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Fluorescence microscopy analysis of dust from biomass burning

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Abstract

During the last century, there has been a growing interest for renewable energy. Biomass combustion is a possible way to deal with fossil fuel scarcity and to reduce CO_2 emissions, but some pollutants (e.g., dust) are emitted nevertheless. New efficient approaches of dust characterization have to be developed in order to know better the impacts of dust on global environment and health. Fluorescence microscopy is a fast and relatively cheap method that could, if related to chemical content or toxicological data, characterize dust.

In a first attempt, fluorescence spectroscopy was applied to two dust samples, which were collected in a multicyclone and resulted from combustion of miscanthus (an energy crop) and wood chips. The collected particles were mounted between a standard slide and a coverslip. On a confocal microscope equipped for fluorescence spectroscopy, 32 images are acquired on the same field of observation corresponding to different fluorescent emission wavelengths (excitation at 405 nm; emission from 413.9 nm to 689.8 nm with steps of 8.9 nm). The ImageJ software was used to analyze single-particle and whole-sample fluorescence spectra. These plots are related to fluorescence of some components of dust, as found in the literature (Carletti et al., 2010).

These results allow the choice of the set of filters and then wide-field fluorescence microscopy can be useful to characterize dust. On the same field of observation, white light collected and processed images allowed us to define the equivalent surface of particles. Meanwhile, on fluorescence images the surface and the fluorescence intensity were quantified. Both the percentages and densities of fluorescence (per particle surface) were measured. To ensure dust sample representativeness many fields per sample were collected.

Subsequently, twelve different samples resulting from biomass combustion were investigated using this protocol. The dust samples were collected either with flue gas treatment devices (Multicyclone, Bag House filter, Electrostatic Precipitator) or with a DEKATI DGI impactor ($PM_{2.5}$). The biomass boilers had the following nominal outputs: 40 kW, 400 kW, 2.8 MW, 8MW, 17.3 MW. They

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combusted both wood chip mixtures and miscanthus. In order to make sense of this information on fluorescence, it will be attempted to correlate the measurements to chemical components of dust: PolyAromatic Hydrocarbons (PAH), Humic-Like Substances (HULIS), non-combusted matter, and fluorescent minerals.

Keywords: Ultrafine particles; PM2.5; fly ashes; biomass combustion; HULIS; HAP; fluorescence analysis.

1. Introduction

Alternative energies like biomass burning emits some pollutants like dust that might affect the environment and human health. New characterisation methods have to be sorted out in order to face the challenging behaviours of these kind of materials. Electronic and Optical Microscopy has widely been used (e.g., Coudray et al., 2008) for the observation and characterization of dust from biomass burning. Fluorescence Microscopy is a realtively cheap and fast method that could be used as and analytical method to define some parameters (morphological data and fluorescence emission) of dust from biomass burning.The study will focus on burning and collection of this dust; chemical analysis, electron microscopy observations and setting up a protocol for Fluorescence Microscopy analysis.

2. Experimental procedures

2.1 Combustion tests

In this study, heat was mainly produced by the combustion of miscanthus, wood chips, and corn cobs as co-fuel. The following boilers introduced in this paragraph have a moving stepped grate. Combustion tests at laboratory scale were performed in a multi-fuels boiler (HKRST/V-FSK) supplied by REKA (Aars, Denmark). Performance of the boiler ranged from 30 to 40 kW and combusted miscanthus for this study. The municipality of Ammertzwiller (Haut-Rhin, France) has installed in 2008 a biomass boiler of 400 kW that was purchased from Köb-Pyrot (Germany). They combusted wood chips and miscanthus (miscanthus was burned with 2% $Ca(OH)_2$). A multi-cyclone from the same supplier lowers particulate emissions. This dust sampling was used to provide this study. The city of Rixheim (Haut-Rhin, France) in association with Mulhouse Alsace Agglomeriation (M2A) has installed in 2008 a biomass boiler combusting wood chips and purchased from COMPTE.R. (France). The first flue gas treatment is a multi-cyclone and the filtered dust wasn't be used for this study. The terminal flue gas treatment is a Bag House filter from LÜHR (Germany) and provides material that was used for this study. The SCCU is exploiting in Colmar (Haut-Rhin, France) a biomass boiler from URBAS (Austria) that combusted wood chips. The first flue gas treatment is a multi-cyclone and the filtered dust hasn't be used for this study. The terminal flue gas treatment is an electrostatic precipitator from URBAS and provides material that was used for this study.

The EBM thermique company is exploiting in Saint-Louis (Haut-Rhin, France) a biomass boiler from URBAS (Austria) that combusted wood chips with 2% corn cobs as co-fuel. The boiler has a fluidised bed technology. The only flue gas treatment is an electrostatic precipitator from URBAS and provides material that was used for this study.

2.2 $PM_{2.5}$ collection

A Dekati Gravimetric Impactor (DGI) manufactured by DEKATI Ltd. (Tampere, Finland) was used to collect particles from the gas exhaust at laboratory scale. This system allows the collection of two size fractions of particles ranging from 0.4 µm to 2.5 µm in function of their aerodynamic equivalent cut-off diameter (D_{ae}) at 50% efficiency at nominal air flow rate of 100 Lpm. Particulates bigger than 2.5 µm and smaller than 0.4 µm are collected respectively through impaction plate and terminal filter stage.

2.3 Chemical analyses

Polycyclic Aromatic Hydrocarbons (PAH) adsorbed on particulate matter and cyclone ashes were extracted with acetonitrile by means of an Accelerated Solvent Extractor (ASE 300, Dionex). Procedure was detailed by Liaud et al. (2015). Residual carbon presents in ashes was determined by thermogravimetric analysis according the standard protocol NF EN 14775.

Water Soluble Organic Carbon (WSOC) and water soluble Humic-Like Substances (HULIS) were analysed using protocols previously detailed by Baduel et al. (Baduel et al., 2009). Results of these analyses will be given in a global table with results of Fluorescence Microscopy analysis (Table 1).

2.4 Scanning Electron Microscopy (SEM)

A series of SEM observations (FEI model Quanta 400) were made directly onto glass coverslips placed onto the DGI impactor plates following methods introduced in previous studies with other impactors (Coudray et al., 2008) . They were made on $PM_{2.5-1}$ and $PM_{1-0.4}$ during miscanthus combustion at laboratory scale. Even after only 20 seconds of impaction and not directly close to impaction spots, the particles tend to agglomerate. This agglomeration is hard to break as shown in the images from re-suspension (Fig. 1). Also, in order to remove aggregation during impaction, particles have been recovered from impactor plate, re-suspended in isopropanol, mixed using sonication method, quickly dried, and observed.

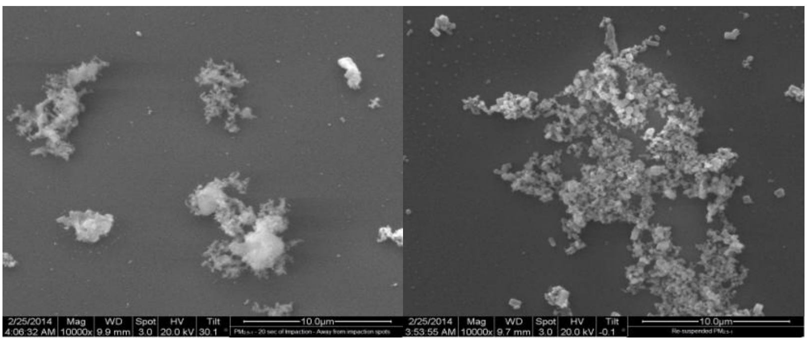

Fig. 1. SEM Observation of $PM_{2.5}$ from miscanthus burning onto impaction plate (left) and re-suspended (right).

SEM observations enabled to have very good morphological information about PM2.5. SEM can also be coupled with EDS spectra in order to have information on atomic composition of fine particles, nevertheless, it is also a cost-effective and time-consuming method and no information about carbonaceous molecules is obtained. Fluorescence microscopy allows to obtain the fluorescence emission within relatively cheap and fast method; this propriety of particles could lead to characterize some chemical content of dust. In order to approach Beer-Lambert law, the linear correlations of fluorescence and chemical content in this paper consider fluorophores as carbonaceous content of dust like residual carbon, WSOC, HULIS and PAH.

2.5 Spectroscopic observations – Fluorescence microscopy analysis

Fly ashes (100-500 µg) are placed between microscope slide (Thermo Scientific™ 2951R) and coverslip (Roth® 0657.2) using Eukitt® (Fluka 03989) mounting medium. Spectroscopic measurements were performed on 2 fly ashes samples using a confocal microscope, sample is excited at 405 nm and emission observed from 413.9 nm to 689.9 nm. The two objectives of this first study were to determine the set of filters for fluorescence microscopy analysis and also to cross out the possibility of differences of fluorescence emissions from different types of particles within same sample of dust. Microscope slide from miscanthus and forest residues burning were sifted and observed according to the previous method using the confocal microscope.

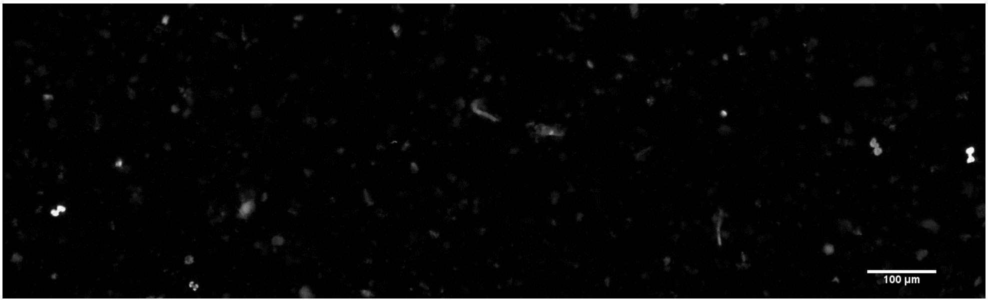

Fig. 1. A wide field microscope, fluorescence emitted at 468 nm of Cyclone Ashes from Miscanthus burning.

Fluorescence intensity was evaluated at different wavelength on the total field but also for single particles (having different shapes) measurements:

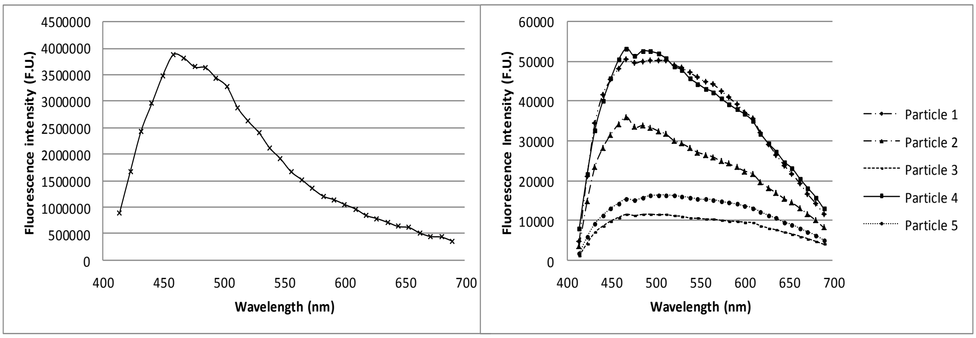

Fig. 2. Bulk wide field particles (left) and single particle (right) fluorescence emission spectra (413- 689 nm).

It seems like every particles exhibit the same fluorescence spectra, with different intensity of fluorescence emitted. The following set of filters was consequently fixed for fluorescence observations: $\lambda_{excitation}$= 420-440 nm; $\lambda_{observation}$ >475 nm. Fluorescence microscopy analysis was performed using an OLYMPUS BX51 wide field microscope modified to acquire 3D images using computational optical sectioning, as described by previous users (Maalouf et al., 2011). Acquisitions are possible through white light or fluorescence mode.

For all acquisitions, a 10X objective with a numerical aperture of 0.25 is used, the measured lateral resolution is 1.5 µm at 600 nm using MetroloJ protocol (almost the same using white light mode). The images are acquired using a cooled CCD (CoolSnap HQ2) camera having a sampling rate of 0.648 µm in the objective plane, and digitized using a 14 bits depth (16 bits images). In order to represent each sample, 10 wide field images (902 µm × 674 µm) are taken using both fluorescence and white light mode.

The objective of microscopy analysis is to determine the shape of the fly ashes and to estimate the part of fluorescence among all of the particles. Using classical image processing protocol (Rolling ball Subtract background, automatic threshold, and binary filter), an estimation of fluorescent areas is possible on mask image. Then, white light observations are also processed using similar protocol (only thresholding differs, ImageJ *Default* threshold) allowing to determine a percentage of fluorescence area. Fluorescence magnitude was estimated using integrated densities of fluorescence per particle areas. The principle is to multiply original fluorescence image with mask image in order to quantify energy of fluorescence emitted, proportional to grey levels (arbitrary unit). Problem of not constant background with different sampling procedure is solved when removing background of images with rolling ball subtraction (Fig. 4).

The non-shaded area under the curve on the processed image (right) is representing the integrated density of fluorescence emitted by dust from biomass burning. Next step will be to relate this analytical result with other properties of same dust.

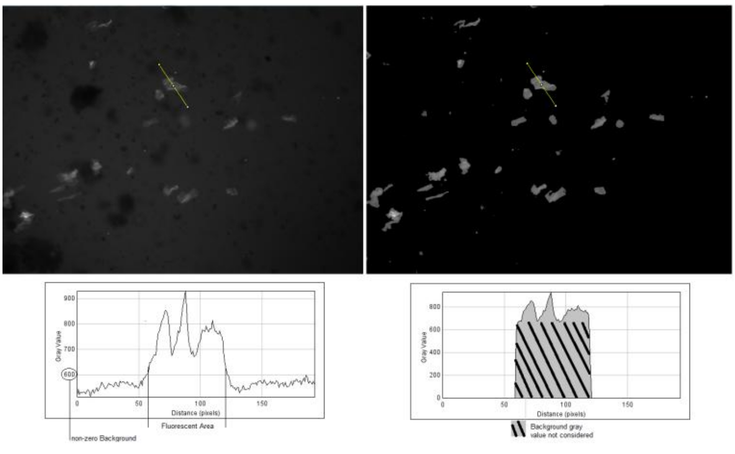

Fig. 4. Raw fluorescent images (left) and processed fluorescence areas (right).

3. Numerical results and correlations

The following results have been obtained (Table 1):

Table 1:Chemical and microscope analysis of fly ashes.

Fuel and boiler power – Particle size or collection mode	Residual carbon (%WT)	WSOC (mg/g)	HULIS (µg/g)	PAH (µg/g)	Fluorescence Intensity (F.U/part)
Miscanthus 40kW (>2.5 µm)	**17.8**	4.95	281	**30.8**	**8.16**
Miscanthus 40kW (1-2.5µm)	**6.54**	5.23	500	**9.04**	**2.41**
Miscanthus 40kW (0.4-1 µm)	**2.92**	1.21	95	**2.46**	**0.94**
Forest residues 400 kW -Cyclone	9.46	2.17	116	4.97	37.5
Miscanthus 400 kW - Cyclone	20.5	1.72	491	1.84	36.7
Forest residues 2.8 MW – Baghouse	7.29	1.61	155	1.79	6.83
Forest residues 8 MW - ESP[a]	11.0	2.86	101	0.79	1.15
Forest residues + 2% Corncobs 17.3 MW - Baghouse	10.9	4.34	121	0.34	8.04

a. Electro Static Precipitator

Global correlations are not possible because of variable fuel, boiler technology, boiler power and dust collection procedure. We found very good correlation between Residual carbon and sum of PAH content (coefficient of determination R^2=0.9999).We also found very good correlation between Residual carbon and fluorescence intensity per particle area (coefficient of determination R^2 = 0,9983).

4. Conclusions

Fluorescence of different dusts from biomass burning has been exhibited under different boiler conditions. A good correlation between Residual carbon, PAH and Fluorescence Intensity has been achieved within 3 samples. These preliminary results could lead to a use Fluorescence Microscopy as a fast and cheap method to analyse PAH in dust.

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