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A new method for assessing the contribution of Primary Biological Atmospheric Particles to the mass concentration of the atmospheric aerosol

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ABSTRACT

Primary Biologic Atmospheric Particles (PBAPs) constitute an interesting and poorly investigated component of the atmospheric aerosol. We have developed and validated a method for evaluating the contribution of overall PBAPs to the mass concentration of atmospheric particulate matter (PM). The method is based on PM sampling on polycarbonate filters, staining of the collected particles with propidium iodide, observation at epifluorescence microscope and calculation of the bioaerosol mass using a digital image analysis software. The method has been also adapted to the observation and quantification of size-segregated aerosol samples collected by multi-stage impactors.

Each step of the procedure has been individually validated. The relative repeatability of the method, calculated on 10 pairs of atmospheric PM samples collected side-by-side, was 16%.

The method has been applied to real atmospheric samples collected in the vicinity of Rome, Italy. Size distribution measurements revealed that PBAPs was mainly in the coarse fraction of PM, with maxima in the range 5.6–10 μm. 24-h samples collected during different period of the year have shown that the concentration of bioaerosol was in the range 0.18–5.3 μg m^{-3} (N = 20), with a contribution to the organic matter in PM_{10} in the range 0.5–31% and to the total mass concentration of PM_{10} in the range 0.3–18%.

The possibility to determine the concentration of total PBAPs in PM opens up interesting perspectives in terms of studying the health effects of these components and of increasing our knowledge about the composition of the organic fraction of the atmospheric aerosol.

1. Introduction

In the last two decades much attention has been paid to improve our understanding of the chemical composition of atmospheric particulate matter (PM). The inorganic components of PM (elements and ionic species) have been individually determined in most of the studies about the atmospheric aerosol composition, as they are relatively low in number and easy to quantify. These investigations resulted in a detailed knowledge of PM inorganic components, their concentration range, size distribution, major sources and, very recently, specific health effects (Viana et al., 2008; Putaud et al., 2010; Lu et al., 2015, and cited therein).

A very different situation occurs for the thousands of different carbon-containing components. In their review about the source apportionment of particulate matter in Europe, Viana et al. (2008) underline the "almost complete absence of data on speciation of organic aerosols". Most of the scientific studies, in fact, limit to the determination of elemental carbon (EC) and of organic carbon (OC) as a whole, generally carried out by thermo-optical methods. Although this approach cannot offer insight into the sources of this important fraction of PM, it is often used to obtain the mass closure that is the quantitative correspondence between the gravimetric mass and the sum of individual chemical determinations (Sillanpa et al., 2006; Mantas et al., 2014; Perrino et al., 2014, among others).

Other, more complex, approaches include a partial chemical speciation of OC that is the determination of some classes of organic components; at the state of the art, a complete speciation of all organic components of PM is very far from the actual technical possibilities. Often, the organic components to be individually measured are chosen because of their toxicity. Specific analytical methods have been developed for organic micro- and trace PM components that have been recognized to be responsible for severe health effects (e.g. polycyclic aromatic hydrocarbons, dioxins, polychlorobiphenyls). Much attention has also been paid to species that are considered as reliable specific tracers of PM sources: this is the case, among others, of levoglucosan for hard wood combustion (Simoneit, 2002), cholesterol for meat cooking (Rogge et al., 1991), nicotine for cigarette smoke (Daisey, 1999). Nevertheless, the most comprehensive studies were hardly able to speciate 30% of the total organic PM mass (Neusüss et al., 2000).

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A relevant fraction of organic PM is constituted by primary biological aerosol particles (PBAP or bioaerosol), that is all kind of particles derived from biological organisms, including dead or alive microorganisms and fragments of biological material (viruses, bacteria, fungal spores and fragments, airborne algae, pollen grains and fragments, plant debris, insect fragments, fur fibres and skin fragments from animals and humans).

Although the occurrence of airborne biological particles in atmospheric PM samples had been recognized since the second half of the last century, these components received less attention than the other PM species and their quantitative contribution to PM mass was widely underestimated. This is probably due to the traditional pairing of atmospheric sciences with analytical chemistry and to the lack of an interdisciplinary approach able to include chemistry, biology and biotechnology. PBAP has been significantly addressed by quantitative studies about PM only in the last decade, with a recognized increasing importance of their mass contribution and of their role in climate, medical and atmospheric pollution issues (Despres et al., 2012).

A variety of methods for bioaerosol detection have been developed. Some of the oldest ones, often thought to evaluate the quality of air from the point of view of pathogenicity or allergenicity, were aimed to measure specific viable species (bacteria, fungi, algae). These studies were generally culture-based and able to determine bioaerosol concentration in terms of number of colony-forming units (CFU m^{-3}) and not to yield a quantitative response in terms of mass concentration (Menetrez et al., 2007b; Hsu et al., 2012; Lee et al., 2012, among others). Besides, most of the environmental PBAPs are non-viable or non-culturable (only about 17% of known fungal spores and about 1% of bacteria can be grown in culture) and the results obtained by cultivation methods drastically underestimate their variety and air concentration (Bridge and Spooner, 2001; Chi and Li, 2007; Fierer et al., 2008).

Some other methods focus on a specific class of bioaerosol and consist in the identification and determination of a specific chemical tracer of that class. This is the case, among others, of cellulose for plant debris (Puxbaum and Tenze-Kunit, 2003), ergosterol for fungal spores (Di Filippo et al., 2013), muramic acid for bacteria (Bal and Larsson, 2000), dipicolinic acid for bacterial spores (Li et al., 2004), phospholipids for fungal cells, bacteria and pollen (Pankhurst et al., 2012; Womiloju et al., 2003). Although very reliable and sensitive, these methods rely on the use of a numerical factor to convert the tracer amount into carbon mass and thus to evaluate the quantitative contribution of this type of bioaerosol to total PM. These factors are generally species-dependent and inevitably imprecise; moreover, the quantification of the overall bioaerosol content of atmospheric PM would require the time-consuming and costly determination of many different tracers on each sample.

More recently, molecular biology techniques such as polymerase chain reaction (PCR) or fluorescent in-situ hybridization (FISH) have been applied to atmospheric samples. PCR consists in the selection of a DNA sequence characteristic of a specific microorganism or group of microorganisms; the sequence is then amplified and identified by comparison with existing databases (Boreson et al., 2004; Peccia and Hernandez, 2006). An improved version of the technique, quantitative PCR (qPCR), has also the ability to provide quantitative information (An et al., 2006; P. Blais-Lecours et al., 2015). FISH consists in the specific hybridization of complementary nucleic acid sequences via fluorescent labelled probes (Moter and Göbel, 2000). Finally, metagenomics approaches, among which the Next Generation Sequencing (NGS) platforms, have been used to characterize the total genetic components of bioaerosol samples (Blais-Lecours et al., 2015, and cited therein). The efficiency and popularity of these techniques, able to reliably identify single biological aerosol particles on a species or genus level, have enormously increased during recent years. However, a limited number of application to atmospheric bioaerosol are reported in the scientific literature, and, to our knowledge, none was able to carry out a complete identification of the wide variety of PBAPs contained in a single atmospheric PM sample.

Only a few attempts have been made to evaluate the overall bioaerosol content of PM independently of type, viability, cultivability and fragmentation of the individual particles and to assess its contribution to the atmospheric concentration of PM. Some of these rely on complex and expensive on-line instruments developed in response to biowarfare agents, such as the Ultraviolet Aerodynamic Particles Sizer (Huffman et al., 2012) and the Waveband Integrated Bioaerosol Sensor (WIBS), based on the UV light-induced fluorescence method (Toprak and Schnaiter, 2013). Another interesting method involve the determination of the total protein mass in the sample by reaction with the NanoOrange agent and the measurement of the resulting level of fluorescence (Menetrez et al., 2007a, 2009). This latter procedure has the important advantage of determining a non-specific tracer of any type of bioaerosol, but the evaluation of the total PBAP content of atmospheric PM cannot be performed unless a reliable protein-to-total mass conversion factor for each bioaerosol type is determined. Some attempts to characterize all types of bioaerosol particles have been also carried out by using scanning electron microscopy coupled to energy-dispersion X-ray spectroscopy (SEM/EDX; Coz et al., 2010). Given the great variety of PBAPs in structures and types, however, the use of this technique requires a manual classification of the acquired images, which makes the method not suitable for routine analyses.

We report in this paper the validation of a simple method for the determination of bioaerosol contribution to the mass concentration of atmospheric PM. It includes sampling on polycarbonate filters, staining the sample with an appropriate fluorochrome, inspection of the sample by epifluorescence microscopy and calculation of the bioaerosol mass with the aid of a digital image analysis software. The method has been applied to PM_{10} samples collected over a 2-year period in the peri-urban area of Rome, Italy and, with appropriate adjustments, to size-segregated samples collected on a multi-stage impactor. Side-by-side determinations of OC and of the PM mass for the calculation of bioaerosol contribution to organic matter and to PM mass have been also carried out.

2. Experimental

2.1. Sampling site and equipment

PM samplings were carried out during the period 2013–2015 at the facilities of the Institute of Atmospheric Pollution Research in Montelibretti, a peri-urban area at about 25 km from Rome (42°06′13.2″N, 12°37′48.0″E, 48 m a.s.l.). The site is located in a green area with trees and bushes, about 50 m from the nearest local road and 500 m from a busy road.

Sampling of atmospheric PM devoted to PBAP determination was performed on 47 mm polycarbonate filters, 0.8 μm pore size (Isopore membrane filters, MILLIPORE, Mi-IT). 24-h samples were collected from midnight to midnight by means of a Silent Sampler (FAI Instruments, Fonte Nuova, Rm-IT) operating at the flow rate of 10 l min^{-1} and equipped with a PM_{10} impactor. This value of the flowrate was chosen so as to complete the 24-h sampling period without running into excessive pressure drop across the filter.

Additional instruments were used to determine the mass concentration of PM_{10} and to collect samples aimed at determining OC. Following the same sampling schedule, daily concentration of PM_{10} was determined using an automated beta attenuation monitor (SWAM 5a, FAI Instruments, Fonte Nuova, Rm-IT) operating at the flow rate of 2.3 m^3 h^{-1} and equipped with Teflon membrane filters (TEFLO, 47 mm, 2.0 μm pore size, PALL Life Sciences); samples aimed at determining the daily concentration of EC and OC were collected on quartz fibre filters (TISSUQUARTZ 2500QAT, 47 mm, PALL Life Sciences) by means of an additional Silent Sampler.

A series of twenty 24-h samples aimed to determine the concentration of PBAPs was taken approximately once a month from May 2013 to April 2015; OC and PM_{10} concentration was measured as well.