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Comparison of Fiber Counting by Monitor Screen and Eyepieces of Phase Contrast Microscopy

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Abstract

***Background*:** These minerals have been extensively used in industrial products such as cement-asbestos sheet and pipe, brake shoe, clutch, insulation materials, etc. Occupational and non - occupational exposures to this carcinogenic material have caused to develop several methods to evaluate airborne asbestos fibers.

***Materials and Methods*:** In this study, multiple microscopic method of determining the type and concentration of asbestos fibers has been used in an industry. The forty five personal samples on membrane filters (MCE) were collected of different processes of a manufacturing factory of cement-asbestos sheet. The half of each filter was prepared and then fibers counting were accomplished by ocular PCM and LCD images methods. Another part of filters was used for identification of asbestos fibers elements and types by scanning electron microscope method.

***Results*:** Fibers concentration range were determined 0.009-0.243 fibers/cc by direct counting method (Ocular PCM), while by indirect method (LCD Images), results were 0.00-0.117 fibers/cc and statistical tests showed significant difference (p<0.02). Study of elemental composition of fibers by scanning electron microscope confirmed that, the majority of fibers were chrysotile. Study of elemental composition of fibers by scanning electron microscope confirmed that majority of fibers are chrysotile.

***Conclusion*:** Due to limitation of study, use of 1.3 megapixels in indirect method, PCM direct method remains one the best methods of Asbestos fibers counting in Iran.

Introduction

Because of acoustic performance, high shearing strength, high dielectric strength and also temperature and chemical resistance, asbestos became well-known among industries since the end of 19th century. Depending on their shape, asbestos is divided into two main categories; amphibole and serpentine, which each category is composed of one or more minerals. Amphibole categories include Crocidolite, Anthophyllite, Amosite, Anthophyllite, Actinolite, Tremolite and latter category consists of Chrysotile [1]. These minerals have been extensively used in industrial products such as cement-asbestos sheet and pipe, brake shoe, clutch, insulation materials, etc [2]. Adverse health effects of exposure to asbestos fibers have become obvious based on animal experiments and epidemiological data. Diverse studies are shown that asbestos can cause gastrointestinal cancer, laryngeal cancer, pleural effusion, pleural plaques, lung cancer, mesotelioma as well as accelerated pulmonary fibrosis (asbestosis) [3]. Since the asbestos is a heterogeneous material chemically, its recognition and analysis remain difficult. For this, diverse equipments such as Phase contrast microscopy (PCM), electronic microscopy, and X-Ray diffraction are used. PCM is the simplest method which used for measurement of asbestos fiber concentration and is known as NIOSH (National Institute for Occupational Safety and Health) method No 7400 [4]. In this method samples are counted using PCM (Magnitude 400x) and the result is reported as the number of fiber per milliliter of air (f/cc). Each fiber longer than 5 μm as well as fibers with a length-to-width ratio equal to or greater than 3:1 are counted as one fiber [5]. NOISH 7400 is a visual method for fiber counting based on morphological characteristics, so different factors such as strength of sight affect the accuracy and precision of this method [6]. Many studies have evaluated the ability of different methods on measurement of asbestos fibers and results compassion in past years. Compassion of asbestos fiber counting on monitor screen (indirect method) and eyepieces of PCM (direct method) has done in one study. It has used 30 samples which were collected from vicinity of a cement-asbestos sheet factory and counted by NIOSH 7400, direct PCM (Magnitude 400x) and indirect method by means of TV screen [7]. Another study was used different microscopic methods such as optical microscopy (op), pcm, polarized microscopy (plm), transmission electron microscopy (tem), scanning electron microscopy (SEM) for determination of asbestos fiber concentration

and detection type of them. Ratio between mean concentration of OP and PCM method was calculated 2.39, and between TEM and PCM methods was 3.87 also between TEM and OP methods was computed 1.62 [8]. NIOSH method 7400 is not able to distinguish between asbestos and non-asbestos fibers; recently, SEM which uses X-ray emission is employed to determine type and amount of minerals which exist in asbestos samples [9]. Measurement of occupational exposure to airborne asbestos fibers needs cost-benefit and scientific methods. Currently just PCM can be used for asbestos fiber counting in Iran, unfortunately. Because of limitations such as being time-consuming and possibility of counting error, are made PCM less favorite method for asbestos fiber counting. Since little studies have been done on the asbestos fiber counting by different method in Iran, it seems that doing this study is necessary.

Materials and Methods

The aim of this study was to count asbestos fibers by PCM using direct (through the eyepieces of PCM) and indirect (through the LCD Images) way and comparing the results. Considering past studies and also pilot sampling before main sampling as well as number of workers in each process, at least seven samples was taken of each process. In fact, after pilot sampling it has been shown that variation of standard deviation in concentration of asbestos fiber is negligible for sample size above seven. Regarding the used method in this study, 45 personal samples were collected from different process of a cement-asbestos sheet manufacture which located in Kerman province, Iran. Sampling period was at least 60 min per sample. In this manufacture 120 employees were working and annual product of the manufacture is 60 thousand ton. The average age and tenure of employees were 41 (29-56) and 15 (7-17) years, respectively. Personal sampling was done from processes including feeding raw materials, mixing, molding, downloading, cutting, perforating and storehouse. Used materials are asbestos (20%) and cement (80%) and producing of cement-asbestos sheet is done in wet process. Samples were collected using 25 MCE filter contained in an electrically conductive cassette assembly that includes a 50-mm extension cowl and personal sampling pump (SKC-Model 224-PCMTX8). Sample preparation was done according to NIOSH method No 7400. In this method a half of a filter is placed on a glass slide and an acetone flash vaporization system is used for clearing filters on glass slides. Subsequently a Walton-Beckett graticule is placed on eyepieces of PCM (in order to measure length, fiber diameter and counting fibers) and a micrometer is used to calibrate the graticule. Any fiber longer than 5 μm and length-to-width ratio equal to or greater than 3:1 is accounted as a fiber. At first fiber counting was done, using PCM at 400X magnification (direct method) and then a digital camera was mounted on one eyepiece of PCM (1.3 Megapixel resolution) and an output port of camera was plugged to a laptop computer (Fig. 1) which already had DinoCapture 2.0 software and it was used for fibers counting (indirect method). For determination the components of the manufacture's asbestos fiber using SEM, another half of the filter was used. SEM can evaluate type and components of fibers [10]. Each sample was counted in the lab of Occupational Health Department of Tehran University of Medical Sciences while the illumination level was 350-500 lux. According to the used method, the following formula was applied to the fiber density on the filter (fibers/mm²) was calculated by dividing the average fiber count per graticule field F/n_f , minus the mean field blank count per graticule field, B/n_b , by the graticule field area, A_f, (approx. 0.00785 mm²):

$$E = \left(\frac{\frac{F}{n_f} - \frac{B}{n_b}}{A_f} \right) \ , \ {fibers}/{mm^2}$$

Subsequently the concentration, (fibers/cc), of fibers in the air volume sampled, (L), using the effective collection area of the filter, (approx. 385 mm² for a 25-mm filter) was calculated:

$$C = \frac{E(f/mm^2) \times A_c(mm^2)}{V(lit) \times \frac{10^3 \ cm^3}{lit}}$$

Statistical analysis was done using SPSS-16 and *t*-test was performed to compare mean of concentration in direct and indirect methods.

Results

All 45 samples were evaluated one by one using direct and indirect methods. Geometric mean of fibers concentration in the air which measured by direct and indirect methods is shown in table 1. The range of asbestos fiber concentration was calculated 0.009-0.243 fibers/cc in direct method whereas it was 0.000-0.117 fibers/cc by indirect method. Independent sample *t*-test was shown that there is a significant difference between mean concentrations of direct and indirect asbestos fiber counting (p=0.02). As it can be observed, there is a significant difference with respect to computed concentration level between direct and indirect PCM methods whereas in the study of Mao et al which was done in the same field, a significant difference between mean concentration level of these methods wasn't revealed (p=0.32) [7].

The ratio of direct PCM method to indirect was 1.76. Subtraction of fiber concentration level of direct from indirect PCM was computed 0.001-0.153 and the bias of indirect to direct PCM was calculated from 22.46 to 100 percent which revealed that measured concentration of fibers by indirect method is by far less than direct method. Therefore, the direct PCM method was selected for the evaluation asbestos fiber concentration level so that the highest asbestos fiber concentration level was 0.16±1.02

fibers/cc that was related to Feeding raw material process. This concentration level is higher than Threshold Limit Value (TLV) which recommended by American Conference of Governmental Industrial Hygienists (ACGIH) which is 0.1 fibers/cc now [11].

Figure 1. Setting of Indirect asbestos fiber counting

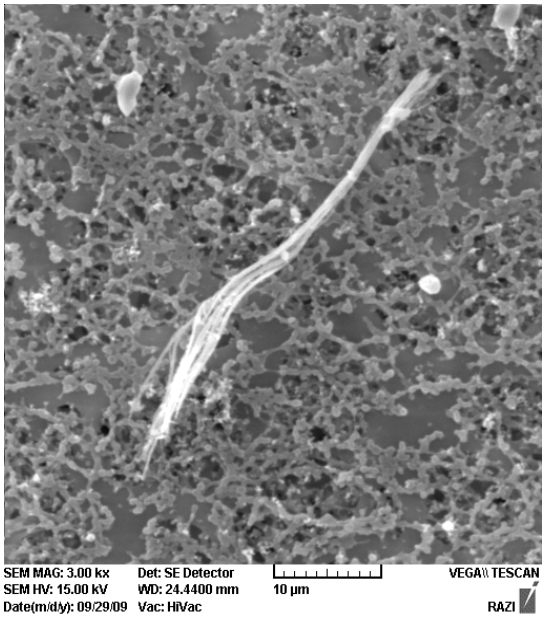

Figure 2. Scanning electron microscope (SEM) image collected of chrysotile fiber

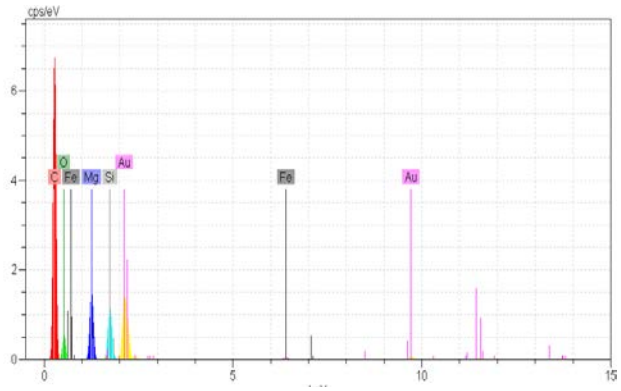

Figure 3. Energy-dispersive spectrometry (EDS) spectrum collected from the air chrysotile fiber

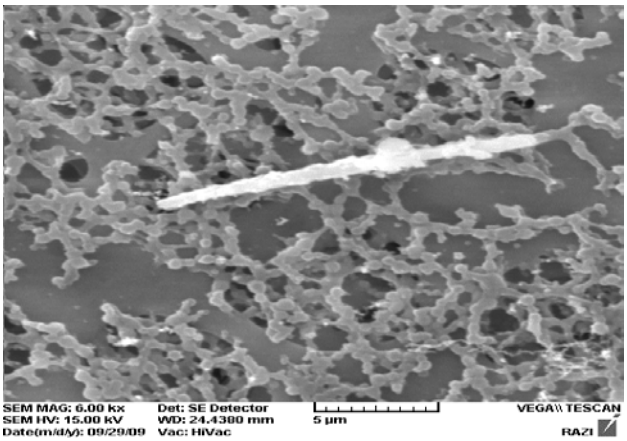

Figure 4. SEM image collected from the air anthophyllite fiber

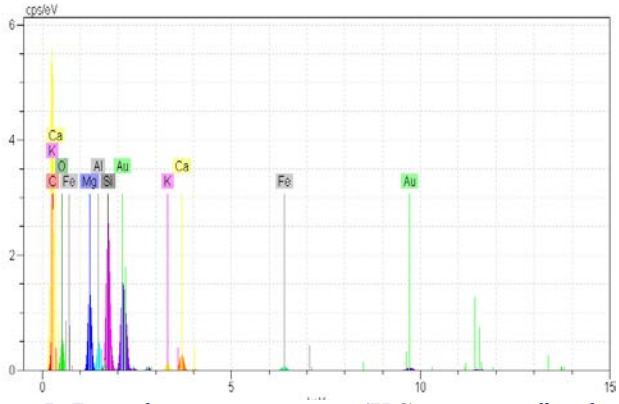

Figure 5. Energy-dispersive spectrometry (EDS) spectrum collected from the air Tremolite fiber

Table 1. Mean and Standard Deviation of airborne asbestos fiber concentration (fibers/cc) by Direct & Indirect counting method

Process	Sample size	Geometric mean concentration by direct method	Geometric mean concentration by indirect method	Samples above TLV*(%)
Feeding raw material	7	0.17 ± 1.02	0.08 ± 0.51	57
Mixing	7	0.02 ± 1.02	0.01 ± 0.50	0
Molding	8	0.06 ± 1.17	0.03 ± 0.51	0
Downloading	7	0.48 ± 1.01	0.04 ± 0.50	0
Cutting& perforating	9	0.1 ± 1.23	0.05 ± 0.53	33
Storehouse	7	0.03 ± 1.11	0.01 ± 0.50	0
Total	45	0.06 ± 1.36	0.04 ± .078	16

*Recommended threshold limit value by the American Conference of Governmental Industrial Hygienists (ACGIH) and Iranian Committee of Occupational Health is 0.1 fibers/cc

A significant difference was detected between this concentration and concentration levels of other processes (p=0.034). As well as the concentration level in mixing process was less than others (0.02±1.02 fibers/cc). This finding is against a study which was performed in a brake-shoe and clutch manufacturer so that in dry processes of that factory the mean concentration of asbestos fiber (0.63-0.87 fibers/cc) was much more than TLV (0.1 fibers/cc) [2, 12]. Results showed that in 15 percent of samples (7 samples) the concentration levels were above the TLV. Figures 2 to 5 are illustrating images and analysis of asbestos fiber by SEM which approves that Chrysotile is dominant type of asbestos in the field, they support those fibers such as Tremolite exists in the workplace environment.

Discussion

In this study which was done for the first time in Iran, same samples were analyzed by eyepieces of PCM (direct method) and LCD monitors which was connected to the PCM (indirect method). There was a significant difference between mean concentrations of direct and indirect asbestos fiber counting, statistically whereas in the study of Mao et al. which was done in the same field, a significant difference between mean concentration level of these methods wasn't revealed and the reported mean bias in both method was 7.7±8%. Relative standard deviation (RSD) was computed 0.4 which is the same as theoretical RSD of NIOSH method No. 7400 [7]. In this study it seems that recognizing and counting of Chrysotile fibers by LCD monitor are more difficult than eyepieces of PCM. Because the survey of airborne asbestos fibers by PCM method is done properly in samples with fiber density above a 100 fibers/mm^2, so fiber density higher than the studied cases could result in more precise comparison. Despite the difference between the results of direct and indirect method, advantages of using LCD monitor (indirect method) are: less stress on eyes because of bigger screen than eyepieces of PCM; changing operator posture more easily and decreasing the risk of musculoskeletal disorders experience and also teaching more students or operators simultaneously. Due to these advantages, it seems necessary that more studies should be focused on improving indirect methods. It is worth to mention that, normally some mines of chrysotile consist of little amount of Termolite which is not recognizable by routine analysis [1]. Similarly, it should be stated that in some process of producing sheet products, more dangerous asbestos such as Crocidolite and Amosite are used because of increasing the concentration of the products or dewatering of them [13]. Considering the limitation of the study, using a 1.3 megapixel camera, direct PCM method continue to be one of the best fiber counting methods in Iran.

To establishing a more convenient and effective method, improving the quality of image of monitor screen should be more considered in the future.

Even though the geometric mean of 85 percent of processes were below the TLV (0.1 fibers/cc), but results of SEM reveal that import chrysotile asbestos contain Amphibole type of asbestos such as Actinolite and Tremolite which have higher risk of asbestos related cancer.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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References

1. Kakooei H, Yunesian M, Marioryad H and Azam K. Assessment of airborne asbestos fiber concentrations in Urban area of Tehran, Iran. Air Qual Atmos Health 2009; 2(1): 39-45.
2. Kakooei H, Marioryad H. Evaluation of exposure to the airborne asbestos in an automobile brake and clutch manufacturing industry in Iran. Regular Toxicol Pharmacol 2010; 56(2): 143-147.
3. de Las Casas MP, Fernandez Infante B. [Occupational lung cancer] Spanish [Abstract]. An Sist Sanit Navar 2005; 28 Suppl 1: 101-6.
4. IARC monographs on the evaluation of the carcinogenic risk of chemicals toman: Asbestos. IARC Monogr Eval Carcinog Risk Chem Man 1977; 14: 1-106.
5. Baron P. Asbestos Analysis-NIOSH Method 7400. Appl Ind Hyg1987; 2(6):8-10.
6. Paik NW, Levin SP, Schork MA. Intracounter, intralaboratory, and interlaboratory variation of asbestos counting with phase-contrast microscopy. Appl Occup Environ Hyg 1994; 9(7): 510-516.
7. Mao IF, Yeh HR, Chen ML. Comparison of fiber counting by TV screen and eyepieces of phase contrast microscopy. AIHA J (Fairfax, Va) 2002; 63(6): 756-61.
8. Kohyama N, Kurimori S. A total sample preparation method for the measurement of airborne asbestos and other fibers by optical and electron microscopy. Ind Health 1996; 34(3): 185-203.
9. Asbestos International Association [AIA]. Method for the determination of airborne asbestos fibers and other inorganic fibers by scanning electron microscopy. AIA Health and Safety Publ RTM no2. London: AIA; 1984.
10. ISO 14966:2002. Ambient air determination of numerical concentration of inorganic fibrous particles-scanning electron microscopy method. International Organization for Standardization. 2005.
11. American Conference of Governmental Industrial Hygienists [ACGIH], Threshold limit values for chemical substances and physical agents and biological indices. Cincinnati: ACGIH; 2009.

12. Kakooei H, Sameti M, Kakooei AA. Asbestos exposure during routine brake lining manufacture. Ind Health 2007; 45(6): 787-792.

13. Agency for Toxic Substances Disease Registry [ASTDR]. Toxicological profile for asbestos (Update). US Department of Health and Human Services: Public Health Service Atlanta; 2001.

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