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Airborne fungi in biofuel wood chip storage sites

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ABSTRACT

An experimental biofuel wood chip storage site was studied, as a potential fungal "reservoir," by means of quantitative and qualitative assessments of airborne fungal spores.

Fungal load in the bio-aerosol, determined through active and passive methods, declined with the distance from wood piles. Occupational exposure was comparatively evaluated when two specific operational tasks, manual and mechanized handling, were performed. Under the conditions tested, the manual operators were more exposed to the airborne fungal spores (4864 ± 580 CFU m^{-3} air). The collected spores were identified as belonging to species of the genera *Alternaria*, *Aspergillus*, *Penicillium*, *Fusarium*, *Cladosporium, Pleospora, Cochliobolus*, *Epicoccum, Absidia,* and *Trichoderma.* Most prevalent were the genera *Alternaria* and *Cladosporium*, with the highest percentages of occurrence (30 and 12%, respectively). To the best of our knowledge, this is the first work reporting the identification through molecular methods of airborne fungi released during the handling of wood chip biofuel biomass.

1. Introduction

The demand for renewable energy sources, such as biomass, is steadily increasing worldwide due to recent policies aimed at reducing fossil fuel consumption (Noll and Jirjis, 2012). The use of energy crops, woody species, and forestry and agricultural residues for energy supply is estimated at between 2 and 10% of primary global net production (Smeets et al., 2007; IEA, 2012). Fast-growing wood species such as pine and poplar, are preferred for biomass-based heat and/or electricity production (Abbasi and Abbasi, 2010). However, depending on geographic location and season, the available wood does not always meet the demand for energy production and there may be periods of the year when the demand for energy cannot be directly satisfied (Noll et al., 2010; Noll and Jirjis, 2012), thus necessitating the storage of large quantities of biomass. In heating plants, the storage of comminuted wood (processed mechanically to reduce the particle size) generally occurs in outdoor piles. Therefore large-scale outdoor storage of wood (meaning thousands of tons of materials) is becoming an increasingly important issue.

During the storage time, microbial wood degradation occurs, involving the action of a broad spectrum of fungal species that may also have complementary functions (Noll and Jirjis, 2012; Suchomel et al., 2012). Hence, large-scale biomass storage sites may represent a "reservoir" of fungal spores, similarly to composting and recycling plants (O'Gorman, 2011). Moving large quantities of wood materials leads to the release into the air of a high concentration of spores that may then be inhaled and ingested by workers and/or deposited onto their skin and eyes (Grisoli et al., 2009). Since airborne mould spores have a diameter of only 2–10 μm and are ubiquitous, they can easily penetrate into the lower airways of the human respiratory tract (O'Gorman, 2011), causing severe diseases like invasive pulmonary infection, or contributing to allergic sinusitis and allergic broncho-pulmonary diseases (Cornet et al., 2002).

In biofuel plants there is a known risk associated with handling wood and wood chips (Madsen, 2006; Madsen et al., 2009); however, regulatory occupational exposure limits (OEL) are not set for airborne biological agents, nor are specific recommendations (related to biofuel plants) for bio-aerosol-related health risk management provided. To do this, a basic knowledge of bio-aerosol

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composition and, in particular, of airborne fungal spores, would be required.

The objective of this study was to investigate the airborne fungi collected in the proximity of biomass chip piles stored in outdoor environments and to evaluate workers' exposure levels to fungal spores from the bio-aerosol. The viable fungal component of the bio-aerosol was evaluated both quantitatively and qualitatively in order to assess its possible harmfulness.

2. Materials and methods

2.1. Sampling site and movement activities

The study was carried out in the experimental storage plant of the Research Center on Agriculture (CRA-ING Monterotondo Rome, Italy); all sampling procedures took place in September 2012. The storage site had a total area of 6000 m^2, where piles of poplar chips were stored for six months, from March to September 2012. The piles had a mean volume of 117 m^3, an average height of 4 m, and a weight of about 40 tons.

At the time of sampling, the internal temperature of the piles was about 30 °C, the moisture content of the wood chips was approximately 30%, and the bulk density was about 400 kg m^{-3}.

The wood chips were handled using a cabin vehicle equipped with orange peel grab (Colmar 301, Italy). The biomass was then loaded on a truck and transported to another area of the research center in order to carry out the operations generally performed in heating plants.

2.2. Meteorological measurements

The temperature, humidity, wind, and rainfall were monitored at 30-min intervals during the entire storage time by an automatic recording device (SIAP-MICROS DA 9000 Davis, CA), located in the same area.

2.3. Environmental sampling procedures

Fungal spores, mycelial fragments, and dust quantifications were carried out during the handling of six wood piles. Particulate quantification was performed using the sampling device BRAVO m2 (TECORA, 94134 Fontenay sous Bois Cedex, France), with a flow rate of 15 L/min. Each device was equipped with a Fluoropore membrane filter (FHLP04700; Millipore, MA, USA) with a pore size of 0.45 μm. Both active (Grisoli et al., 2009; O'Gorman, 2011) and passive (Grisoli et al., 2009; Yassin and Almouqatea, 2010) methods were carried out during ordinary work days as shown in Fig. 1.

2.3.1. Growth media and culture conditions

Rose Bengal agar (RBA) (HiMedia, Mumbai, India), supplemented with chloramphenicol (0.01% w/vol) was used as the culture medium. The RBA plates were incubated in the dark at 30 °C and 60% relative humidity. Fungal colonies were counted after 48 h of incubation and, to avoid underestimation of fungal concentration due to the omission of slow-growing strains, after an additional 24 h.

2.3.2. Active method

Quantitative data were collected in duplicate using the stationary air sampling device mentioned above that aspirated 8 L of air per minute for 15 min. The sampler was disinfected with 70% rubbing alcohol, and dried before loading a sterile cellulose acetate filter of 47 mm diameter and 0.22 μm pore size. The filter was then placed in a petri dish with RBA and incubated for 72 h at 30 °C.

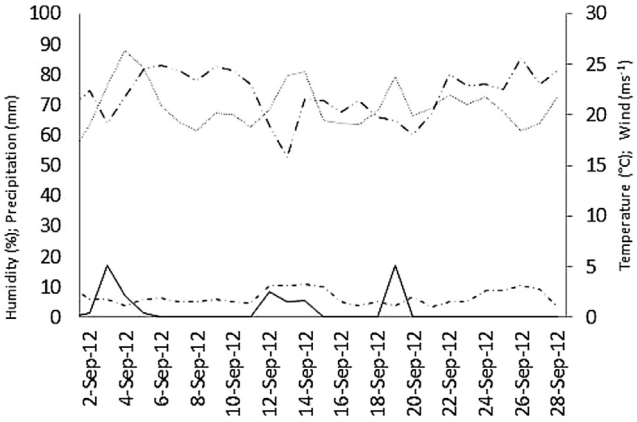

Fig. 1. Meteorological parameters registered during the experiment: air humidity (·····); precipitation (——); mean daily temperatures (·····); wind speed (– · –).

Results are expressed as numbers of colony-forming units for cubic meter of air (CFU m^{-3}).

2.3.3. Passive method

Petri dishes (90 mm) with RBA were exposed to air on a tripod at a height of 1.5 m above ground for 1 h. The plates were then incubated at 30 °C as described above. Counts of fungal colonies were expressed as colony-forming units per petri plate per hour of exposure. Colonies with different morphology were isolated in pure culture and identified. The stock cultures of the various isolates were preserved at the University of Tuscia Microbiology Laboratory (DIBAF), Viterbo, Italy.

2.4. Personnel sampling procedure

Worker exposure level, during handling of the piles, was determined by epidermal and operator breathing sampling (dust and spores).

2.4.1. Epidermal sampling

After 1 h of handling procedures, a sterile swab was pre-moistened in sterile buffer solution (50 mM Tris buffer pH 8.0, 1 mM EDTA, and 0.5% Tween-20) and then rubbed and rolled on a 2-cm^2 skin sampling area close to the eyes of the operators. The skin surface was measured with a ruler and marked. The swab was subsequently rubbed onto the agar growth medium (RBA). Epidermal sampling before exposure was taken as the control.

2.4.2. Breathing sampling

Personnel with different tasks were monitored at 20-min intervals by means of a mobile air personal sampler (EGO PLUS TT – PF 11221, Zambelli s.r.l., Italy), equipped with Dorr-Oliver cyclone, designed to separate the respirable fraction of airborne dust from the non-respirable fraction. The device had a flow rate of 4.3 L min^{-1} and was equipped with a sterile cellulose acetate filter with a diameter of 37 mm and a pore size of 0.22 μm. For the airborne particulate, the same device was equipped with a Fluoropore membrane filter (FHLP03700, Millipore), with a pore size of 0.45 μm.

2.5. Fungal identification

Genomic DNA (gDNA) of isolated strains was extracted according to the method of Dellaporta et al. (1983). Amplification of the