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

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ARTICLE INFO

Article history:

Received 3 November 2015

Revised 26 February 2016

Accepted 29 February 2016

Available online 29 April 2016

Keywords:

Wheat

Aspergillosis

Emilia-Romagna

Morphology

SEM-EDS

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 (AGRIT, 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).

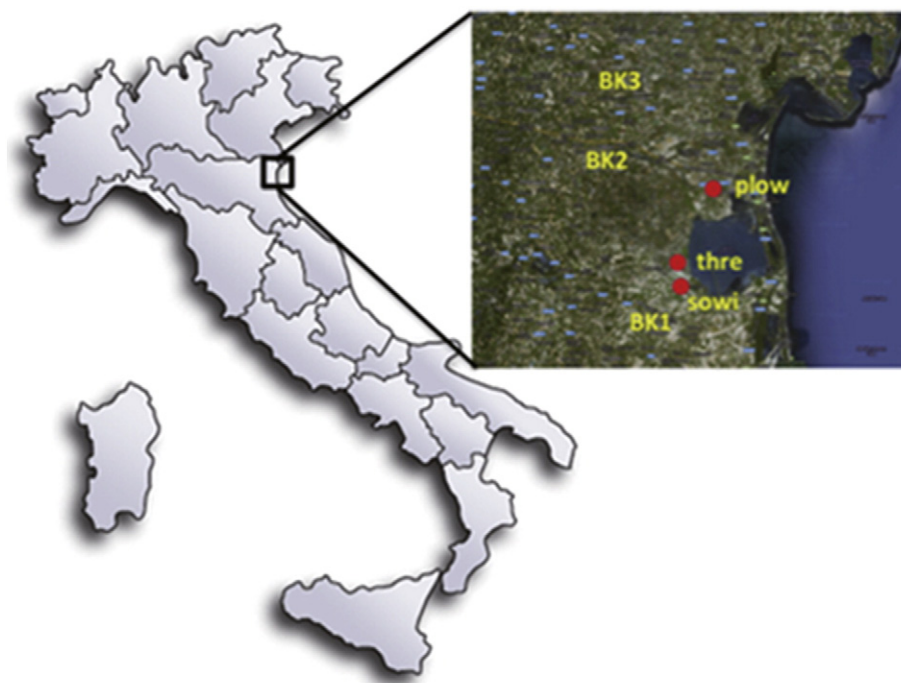


Fig. 1 – Map of sampling sites (thre, threshing; plow, plowing; sowi, sowing) and of the three aerosol monitoring stations (BK1, BK2, BK3).

1.2. Sampling

The emitted dust plume was sampled with a Marple eight-stage cascade impactor (Model 298, Graseby Andersen Samplers, Inc., Atlanta, Georgia). Two quartz fiber filter types were used as collection substrates, the impactor substrate SKC filter 225–1826 1.2 μm R-100 (SKC, Inc., USA) and the open face Pall Tissuquartz filter 2500 QAT-UP Ø34 mm (Sigma-Aldrich, Mo, USA). The eight stages of the impactor had SKC filters with the following aerodynamic equivalent particle diameters (AED): 0.52–0.93, 0.93–1.55, 1.55–3.5, 3.5–6, 6–9.8, 9.8–14.8, 14.8–21.3, >21.3 μm . The final particles with AED <0.52 μm were collected by the PALL, used as final filter. An open filter holder equipped with a cylindrical apron was used to collect total suspended particles (TSP) on a polytetrafluoroethylene (PTFE) membrane (Pall teflo w/ring pore size 1.0 μm Ø47 mm R2PL047, Pall Corp., USA). Samplers were placed within the dust plume by a telescopic pole mounted on a vehicle. An air sampling pump SKC Airchek 2000 (SKC, Inc., USA) was used with a flow rate of 2 l/min. The pump was compensated for losses up to 1000 mm H₂O to maintain a constant sampling flow rate. The aerosol was sampled into the dust plume following the agricultural machine only downwind during the three chosen activities. The distance (5.7 m for threshing and 2 m for plowing and sowing) between the impactors and the emission point (agricultural machine) was kept constant to avoid as much as possible the aerosol dispersal during sampling. The number of PM particles analyzed was 750 (5 repeated analyses of 150 particles each) respectively for threshing and plowing, and 300 (2 repeated analyses of 150 particles each) for sowing.

The meteorological data (temperature, humidity, wind direction and speed) were provided by the weather station of ARPA (Argenta, Ferrara, Italy) (44° 36' 01.24" N–12° 04' 37.72" E) as previously described (Telloli et al., 2014).

1.3. Analyses

After collection, all samples (sealed with aluminum foil) were transported in a refrigerated cooler to the laboratory for the study of air quality of the Technical Unit for Modeling, Methods and Technologies for Environmental Assessment (UTVALAMB, Italian National Agency for New Technologies, Energy and Sustainable Economic Development (ENEA), Bologna, Italy). The filters were maintained under sterile conditions, placed in plastic Petri dishes and stored at –25°C at the Department of Earth Sciences, University of Ferrara (Ferrara, Italy) until microscopical analyses.

Samples collected on SKC and Pall Tissuquartz filters were first observed under a stereomicroscope SZ6745TR (Optika, Ponteranica, Bergamo, Italy) (total magnification 90×) equipped with a webcam Moticam 5.0 Mp (Motic Asia, Hong Kong), for a preliminary analysis of PM. Afterwards they were observed under a polarizing microscope with incident and transmitted illumination B-600 POL-I (Optika) equipped with a webcam Moticam 5.0 Mp (Motic Asia). All observations were conducted at the Laboratory of the Department of Earth Sciences, University of Ferrara (Ferrara, Italy). Images were displayed by the program Moticam Image Plus 2.0 (Motic Asia).

The PTFE membranes were used for analyses by SEM–EDS at the Laboratory of Applied Ecology of the Department of Life Sciences and Biotechnology of the University of Ferrara (Ferrara, Italy), to characterize morphology and composition

in chemical elements of collected aerosol particles. The analyses were performed by a SEM Zeiss AG-EVO® 40 Series — EVISA (Carl Zeiss AG, Oberkochen, Germany) equipped with an EDS INCA 300 (Oxford Instruments, Abingdon, United Kingdom) for X-ray microanalysis. Images were displayed by the program SmartSEM (Zeiss). The particle size and the surface morphology of sampled aerosol particles were investigated in high resolution mode with a working voltage of 20 kV, choosing to analyze the particles between 1 and 10 μm . High resolution images of particles were obtained by regulation of vacuum inside the instrument vacuum chamber. The SEM-EDS technique is often employed to identify airborne particulate deposits and biological materials (Haapala, 1998). In EDS, the X-ray detector measures the number of emitted X-rays as a function of their energy: since chemical elements have a characteristic energy, the EDS spectrum can be used to identify the amount of a given chemical in a sample (Frankel and Aitken, 1970). This technique may characterize the chemical composition of particles with diameter larger than 1 μm (Wilkinson et al., 2011). The SEM technique can be employed in different studies of the atmosphere (De La Campa et al., 2007; Fromme et al., 2008; Campos-Ramos et al., 2009). Measurements for SEM-EDS were performed on TSP samples collected on PTFE membranes. Portions of the filters were mounted on aluminum support “stubs” with double-sided tape with a graphite-based electrical conduction. Some samples were coated with a thin layer of gold (coating) film by electric arc high vacuum method and then analyzed by SEM. The full characterization of the fine particles was prevented by the detection limit of the instrument and by the difficulty to identify the small particles within the web of PTFE membranes (Conner and Williams, 2004; Casuccio et al., 2004).

The morphological and chemical compositions by SEM-EDS and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) analysis for the terrigenous fraction are described in detail in Telloli et al. (2014).

1.4. PM particles identification criteria

The identification of pollen was based on the handbook by Hesse et al. (2009). The identification of fungal spores and other structures was based on Käärik et al. (1983), choosing the size identification limit of 1 μm .

2. Results

2.1. Optical microscopy

The PM particles collected on teflo filters were first observed by stereomicroscopy (Fig. 2) and then prepared for SEM-EDS. At the stereomicroscope, the organic particles on a teflo filter larger than 10 μm were crushed vegetable parts (Fig. 2).

All SKC quartz filters (AED 0.52–0.93, 0.93–1.55, 1.55–3.5, 3.5–6, 6–9.8, 9.8–14.8, 14.8–21.3, >21.3 μm) and the final Pall Tissuquartz filters (AED < 0.52 μm) were first analyzed by stereomicroscopy and then by optical microscopy. Fig. 3a shows an example of SKC filter (threshing) observed under a stereomicroscope: PM particles had different sizes and the colors varied from yellow to brown to dark black. A preliminary analysis revealed that in these samples the amount of vegetable fragments, easily recognizable for their rather elongated shape and brownish color, was about one third of the particles (Fig. 3a). The other PM particles of organic origin were more or less roundish and their color varied from light brown to dark brown and black; some were isolated and others gathered in clusters. These particles could be clearly identified only by SEM-EDS.

When SKC filters were observed under the optical microscope, only the stages with AED 0.52–0.93, 0.93–1.55, 1.55–3.5, 3.5–6 and 6–9.8 showed particles with size less than 10 μm . Fig. 3b shows an example of the impactor stage 0.52–0.93 μm .



Fig. 2 – Organic particles on a teflon (open face) filter. Inlay: magnification of a detail of the filter. Inlay scale bar: 500 μm . An arrow highlights crushed vegetable parts.

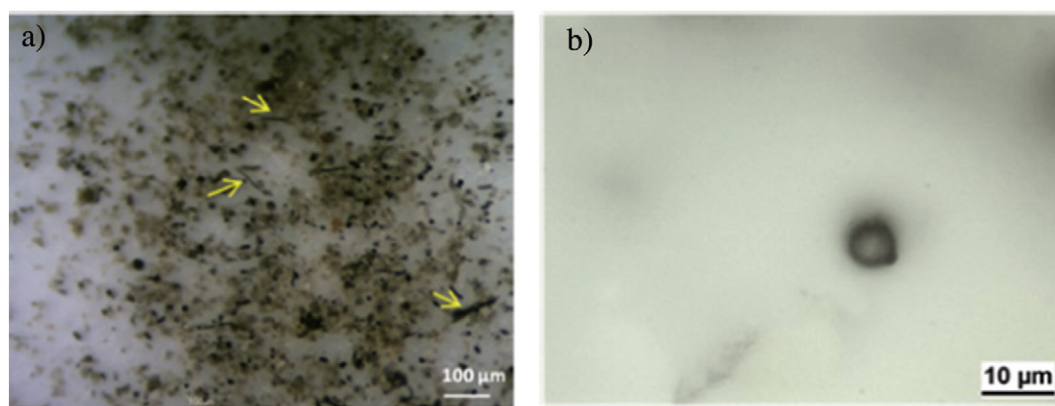


Fig. 3 – Optical microscopy observations of SKC (impactor substrate) filters. (a) filter observed by stereomicroscopy at magnification 90× showing PM particles of different sizes and colors (impactor stage >21.3 μm). An arrow highlights crushed vegetable parts. White scale bar on the left: 100 μm; (b) PM particle less than 10 μm on a filter observed at 500× (impactor stage 0.52–0.93 μm). PM: particulate matter.

This size is relevant for human health, since PM <10 μm is classified as inhalable coarse particles that could penetrate the human respiratory tract and create serious epidemiological risks (Von Klot et al., 2005).

2.2. SEM and SEM-EDS analyses

The organic particles collected on teflo filters respectively during threshing, plowing and sowing were observed by SEM-EDS after metallization with gold. The EDS microanalysis confirms the organic particles observed through the chemical composition. Organic particles observed refer only to bioaerosols, excluding combustion and semi-volatile PM. This was confirmed by Organic Carbon/Elemental Carbon (OCEC) analysis described in Telloli et al. (2014). At SEM the majority of PM particles identified were composed of fungal spores, single or aggregated, with variable shape (spherical, ellipsoidal, pyriform, etc.). The size of these spores varied from 1 to 300 μm, but the majority of them were within 3 to 30 μm. Since the organic particles most relevant for human health have size ≤10 μm, the results concern these types of particles.

2.2.1. Threshing

During threshing the organic particles collected were mostly fungal spores, but there were also bacteria, plant fragments and pollen grains (Fig. 4).

Fig. 4a shows aggregates of conidiospores of *Aspergillus* sp. (Ascomycota Eurotiales), a highly aerobic mold growing in almost all oxygen-rich environments, usually on the surface of substrates such as wheat. The genus is identified for the morphology of conidiospores and conidia, one of which is shown in detail in Fig. 4b. Fig. 4c shows an association of conidia of *Aspergillus* with a spore of another fungus belonging to the family Pucciniaceae (Basidiomycota Pucciniales). The spore is recognizable by its surface ornamentation with irregular verrucous tubercles (Fig. 4D, E) (Käärik et al., 1983). Pucciniaceae are a group of rust fungi causing cereal plant diseases.

The spores in Fig. 4f are difficult to identify, but probably represent spores of Trichocomaceae, a cosmopolitan family of Ascomycota (Eurotiales), based on Käärik et al. (1983).

Fig. 4g shows two spores of another fungus from the division Ascomycota, *Alternaria* sp. (Pleosporales). The spores of this genus were identified by their typical pear shape and the presence of septa crossing in both directions.

Fig. 4h and i shows pollen of Poaceae (Liliopsida Poales) and Fig. 4i shows a pollen grain from *Triticum* sp. The pollen grains of this plant family have spherical or oval shape and only one opening.

Other organic particles found in the threshing dust plume were colonies of bacteria (Fig. 4m) and vegetable fragments (Fig. 4n).

2.2.2. Plowing

During plowing the organic particles collected were mostly fungal spores, but there were also some pollen grains, bacteria and plant fragments (Fig. 5a–h). Fungal spores and pollen grains were different from those collected during threshing.

Fig. 5a shows a spore chain of *Aspergillus* Section *Nigri* (Silva et al., 2011), ubiquitous in soil and decaying organic matter (Samson et al., 2004), where it plays a key role in carbon and nitrogen recycling but causes a disease (“black mold”) on fruits and vegetables. Fig. 5b–e shows fungal spores and hyphae with different morphologies and ornamentations, which could not be clearly identified.

Among the pollen grains that could be identified, one clearly belonged to the genus *Amaranthus*, family Amaranthaceae (Magnoliopsida Caryophyllales) (Fig. 5f) (Hesse et al., 2009). These pollen grains are spheroidal with 30–65 pores and thin, granular exine. Another pollen grain, shown in Fig. 5g, was tentatively identified as belonging to the family Poaceae.

Frequent vegetable fragments similar to that shown in Fig. 5h and bacterial colonies similar to those seen in Fig. 5b and e were also found.

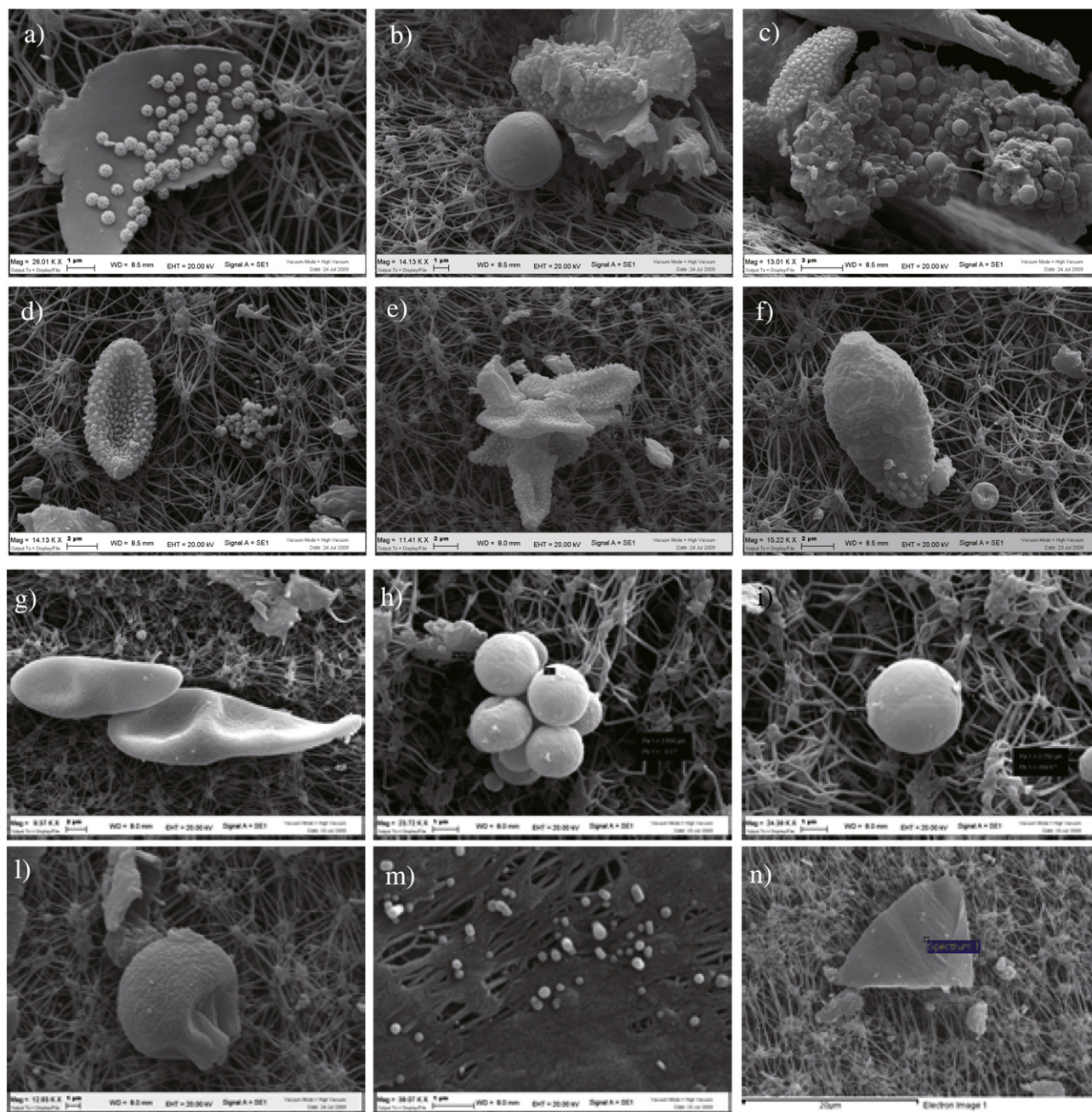


Fig. 4 – Examples of organic particles collected during threshing and observed at SEM. (a) conidiospores and conidia of *Aspergillus* sp.; (b) detail of *Aspergillus* sp.; (c) association of conidia of *Aspergillus* with a spore belonging to the family Pucciniaceae; (d) and E spores of the family Pucciniaceae; (f) a spore of the family Trichocomaceae; (g) two spores of *Alternaria* sp.; (h) and (l) pollen grains of the family Poaceae; (l) a pollen grain of *Triticum* sp.; (m) colonies of bacteria; (n) vegetable fragments. SEM: scanning electron microscope.

2.2.3. Sowing

During sowing the organic particles collected were only composed of fungal spores (Fig. 6a–f).

Fig. 6a shows a spore chain of *Aspergillus* Section *Nigri* (previously found during plowing) and Fig. 6b shows another species of the genus, *Aspergillus fumigatus*, identified by the cup-shaped morphology of spores (Cramer et al., 2008). Fig. 6c and d shows other spores belonging to the genus

Aspergillus whose species could not be identified. Fig. 6e probably shows conidiophores of *Stemphylium* sp. (Ascomycota Pleosporales), a genus including species which parasitize plants. Fig. 6f shows a radulasporer with smooth wall, identified as belonging to the genus *Arthrinium* sp. (Ascomycota Apiosporaceae) based on Käärik et al. (1983). This cosmopolitan genus of filamentous fungi is common in plant debris and soil.

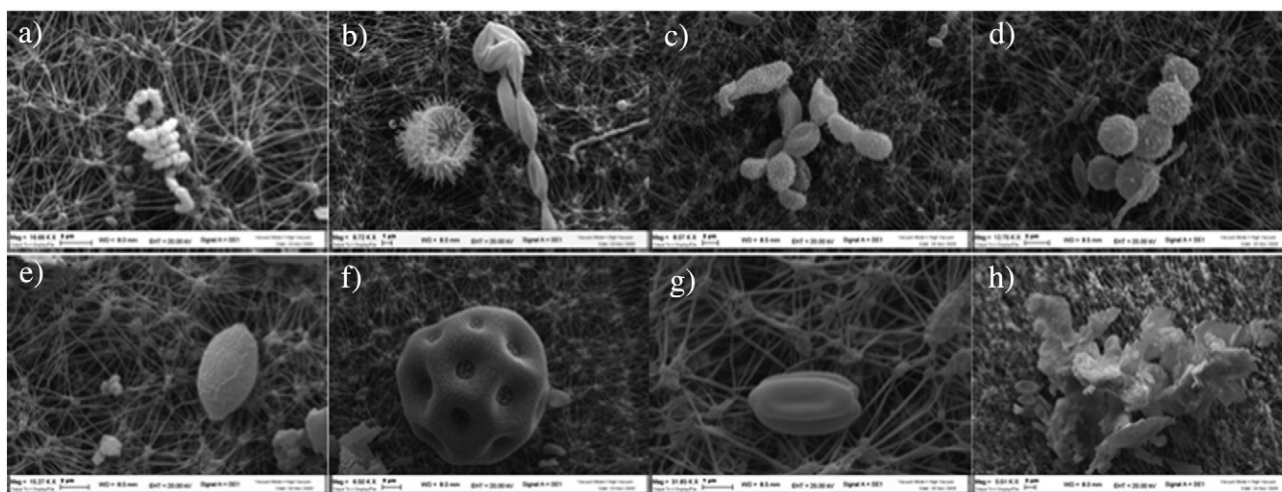


Fig. 5 – Examples of organic particles collected during plowing and observed at SEM. (a) chain of spores of *Aspergillus* Section *Nigri*; (b), (c), (d), (e) unidentified fungal spores and hyphae; (f) a pollen grain of *Amaranthus* sp.; (g) a pollen grain of the family Poaceae; (h) vegetable fragment.

3. Discussion

The PM particles collected during threshing, plowing and sowing activities, analyzed by SEM, were divided into organic and inorganic particles based on EDS analysis. The percentage of organic material was then calculated by counting and resulted high in all three agricultural operations, respectively amounting to 55% during threshing and 45% during plowing. In the samples collected during sowing the organic contribution was the highest (over 60%), probably because of humidity favoring the proliferation of molds and bacteria. The high presence of organic materials in the samples collected during

the three agricultural operations was confirmed by previous data on particulate organic matter obtained by analysis of organic carbon with a thermal optical analyzer (Telloli et al., 2014).

The majority of PM particles collected during the three agricultural activities were fungal spores: the pollen grains, the bacterial colonies and the plant fragments were rare (Figs. 4, 5, 6). The most frequent fungal taxa were the genus *Aspergillus* sp. (Ascomycota), and the family Pucciniaceae (Basidiomycota) and Trichocomaceae (Ascomycota). Occasionally, *Alternaria* sp. (Pleosporales) was found in threshing and the genera *Stemphylium* sp. (Ascomycota Pleosporales) and *Arthrinium* sp. (Ascomycota Apiosporaceae) in sowing.

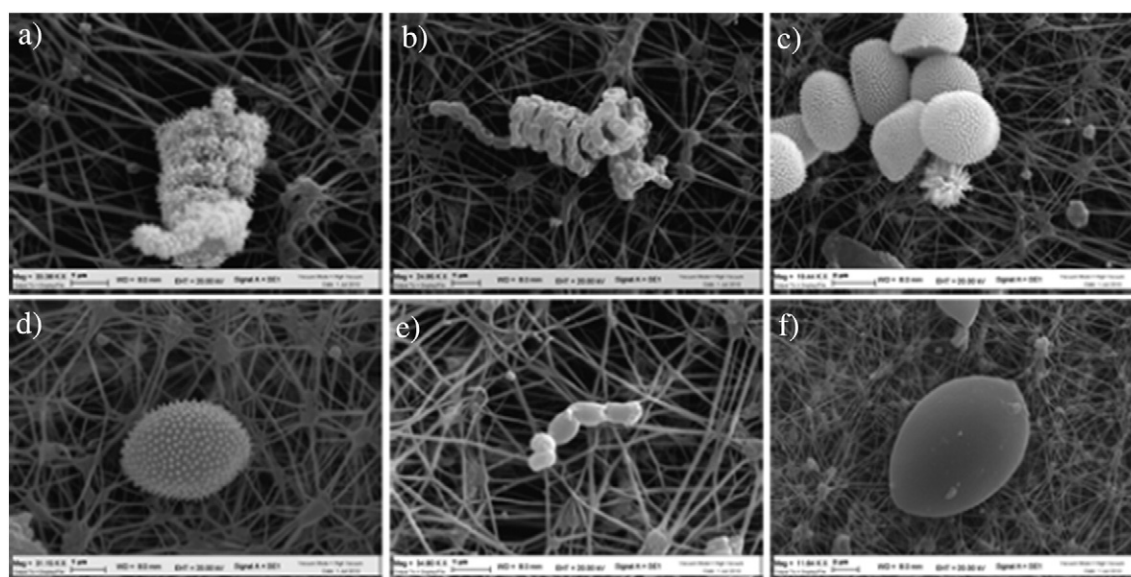


Fig. 6 – Examples of organic particles collected during sowing and observed at SEM. (a) chain of spores of *Aspergillus* Section *Nigri*; (b) chain of spores of *Aspergillus fumigatus*; (c) and (d) spores of *Aspergillus* sp.; (e) chain of spores of *Stemphylium* sp.; (f) a spore of *Arthrinium* sp.

During sowing the wheat was treated with 5 ml/Kg of triticonazole–guazatine 1:1, an antifungal selective for *Fusarium* sp., *Parastagonospora nodorum* and *Tilletia* sp. We cannot rule out the fact that the treatment could have exerted a selective action on fungal species detected.

Pollen grains were found only in samples from threshing and plowing and mostly belonged to Poaceae (Fig. 4h, i, l, f, g): in threshing it was possible to identify the genus *Triticum* sp. (Fig. 4l), while in plowing pollen grains from the family Amaranthaceae, genus *Amaranthus* sp. could be identified (Fig. 5f).

ARPA Emilia-Romagna publishes monthly bulletins concerning the presence of fungal spores from plant pathogens and pollen grains from potentially allergenic plants (ARPA Emilia-Romagna, 2009). These data support the fact that *Alternaria* sp. was frequent during the sampling period of threshing and during sowing *Stemphylium* sp. could be present. Concerning the genus *Aspergillus* and its species we detected, no data are included in the ARPA bulletins.

About pollen grains, the ARPA bulletins provide forecasts of flowering time and pollen air diffusion of native vegetation based on the average vegetative growth detected in the previous year (Table 1). The ARPA data are in agreement with the fact that pollen from Poaceae was frequent during threshing, somewhat less during plowing, and absent during sowing. According to ARPA data, pollen from the family Amaranthaceae had a low concentration during threshing and plowing and this is in agreement with our results which identified *Amaranthus* pollen only in plowing.

In the Emilia-Romagna region (Northern Italy) the main factors affecting the presence of fungal spores and/or pollen in airborne PM are temperature and relative humidity: generally the concentration of fungal spores is higher in rural areas (in relation to agricultural activities) in comparison to urban ones (Dorner and Horn, 2007; Atehnkeng et al., 2008). Temperature and humidity are known to promote fungal and bacterial proliferation (Lamonaca et al., 2014; Pakpour et al., 2015). For example, the growth of the genus *Alternaria* is favored by an air temperature of 18–32°C, a relative humidity higher than 65% and by calm wind conditions (Rodriguez-Rojo et al., 2005). A previous study confirmed that the first condition (high temperature) occurred during threshing and plowing activities, the second one (humidity) during sowing, and the third one (calm wind) during all activities (Tello et al., 2014).

Figs. 4, 5 and 6 show that the organic matter sampled during threshing had a more crushed structure when compared to samples from the two other agricultural activities. Palynological studies (Hesse et al., 2009) support the fact that crushed particles (dry particles) are typical of environments

with scarcity of water (June was a dry month during sampling in 2009). On the contrary, non-crushed particles with a more rounded morphology are typically found when soils are highly moisturized during the sampling period (October for plowing and November for sowing) (Nakagawa et al., 2000; Kim, 2007). In regions where temperatures are consistently high, there is a significant loss of water through evapotranspiration, so even with high rainfall only some water is available to plants or to geomorphological processes. Aridity is a climate condition caused by the simultaneous lack of rain (200–400 mm) and strong evaporation. About 41% of the surface of the planet is characterized by hyper-arid, arid or semi-arid lands (Feng and Fu, 2013). According to precipitation/potential evapotranspiration index (De Martonne, 1926) the aridity index range of the East Po Valley from 1960 to 2008 is 0.65–1.00 (Costantini et al., 2013). These data support the presence of crushed particles during threshing in summer.

Fig. 7 shows the trend of annual mean temperature (°C) and the concentration of rainfall (mm) during the year 2009 in the sampling area (ARPA Emilia-Romagna, 2015b). It is interesting to notice the high concentration of heavy rains in August 2009, an anomalous condition when compared to the previous years. These heavy rains favored the proliferation of fungi in the plowing period. Soil moisture therefore represented a relevant factor for agricultural emissions, requiring further analyses in targeted monitoring campaigns.

3.1. Medical implications

Fungal spores and pollen are carried by wind over large distances and, being part of PM inhalable by humans, they may reach the deep airways, causing medical symptoms (Gangl et al., 2015; Fukutomi and Taniguchi, 2015). According to bulletins published online by the Rete Italiana di Monitoraggio Aerobiologico POLLnet in Emilia-Romagna (Northern Italy) fungal spores are by far the most frequent component of organic particulate matter (POLLnet Emilia-Romagna, 2015).

The fungal spores most commonly responsible for respiratory allergies in Emilia-Romagna are those of the genera *Alternaria*, *Cladosporium*, *Epicoccum*, *Botrytis*, *Stemphylium*, *Aspergillus* sp. (Knutsen et al., 2012; ARPA Emilia-Romagna, 2015c). The sampling of fungal spores during agricultural activities is of considerable medical interest to avoid professional exposure in the critical periods.

Our data show that the amount of fungal spores detected during the three agricultural activities sampled (threshing, plowing and sowing) was always prevalent in comparison to pollen grains and bacteria. The identified genera of fungal spores included *Alternaria* sp., *Stemphylium* sp., *Aspergillus* sp.

Table 1 – Average concentrations of pollen from Poaceae and Amaranthaceae, and spores from *Alternaria* sp. and *Stemphylium* sp. (particle/m-3 air) according to palynological bulletins of ARPA Emilia-Romagna (2009) in the province of Ferrara on sampling periods.

		Poaceae	Amaranthaceae	<i>Alternaria</i>	<i>Stemphylium</i>
Threshing	25 June 2009	>30	Low (0.1–4.9)	519	40
Plowing	7 October 2009	Low (0.6–9.9)	Low (0.1–4.9)	132	36
	8 October 2009	Low (0.6–9.9)	Low (0.1–4.9)	265	22
Sowing	17 November 2009	–	–	2	2

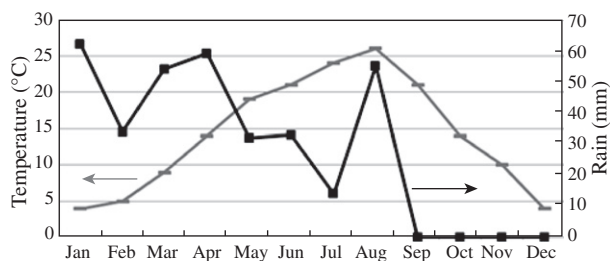


Fig. 7 – Annual mean temperature and rainfall during 2009 in the sampling area (ARPA Emilia-Romagna, 2015b).

and, as expected, the higher prevalence was detected during sowing in November, the month with the highest average rainfall in Emilia-Romagna.

All genera of fungal spores described in our study are associated to medical syndromes. For example, *Alternaria* sp. is an important airborne allergen, producing about ten different allergens (Knutsen et al., 2012) which may cause hay fever, asthma and other allergy related symptoms (Bush and Prochnau, 2004; Knutsen et al., 2012). Exposure to *Alternaria* sp. has been related to persistent asthma in adults and children and potentially fatal episodes of asthma (Salo et al., 2006). *Stemphylium* sp. is reported to be occasionally involved in allergic asthma (Agarwal et al., 1982) but, as other fungal genera, has also been reported to cross react with *Alternaria* sp. (Portnoy et al., 2008).

Some species of the genus *Aspergillus* may cause invasive and non-invasive aspergillosis in humans, animals and insects (Yu et al., 2014; Chabi et al., 2015; Ohashi et al., 2015). The main clinical syndromes caused by *Aspergillus* sp. are allergic bronchopulmonary aspergillosis (Skov et al., 2005; Patterson and Strek, 2010), severe asthma with fungal sensitization (Agarwal, 2011; Hogan and Denning, 2011; Knutsen et al., 2012), aspergilloma (Harman, 2011), invasive pulmonary aspergillosis (Segal and Walsh, 2006; Harman, 2011; O’Gorman, 2011), and chronic necrotizing aspergillosis (Thurnheer et al., 2004). Other diseases caused by *Aspergillus* sp. are primary cutaneous infections and onychomycosis (fungal nail infection) (Van Burik et al., 1998). *Aspergillus* sp. may also be involved in infections of sinus and of the external auditory canal (Rotoli et al., 2001). The species of the genus mostly involved in pulmonary diseases is *A. fumigatus*.

Given the ubiquitous distribution of the genus, the inhalation of fungal spores is a common way of introduction of the pathogen, but in healthy individuals the spores are rapidly destroyed by the immune system. Thus patients at higher risk to develop pulmonary pathologies after exposure to *Aspergillus* conidia are the immunocompromised ones (Lin et al., 2001; Kousha et al., 2011; Fernández et al., 2014). For example, patients undergoing hematopoietic stem-cell transplantation may develop invasive pulmonary aspergillosis with a mortality rate up to 90% (Kousha et al., 2011).

Although *Alternaria* sp. spores are the most frequent component of organic particulate matter in Emilia-Romagna (POLLnet Emilia-Romagna, 2015), in Italy one of the main causes of allergy is pollen from Poaceae (D’Amato et al., 2007). About 20% of people show allergy to pollen from this family

(Peternel et al., 2006; D’Amato et al., 2007). When inhaled, pollen allergens cause abnormal immunoreactions characterized by symptoms similar to those of common cold, such as runny nose, sneezing, itchy nose and eyes, congestion of the nasal mucous membranes, difficulty in breathing, rashes and sometimes tightness in the chest (Puc, 2003).

Sizes were generally small and easily dispersed in the air during sporulation, which occurs from late spring until autumn in Emilia-Romagna; the highest pollen concentrations are usually recorded in late summer.

4. Conclusions

During the sampling period the most frequent components of the organic PM were fungal spores of the genus *Aspergillus* and the pollen grain component (Poaceae and Amaranthaceae) was minimal. Only during sowing, the treatment with antifungal active ingredients and the humidity degree could have affected the sampling of fungal species.

The high presence of fungal spores in samples collected during the three agricultural activities could represent a serious risk of developing allergic diseases for people directly involved in wheat farming; on the contrary, based on our data the pollen grain component does not appear to be significant. It is interesting to notice that very few studies have been published up to date on organic components of particulate matter during agricultural activities in Italy, and especially in Emilia-Romagna, a region in which about 64% of agricultural land and about 81,000 workers are involved in crop farming. Moreover, the close interactions between agricultural areas of cereal farming and urban areas in the Po Valley require further studies to monitor environmental quality and verify the effects of the agronomic cycle on human health.

Acknowledgments

We would like to thank Dr. Maria Rita Bovolenta for her precious help in analyzing samples by SEM-EDS and Professor Laura Cattani for fungal spores and pollen identification.

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