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Fungal spores and pollen in particulate matter collected during agricultural activities in the Po Valley (Italy)

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ABSTRACT

Airborne particulate matter (PM) containing fungal spores and pollen grains was sampled within a monitoring campaign of wheat threshing, plowing and sowing agricultural operations. Fungal spores and pollen grains were detected and identified on morphological basis. No studies were previously available about fungal spore and pollen content in agricultural PM in the Po Valley. Sampling was conducted in a Po Valley farmland in Mezzano (Ferrara, Italy). The organic particles collected were examined by scanning electron microscopy with energy dispersive X-ray spectrometer. Fungal spores and pollen grains were identified when possible at the level of species. The most frequent components of the organic particles sampled were spores of *Aspergillus* sp., which could represent a risk of developing allergies and aspergillosis for crop farmers.

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Introduction

Particulate matter (PM) is one of the most common air pollutants, known for its significant impact on environment (Bell and Holloway, 2007) and human health (Pope et al., 2002; Kelly, 2009). It is a complex mixture of solid and/or liquid particles suspended in air, different in size, composition and origin. Particles in PM are classified according to aerodynamic diameter, namely the diameter of a spherical particle with a density of 1000 kg/m³ and the same settling velocity as an irregular particle. Based on this parameter, PM is classified in inhalable coarse particles (with aerodynamic diameter larger than 2.5 µm), fine particles (with aerodynamic diameter smaller than 2.5 µm) and ultra-fine particles (aerodynamic diameter smaller than 100 µm), according to the International

Organization for Standardization (1995). This standardization was developed mainly to assess health effects of airborne particles in working places and in the environment, and provided definitions and conventions for aerodynamic diameter, inhalable, extra-thoracic, thoracic, tracheo-bronchial and respirable fractions. The PM may be produced by different sources and mechanisms, and contain numerous chemical compounds. They may be emitted from natural (e.g., dust storms, forest fires, and volcanoes) and anthropogenic sources (e.g., car, airplane and naval traffic, domestic coal burning, power plants and industrial processes, and waste incinerators) relevant to public health. These sources are mainly concentrated in urban areas, usually with high population density. Some particles (primary particles) are directly produced from sources such as sea sprays, desert dust, agricultural activities,

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stacks, fires, building sites, unpaved roads and fields. Others (secondary particles) are formed in complex chemical reactions in the atmosphere, such as sulfur dioxide and nitrogen oxides produced from combustion of fossil fuels and volatile organic compounds from power plants, industries and automobiles (Tucker, 2007).

The impact of PM air pollution on morbidity has been examined, providing evidence of detrimental effects on respiratory conditions as well as negative impacts on cardiovascular diseases following both short-term and chronic exposure. Acute (Zanobetti et al., 2000; Atkinson et al., 2001; Halonen et al., 2008) and chronic respiratory effects (Schikowski et al., 2005; Gasparini et al., 2006; Downs et al., 2007; Grigg, 2009) related to environmental PM concentrations include reduced lung function, increased asthma symptoms (Gauderman et al., 2004; Meng et al., 2010) and childhood allergies (Morgenstern et al., 2008; Parker et al., 2009; Gehring et al., 2010). Cardiovascular effects associated with short and/or long-term increase in PM air pollution may cause ischemic heart disease (Tonne et al., 2007; Zanobetti and Schwartz, 2007) and cerebrovascular disease (Hong et al., 2002; Miller et al., 2007). However, the identification of toxic components in PM is a challenging task since PM air pollution is a complex mixture of particles varying in mass, number, size, shape, surface area, chemical composition, as well as reactivity, acidity, solubility and origin. Airborne pollen and fungal spore are part of natural sources of air pollution. Scientists were initially interested in biological characteristics of pollen grains (Kanchan and Chandra, 1980). More recently, the impact of pollen on human health increased from a local issue to a global problem (Behrendt et al., 1997; Emberlin, 1998; Hoffmann et al., 2003). A current hypothesis for this increasing impact is that air pollution may affect directly pollen grains through contamination of flower organs or during air dispersal of pollen grains (Chehregani et al., 2004).

Pollen grains are male reproductive structures of higher plants. Wind and other abiotic and biotic agents transport pollen from anthers to the stigma of the same flower or of a different flower of the same species, enabling sexual reproduction. The wind releases pollen into the atmosphere during the pollination season thus, in suspension with other particles (Colls, 2002), pollen becomes part of the atmospheric aerosol and may enter human airways. Pollen grains are not only a seasonal air pollutant, but their presence and amount in the atmosphere changes according to meteorological (temperature, precipitation, humidity, wind, etc.), biological (physiological state of plants, plant distribution, etc.) and topographic factors (Puc and Wolski, 2002). The air pollutants may adhere to the surface of airborne pollen grains, changing their morphology (Majd et al., 2004; Rezanejad, 2009). Several studies proved that air pollution may load pollen grains, favoring their descent and increasing their percentage in breathable air, thus promoting allergies and asthma in people living in highly polluted areas (Behrendt and Becker, 2001; Chehregani et al., 2003; D'Amato, 2011).

Interactions between pollutants and pollen wall were studied by electron-probe microanalysis (EPMA) to characterize single-particle aerosols (Ro et al., 2005; Worobiec et al., 2007; Guimarães et al., 2012). The interactions between pollen and other polluting materials such as spores of molds and

bacteria were also investigated (Behrendt et al., 1997; Sawidis, 1997; Okuyama et al., 2007). Scanning electron microscope with energy dispersive X-ray spectrometer (SEM-EDS) was used to show accumulation of atmospheric particles on the pollen surface (Majd et al., 2004; Okuyama et al., 2007; Shahali et al., 2009). Guedes et al. (2009) compared by Micro-Raman spectroscopy the presence of PM on the surface of pollen from *Chenopodium* sp. (Magnoliophyta Caryophyllales) collected in an urban area with pollen from the same genus collected in a rural environment.

Following these studies, the PM emitted during three types of agricultural activity (wheat harvest threshing in summer, plowing and wheat sowing in autumn) was sampled and analyzed in a rural area in the eastern Po Valley (Italy). The size distribution, morphology and chemical composition of inorganic components of these agricultural aerosols were previously analyzed by SEM-EDS (Telloli et al., 2014). Here we analyze by SEM-EDS the organic components of the same PM.

The focus of this study was to determine the prevalence of spores and pollen that could cause human respiratory pathologies such as allergies and aspergillosis.

1. Materials and methods

1.1. Field site

Field sampling was carried out in the eastern Po Valley at Mezzano, near Argenta (Ferrara, Italy), during routine agricultural activities of wheat farming in 2009. Wheat chosen at that time was the main crop that was produced in Italy (AGRITECH, 2008). The sampling site was part of the largest rural areas of the Emilia-Romagna region, in the Comacchio Valley near the sea (44° 36' 40.79" N — 12° 04' 10.52" E, −1 m) (Fig. 1). The soil is classified as a silt clay soil, built from terrigenous sediments and transported and deposited by the Po River (Bianchini et al., 2001).

The aerosol was sampled within the dust plume emitted by the agricultural machinery during wheat harvest threshing in summer (from 2:00 to 7:00 pm Coordinated Universal Time (UTC) + 1 on June 25, 2009) and in autumn during plowing (from 12:00 am to 3:00 pm UTC + 1 on July 7, 2009 and from 11:00 to 12:30 am UTC + 1 on July 08, 2009) and sowing (from 12:00 am to 1:30 pm and from 4:15 to 5:30 pm UTC + 1, on November 17, 2009). The sampling time was chosen to minimize the contribution of effects of thermal inversions. The rural area was about 13 km from the Adriatic Sea and distant from main and secondary roads, in order to minimize the contribution of sea spray and anthropogenic pollution from traffic and other combustion sources. The area was also about 55 km from factories or industries. The three aerosol background monitoring stations of Regional Agency for Environmental Protection and Prevention of Emilia-Romagna (Agenzia Regionale Per La Prevenzione E L'Ambiente (ARPA), Ferrara, Italy) located in Ballirana (Alfonsine, Ravenna, Italy) (BK1), Ostellato (Ferrara, Italy) (BK2) and Gherardi (Jolanda di Savoia, Ferrara, Italy) (BK3) (Fig. 1) measured a mean concentration of particulate matter in the range of 2.5 μm ($\text{PM}_{2.5}$) of 8.0 ± 2.6 , 19.8 ± 4.0 and $25.7 \pm 0.6 \mu\text{g}/\text{m}^3$, respectively in the sampling days of threshing, plowing and sowing (ARPA Emilia-Romagna, 2015a).

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