Particle size, size distribution and morphological evaluation of airborne dust particles of diverse woods by Scanning Electron Microscopy and image processing program

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1. Introduction

Occupational exposure to wood dust generated in some woodworking is one of the most important industrial hazard which workers are exposed to. It has been estimated that approximately 3.6 million workers in the European Union are annually exposed to wood dust [1]. In most of wood industry, both hardwoods and softwoods are manufactured. This classification refers not to the hardness of the wood but to the species of tree: softwoods include the gymnosperm or conifers, while hardwoods include the temperate angiosperms. In recent years, the utilization of tropical hardwoods is increased for their characteristics. These types of woods are primarily angiosperms, but also include some gymnosperms that thrive in tropical climates [2].

During processing, wood dust particles can occur over a range of particle sizes. From the standpoint of occupational health dusts are classified as inhalable, respirable and thoracic on the basis of the dimension of their Aerodynamic Equivalent Diameter (AED). The AED of a particle is the diameter of a unit density sphere that would have the identical settling velocity as the particle. Accordingly to this definition the inhalable fraction (<10 μm AED) can be breathed into nose or mouth, the thoracic fraction (<25 μm AED) can penetrate head airways and enter lung airways and the respirable fraction (<10 μm AED) can penetrate beyond terminal bronchioles to gas exchange region [3]. For the above stated, when the dust particle sizes are small they become airborne and pose more serious issues than larger particles that easily settle out.

Repeated exposure to airborne wood dust particles has long been associated with an increased risk of many adverse health effects, such as asthma, chronic bronchitis, emphysema and even irritant dermatitis, contact urticaria and allergic contact dermatitis. [4]. While allergic and non-allergic effects are reported for exposure both to hard and softwoods [4,5], according to IARC classification only hardwood dust is known to be a human carcinogen (Group 1). This conclusion was based on epidemiological evidences showing an high correlation between exposure to hardwood and sino-nasal cancer occurrence [4]. To date, the underlying mechanism and pathways involved in toxicity and carcinogenicity mediated by wood dust remain to be addressed. Several in vitro studies were focused on the inflammatory response modulated by hard and softwood dust exposure. Although hardwood is considered more harmful than softwood, none of these studies showed significant differences between toxicities of hardwood and softwood dusts [6-8].

In order to obtain a correct evaluation of the risk associated to an occupational exposure, the determination of airborne dust is one of the main issues. This goal can be complex since workers are usually exposed not only to several wood dust species simultaneously, but even to different dust concentrations and dimensions. Thus, a better understanding of the size of dusts produced with woodworking is necessary to define the health risks during the work shift. Furthermore, epidemiological data alone are not able to provide useful information about the effects resulting from wood dust exposure. Moreover, the dimensional characterization of dust may be necessary to obtain homogeneous samples of diverse soft-, hard- and tropical hardwoods for the evaluation of the in vitro cytotoxicity in order to
find the basic processes correlated to the exposure damage. For the
above cited, the aim of this research is the determination of size and
size distribution of dust particles obtained via sanding treatment. We
propose the use of a Scanning Electron Microscopy (SEM) image
analysis method given that the use of indirect advanced methods, such
as those involving scattered or diffracted light or laser, assumes the
particle to be spherical, which is not the predominant case with
natural particulate materials. The purpose is to provide a useful tool to
define the type of dust produced during woodworking and, conse-
quently, to develop a prevention as effective as possible. The proposed
method shows a great potential to be considered as a standardized
method of measurement and analysis in order to characterize the
grain size and size distribution of wood particles before carrying out
any in vitro toxicological tests.

2. Materials and methods

2.1. Airborne dust sampling procedure

Dusts from two hardwoods (sessile oak, oak-tree), two tropical
hardwoods (padouk, iroko) and three softwoods (pine, spruce and
larch) were obtained with a (dust collecting) grinding machine with
360-grit sanding paper.

2.2. Sample preparation for SEM analysis

Once collected the diverse samples were prepared for SEM
analysis as follows. 20 mg of each sample was suspended in 20 ml
of IPA (Isopropanol). The suspension was actively stirred, under
sonication, in order to avoid dust aggregates. 0.5 ml of the above
suspension underwent vacuum filtering. The filter was a polycarbon-
ate membrane (Isopore Membrane Filters by Millipore) with a
porosity of 0.4 μm. After the filtration the membrane underwent an
air drying process. The central body of the membrane was then cut
using a scalpel, fixed to the stab and gold coated under vacuum in
order to ensure image quality during the SEM analysis. Images were
acquired using a Scanning Electron Microscope Philips XL 20. The
SEM process conditions are the following: depth of coating 30 nm and
pressure $7 \times 10^{-2}$ mbar. Given that the proposed method of sample
preparation could result in a preferential alignment of the wood
particles, and therefore to an incorrect estimation of the particle
equivalent size, one powder was selected (pine) and collected following
the above described procedure without the application of suction.
The gravity filtration strongly reduces the possibility of preferential
alignment of the particles with respect to the pores of the membrane.
The sample was then analyzed following the same procedure that will
be described in the following sections and the obtained results are
comparable with those obtained with the application of vacuum

Fig. 1. The process of particle dimension measurement using ImageJ.
filtering for the same wood powder. Consequently vacuum filtering was chosen for the analysis of the other powder samples because is faster than gravity filtration. Each wood sample was collected on three different membranes and the result mediated.

2.3. SEM analysis

Each stab was ideally divided into four sectors and the relative images acquired. For each sample four images were acquired, one for each sector of the stab, assuring a minimum quantitative of particles for each sample according to the standard ISO 13322–1. Sample with GSD (Geometric Standard Deviation) of 1.15 needs, in fact, 1460 particles to achieve mass median diameter within 5% error with 95% probability [9].

2.4. Image processing and analysis

The obtained images were processed using the software ImageJ. ImageJ developed at the National Institutes of Health (NIH), USA is a Java-based public domain image processing and analysis program, which is freely available, open source, multithreaded, and platform independent that can be utilized to develop user-coded plugins to suit the specific requirements of any conceived application [10]. The first step in using ImageJ is the image calibration required to correlate the image dimensions in pixel to physical dimensions. The procedure consists in the drawing of a line over the scale bar of the image acquired by the SEM (Fig. 1-(a)) followed by the command “Analyze → Set Scale”. In our case in the Scale window was entered 50 (in μm) into the “Known Distance” box and changed the “Unit of Measurement” box in μm and checked “Global” as can be seen from Fig. 1-(b). Image processing algorithms require a binary image that can be produced by converting the 8-bit image, that can display 28 gray levels, by proper thresholding. The procedure of obtaining the binary image consists in the use of the ImageJ’s command “Threshold…” Such a command by default produces the auto-threshold limits, wherein the lower limit is varied with the image and the higher limit was at the maximum of 255 gray levels. Use this tool to automatically or interactively set lower and upper threshold values, segmenting gray-scale images into features of interest and background as can be seen in Fig. 1-(c). The scale bar was surrounded with rectangular selection and the contents was cleared (“Edit → Clear”). Proceeding with the correct threshold limits creates the required binary image. In fact, if the object is properly covered by thresholding, then accurate size measurement is automatically guaranteed as the image processing method is direct in principle. The procedure of measurement is obtained by running the ImageJ’s “Analyze particles” routine as can be seen in Fig. 1-(d). The options “Exclude on Edges” and “Include Holes” were selected in order to ensure including all whole particles and ignoring holes, if any after thresholding, in the particles of the image during analysis.

Given that wood particle shapes are irregular, assuming them to be of regular geometrical shapes will result in oversimplified approximation. In fact a spherical particle can be described using a single number – the diameter – because every dimension is identical. As seen in Fig. 2, non-spherical particles can be described using multiple length and width measures (horizontal and vertical projections are shown here). These descriptions provide greater accuracy, but also greater complexity. Thus, many techniques (i.e. scattered light, acoustic attenuation, settling rate) make the useful and convenient assumption that every particle is a sphere. The reported value is typically an equivalent spherical diameter. Microscopy or automated image analysis is the only technique that can describe particle size using multiple values for particles with larger aspect ratios as described also by Gomez Yepes and Cremades [11]. An image analysis system as ImageJ is able to describe the non-spherical particle seen in Fig. 2 using the longest and shortest diameters, perimeter, projected area, or again by equivalent spherical diameter.

The measurement of particle sizes varies in complexity depending on the shape of the particle, and the number of particles characterized must be sufficient to ensure an acceptable level of uncertainty in the measured parameters. Additional information on particle size measurement, sample size and data analysis is available in ISO 9276 [12]. For spherical particles, size is defined by the diameter. For irregular particles, as in the present case, a variety of definitions of particle size exist. In this paper the built-in standard measurements of ImageJ’s “Analyze Particles…”, such as diameter of a circle of equal projection area, Feret’s diameter and minimal Feret’s diameter were chosen also on the basis of previous papers found in the literature [13–16]. The diameter of a circle of equal projection area (dEC) is the diameter of a circle that has the same area as the projection area of the particle as can be seen in Fig. 3-(a). The Feret’s diameter (dF) is the longest distance between any two points along the selection boundary, also known as maximum caliper as can be seen in Fig. 3-(b). The minimal Feret’s diameter (Min dF) is the minimal Feret’s diameter calculated after considerations of all possible orientations (0°...180°).

To estimate the accuracy of the machine vision method, a TiO2 powder (Sigma Aldrich, MO, USA), predominantly rutile showing a particle size less than 5 μm, was selected and analyzed using ImageJ (Fig. 4). In order to evaluate the particle size distributions, 1460 particles for each wood dust were analyzed from electron micrographs and the value of the dEC, dF and Min dF are calculated.

3. Results

Seven diverse wood dusts were analyzed. Larch, spruce and pine for the softwoods; oak-tree and sessile oak for the hardwoods and iroko and padouk for the tropical hardwoods. As for regards the mean values of dEC, dF and Min dF in μm, calculated by the above described machine vision method, they are reported in the below table with the relative standard deviation value (Table 1).

For particle size distributions, reported in Fig. 5, 1460 particles for each sample of wood dust were analyzed from electron micrographs for three different membranes. Min dF was chosen, for the visualization of the results, because it can be considered the more relevant dimensional parameter for inhalation between those reported.
4. Discussion

Picture of wood samples, after grinding, taken by electron microscope show different and complex shapes of particles of wood dust (Fig. 6). As described in The Particle Atlas diverse geometric expression could be observed such as cylinder, cone, rectangular prism and sphere [17].

For the above reason we propose, for the evaluation of size particles and size distribution, the use of Scanning Electron Microscopy (SEM) and ImageJ processing program. In fact, the use of indirect advanced methods, such as those involving scattered or diffracted light or laser, assume the particle to be spherical, which is not the predominant case with natural particulate materials as can be seen from Fig. 6.

Results from SEM analysis show that the sample preparation method developed in this research is applicable to the analysis of the collected wood particles. The characterization of such samples from a morphological and dimensional point of view yields information that can help epidemiologists and toxicologists to understand the causes of respiratory illnesses. The SEM research contributes to a significant analysis with regard to morphological characterization of wood dust. In fact, SEM analysis guarantees the acquisition of high resolution images that can effectively represent the physical dimension of dispersed dust particles. Moreover, the SEM/EDX (Energy Dispersive X-ray spectroscopy) analysis of particles can determine whether a more elaborate analysis of highly toxic metals or organic contaminants is necessary or not showing the presence or not in the spectra of relevant chemicals such as for example varnishes and paints. The diverse wood dusts analyzed by EDX in this research confirmed that no toxic substance or chrome is present in the diverse samples. The machine vision method used for the particles size measurements guarantees the accuracy of the measurement; speedy calculations and automated analysis; measurement of non-spherical particles using the longest and shortest diameters, perimeter, projected area, or again by equivalent spherical diameter; particle size distribution analyzed in a sieveless manner; direct method of measurement; custom-made compiled plug-in and cost-effectiveness.

We found in this study that sanding treatment of diverse wood species, from soft- to hard and tropical hardwoods results in the emission of very small particles (diameter less than 20 μm). This is an issue in protecting the health of workers, since the usual respiratory protective of disposable masks can be ineffective in retaining particles of that size. For the above reason the proposed method of determination of size and size distribution of the dust particles can be very useful for designing handling devices and developing management strategies (such as for example prevention, control or isolation). Moreover, the dimensional characterization is the starting point to investigate the cytotoxicity in vitro of wood dusts, since the cytotoxic effect observed

<table>
<thead>
<tr>
<th>Wood essence</th>
<th>Mean d EC [μm]</th>
<th>Mean d F [μm]</th>
<th>Mean Min d F [μm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larch</td>
<td>7.9 ± 1.0</td>
<td>13.8 ± 2.0</td>
<td>7.3 ± 1.1</td>
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<tr>
<td>Spruce</td>
<td>6.3 ± 0.6</td>
<td>11.1 ± 1.1</td>
<td>6.2 ± 0.7</td>
</tr>
<tr>
<td>Pine</td>
<td>5.5 ± 1.9</td>
<td>10.5 ± 1.0</td>
<td>5.7 ± 1.1</td>
</tr>
<tr>
<td>Oak-tree</td>
<td>6.5 ± 0.9</td>
<td>10.7 ± 1.7</td>
<td>6.5 ± 0.9</td>
</tr>
<tr>
<td>Sessile oak</td>
<td>6.5 ± 0.7</td>
<td>12.2 ± 1.1</td>
<td>6.8 ± 0.7</td>
</tr>
<tr>
<td>Idroko</td>
<td>6.6 ± 1.4</td>
<td>12.3 ± 1.8</td>
<td>6.9 ± 0.7</td>
</tr>
<tr>
<td>Padouk</td>
<td>6.7 ± 1.3</td>
<td>12.0 ± 2.0</td>
<td>7.1 ± 1.0</td>
</tr>
</tbody>
</table>

Fig. 3. Diameter definition. (a): diameter of a circle of equal projection area (d EC); (b): Feret’s diameter (d F).

Fig. 4. Scanning electron micrograph of TiO2 dust used in the present study (a) and particle size distribution considering the value of d EC (b).
could be due to the different range of size within the sample. Some recent studies comparing the toxicity of hard and softwoods showed no significant differences in cell viability after exposure to wood dusts; in these studies, most of the particles, regardless of wood species, had same diameters [7,8]. Starting with the homogeneous samples obtained with the method proposed in this article, even our preliminary data confirm that there is no difference in cell viability between soft, hard and tropical wood (data not shown).
Fig. 6. Series of photomicrographs of particles of wood collected by electron microscopy.
5. Conclusions

We propose, for the evaluation of the particle sizes and size distribution, the use of Scanning Electron Microscopy (SEM) and ImageJ processing program. The proposed method is accurate, quick and reproducible. In our opinion the proposed method could be very useful in the dimensional characterization of wood dust samples in order to better investigate the behavior of the particles themselves according to their morphology and dimension in the human respiratory tract.

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References