterium that can be found in atmospheric PM, resistant to antibiotics, detergents, organic solvents, disinfectants and HMs and have a high ability for survival due to the association with PM (Moore et al., 2006). Our data provide evidence of the presence of *Pseudomonas aeruginosa*, which is an opportunistic pathogen with the ability to cause fatal infections in burn patients, ventilator patients, and patients with chronic debility (Griffin et al., 2003). Additionally, this is an important pathogen in the gastrointestinal tract. *Sphingomonas* sp. was also present in atmospheric PM, which corroborates with previous literature (Polymenakou, 2012).

The presence of *Kocuria* spp. demonstrate a risk of causing bacteremia in chronically ill patients with malignancies or other immune-suppressed states (Dunn et al., 2011). At the same time, *Klebsiella pneumoniae* is a health care associated infective organism which can cause pneumonia, bloodstream infections, wound or surgical site infections and meningitis (Sievert et al., 2013). The source of *K. pneumoniae* is vegetables and plant surfaces (Holden et al., 2009).

Leclercia adecarboxylata is a pathogen that causes fever and leukocytosis (Hwang et al., 2014). Although *Ochrobactrum intermedium* is not a

Table 3
Concentrations of total bacteria and concentrations of culturable bacteria associated with deposition at the four sampling sites in Kandy, Sri Lanka.

Sampling site	Number of samples	Concentrations of total bacteria (cells/mL)			Concentrations of culturable bacteria (CFU/mL)		
		Least significant mean	Minimum	Maximum	Least significant mean	Minimum	Maximum
Fire Brigade	5	1.37×10^5	1.06×10^5	6.44×10^5	5.04×10^3	3.00×10^1	2.27×10^4
Police Station	5	1.08×10^5	5.20×10^4	1.15×10^6	5.53×10^3	1.10×10^2	1.06×10^4
Railway Station	5	8.56×10^4	2.40×10^4	1.74×10^5	1.90×10^3	1.00×10^1	5.72×10^3
NIFS	5	3.93×10^5	2.40×10^4	2.36×10^5	1.49×10^3	1.70×10^2	4.38×10^3

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common pathogen, it has been reported to cause bacteremia in patients with cancers and immune-compromised individuals (Apisarnthanarak et al., 2005; Dharne et al., 2008). Hazes of dust events are responsible for the presence of bacteria in the atmosphere which commonly inhabit the soil (Kaushik and Balasubramanian, 2012).

3.3.3. Variation in bacteria in deposited samples

Exiguobacterium sp. and *Ochrobactrum intermedium* were only found in DD (Table S4 in Supplementary Information). *Exiguobacterium* is an antibiotic resistant bacterium, which is capable of surviving under high concentrations of copper (Vos, 2007). However, the presence of these bacteria was relatively low when compared with the other bacteria (3.00×10^1 CFU/mL). Both, *Exiguobacterium* sp. and *Ochrobactrum intermedium* are capable of living under dry conditions. *Leclercia adecarboxylata* and *Sphingomonas* sp. were present only in BD (Table S4 in Supplementary Information).

Pseudomonas monteilii was present only at site P, which is close to vegetation though situated in the city center compared to the other sites. According to the chemical analysis, site P has high concentration of Zn. Therefore, further investigations are needed to confirm their association and capability for surviving with the high concentrations of Zn. *P. montelii* is a rhizospheric bacteria, which can be grown under limited C (Devi and Gkn, 2012). Therefore, rhizosphere could be the source of these bacteria at this site. *Ochrobactrum intermedium* and *Exiguobacterium* sp. were found only at site F, whereas *Leclercia adecarboxylata* and *Sphingomonas* sp. were found only at site R (Table 4). *Ochrobactrum intermedium* is a common inhabitant of rhizosphere, soil and polluted environments (Aujoulat et al., 2014). It can be concluded that PM transported by wind is responsible for the soil inhabitant bacteria at these sites.

When the duration of collection increased in the case of BD, the concentration of culturable bacteria increased, whereas this phenomenon was opposite for DD (Table S5 in Supplementary Information). High moisture levels during WD are responsible for higher concentrations of bacteria in BD relative to DD. Previous studies have shown that the bacterial viability and concentrations depend on the level of moisture content (Abdel Hameed, 2003; Awad et al., 2006). Consequently, respiratory diseases can rise during the rainy season and public health is at risk due to the increased level of viable bacteria and HM contaminated PM in the atmosphere.

4. Conclusions

Dry and wet atmospheric deposition in Kandy City, Sri Lanka, were analyzed for a range of heavy metals and bacterial pollutants. Al and Fe, from geogenic sources, were found to be in significantly higher concentrations in deposition loads compared to the other heavy metals. The other heavy metals investigated, namely, Cr, Mn, Ni, Cu, Zn, Cd and Pb, were attributed to be primarily originating from traffic sources as confirmed by the GAIA analysis undertaken. Wet deposition provided relatively higher contribution to the metal loads than dry deposition. As Kandy City has historical and religious importance, large numbers of tourists and devotees visit the City during weekends. Therefore, the traffic conditions are higher during weekends than weekdays. Consequently, atmospheric metal loadings are relatively higher during weekends. Within the City, at different locations there are variations in metal concentrations depending on the traffic conditions. Considering the patterns of heavy metal loads within the Kandy City, it can be concluded that mitigation strategies such as easing of traffic congestion, providing bypass arrangements and improving fuel and vehicle quality can reduce the pollution of Mn, Pb, Cu, Ni, Cd and Cr, while Zn pollution may be mitigated by phasing out the use of Zn coated roofs as well as improving fuel and vehicle quality.

In relation to the bacterial investigations, the results obtained confirmed that a number of bacteria present in the sampling sites were opportunistic pathogens. The most common bacterial genus present in the particulate matter was *Pseudomonas* and the airborne transmission of these organisms may pose a significant risk to people depending on numerous factors including the number of bacteria associated with the particulate matter, virulence factors of the specific bacterium and the host immunity. No significant difference was observed among culturable bacteria and total bacterial cell count at the sampling sites. Concentrations of culturable bacteria were higher in bulk deposition compared to dry deposition. Atmosphere does not provide any substrate for bacteria to live on and instead they are dispersed by wind and associate with particulate matter or droplets. The major sources of bacteria in atmospheric deposition could be due to plant surfaces and soil. The bacterial species with pigments, ability to form endospores, capable of surviving under low moisture levels, minimal nutrients, high radiation levels and excessive heavy metal levels were abundant among the atmospheric biological fraction. The study confirmed the potential human and ecosystem health risks generated by heavy metals and bacteria from atmospheric deposition in Kandy City, Sri Lanka.

Table 4
Concentrations of identified bacteria at the sampling sites.

Identified organism	Site			
	Fire brigade (CFU/mL)	Police (CFU/mL)	Railway (CFU/mL)	NIFS (CFU/mL)
Kocuria sp.	0	5.94×10^3	3.750×10^3	4.38×10^3
Exiguobacterium sp.	3.00×10^1	0	0	0
Bacillus pumilus	1.10×10^2	0	5.720×10^3	3.00×10^2
Klebsiella pneumoniae	2.02×10^3	0	0	1.70×10^2
Pseudomonas monteilii	0	7.55×10^3	0	0
Ochrobactrum intermedium	2.38×10^3	0	0	0
Leclercia adecarboxylata	0	0	3.00×10^1	0
Sphingomonas sp.	0	0	3.00×10^1	0
Pseudomonas aeruginosa	2.27×10^4	8.65×10^3	0	1.09×10^3

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Appendix A. Supplementary data

Shapiro-Wilk test results for metal loads on dry and wet deposition, data matrix for PROMETHEE analysis, concentrations of different types of culturable bacteria based on the type and duration of collection of particulate matter, bacteria composition associated with atmospheric deposition, concentrations of total and culturable bacteria at different sites, a schematic diagram of a dry and bulk sample collection system, Q-Q plots for wet and dry deposition, Kruskal Willis test results for concentration of total bacteria, Kruskal Willis test results for concentration of culturable bacteria, are provided in the Supplementary Information.

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