NATURALLY OCCURRING ASBESTOS: THE PROBLEM OF QUANTIFICATION

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I hereby declare that, the contents and organisation of this dissertation constitute my own original work and does not compromise in any way the rights of third parties, including those relating to the security of personal data.

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Summary

Asbestos is a known human carcinogen and this is the reason why it is considered one of the most dangerous and complex factors to face during underground excavations and quarries exploitation in ophiolitic rocks, both for workers' safety and for the disposal of contaminated excavated material. The quantitative determination of the content of asbestos in rock matrices is a complex operation that is susceptible to significant errors.

The purpose of this work is to investigate the problem concerning the quantification of the content of Naturally Occurring Asbestos (NOA) within massive samples deriving from excavation activities.

The current regulations do not fully satisfy the analytical requirements and do not propose an unambiguous procedure regarding NOA.

The principal instruments used for the analysis are the Scanning Electron Microscope (SEM) and the Phase Contrast Optical Microscope (PCOM). PCOM is usually considered as a technique useful only for the recognition of asbestiform minerals. This work, in the first part, evaluates the potential use of PCOM also for the quantification of asbestos content. In this thesis the PCOM methodology is validated through repeated analyzes and statistical evaluations. The major criticism attributed to optical microscopy is that of the lower resolution and the consequent possibility of not detecting the finest asbestos fibers. In order to evaluate the effectiveness of the technique even in samples with low concentrations of asbestos 150 samples are analyzed, both with the PCOM and with the Scanning Electron Microscopy (SEM), which is a technology capable of better resolution. Pros and cons of the various technologies and the main sources of analysis errors, such as the discrimination between antigorite and chrysotile and the presence of out of scale objects, are discussed.

One of the topic deeply investigated is the behaviour of tremolite if subjected to grinding. Tremolite crystal habit can be described as asbestiform (fibrous) for

longer and thinner fibers or non-asbestiform (prismatic) for prismatic fragments. The analysis carried out showed that there is not a formation of fibers from a sample with prismatic habit subjected to a grinding process. Moreover there is a decrease of its fibrousness if the grinding is carried out on a fibrous tremolite sample. This is an interesting theme focused on the relationship between the crystalline particle breaking methods and their impact on the respiratory system.

Finally, different kind of natural samples are described and the best methodology for the analysis is suggested.

Acknowledgment

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Foreword

I am not a geologist, nor an expert in petrography, mineralogy or crystallography, I am an environmental engineer. The approach I have towards the asbestos presence in nature is therefore of methodological order, for the study of techniques and results.

Certainly, in these years, I had the opportunity to deepen my knowledge on the subject very much, trying to have a wider and wider knowledge from different point of view.

With my research group, thanks to the consulting activity for the tunneling opera of "Terzo Valico dei Giovi", new challenges and new problems are continuously approached. We have always tried to find the most appropriate solutions to the various emerging needs, sometimes unexpectedly, during an excavation work of this complexity.

We had the opportunity and good fortune to compare ourselves and collaborate constantly with many laboratories of ARPA (Regional Agencies for Environmental Protection), professionals from the private sector, and other research groups from various universities. I think that is necessary to underline that the regulatory framework related to the problem of the Naturally Occurring Asbestos is extremely inadequate both from the analytical point of view and for the strategies and best practice for the managing of the natural material containing asbestos.

The issue is quite recent and, in the last years, the debate about this topic is increasing widely. I hope that this work of thesis could be useful for all the persons which will be involved in this field of interest and analysis as a sort of guideline.

Chapter 1

INTRODUCTION

The purpose of this work is to investigate the problem concerning the quantification of the content of Naturally Occurring Asbestos (NOA) within massive samples deriving from excavation activities.

The determination of the asbestos concentration has many implications involving the planning of the opera, the management of the excavated material, the method of excavation and the health of workers. The current regulations do not fully satisfy the analytical requirements and do not propose an unambiguous procedure regarding NOA.

The Phase Contrast Optical Microscopy (PCOM) is usually considered as a technique useful only for the recognition of asbestiform minerals. This work, in the first part, evaluates the potential use of PCOM also for the quantification of asbestos content. After the validation of the method through repeated analyzes and statistical evaluations, we tried to verify the effectiveness of the technique even in samples with low concentrations of asbestos analysing 150 samples, both with the PCOM and with the Scanning Electron Microscopy (SEM), which is a technology capable of better resolution. Pros and cons of the various technologies and the main sources of analysis errors are discussed.

The variables within the analytical process are manifold. In this thesis one of the topics discussed in detail concerns the behaviour and the reactions to grinding actions of amphiboles with a fibrous or prismatic crystalline habit. In order to assess if this process (which is part of the analytical procedure and simulates stresses present in the excavation activity), involved alterations in the crystalline habit four samples of tremolite with different crystalline habit were analyzed before and after grinding.

Finally, the last chapter, describes all the different types of samples deriving from tunnel excavation, trying to provide the best indications and suggesting the more effective methodology of analysis to adopt in different situations. The opportunity to develop this thesis comes from the asbestos-related monitoring activity that the research group of the Politecnico di Torino (to which I belong) has been carrying out from more than five years for the excavation work of the Terzo Valico dei Giovi: a work that will guarantee a high-speed train connection between Genoa and Milan.

The next flowchart describes all the steps followed for this research. (
Figure 1)
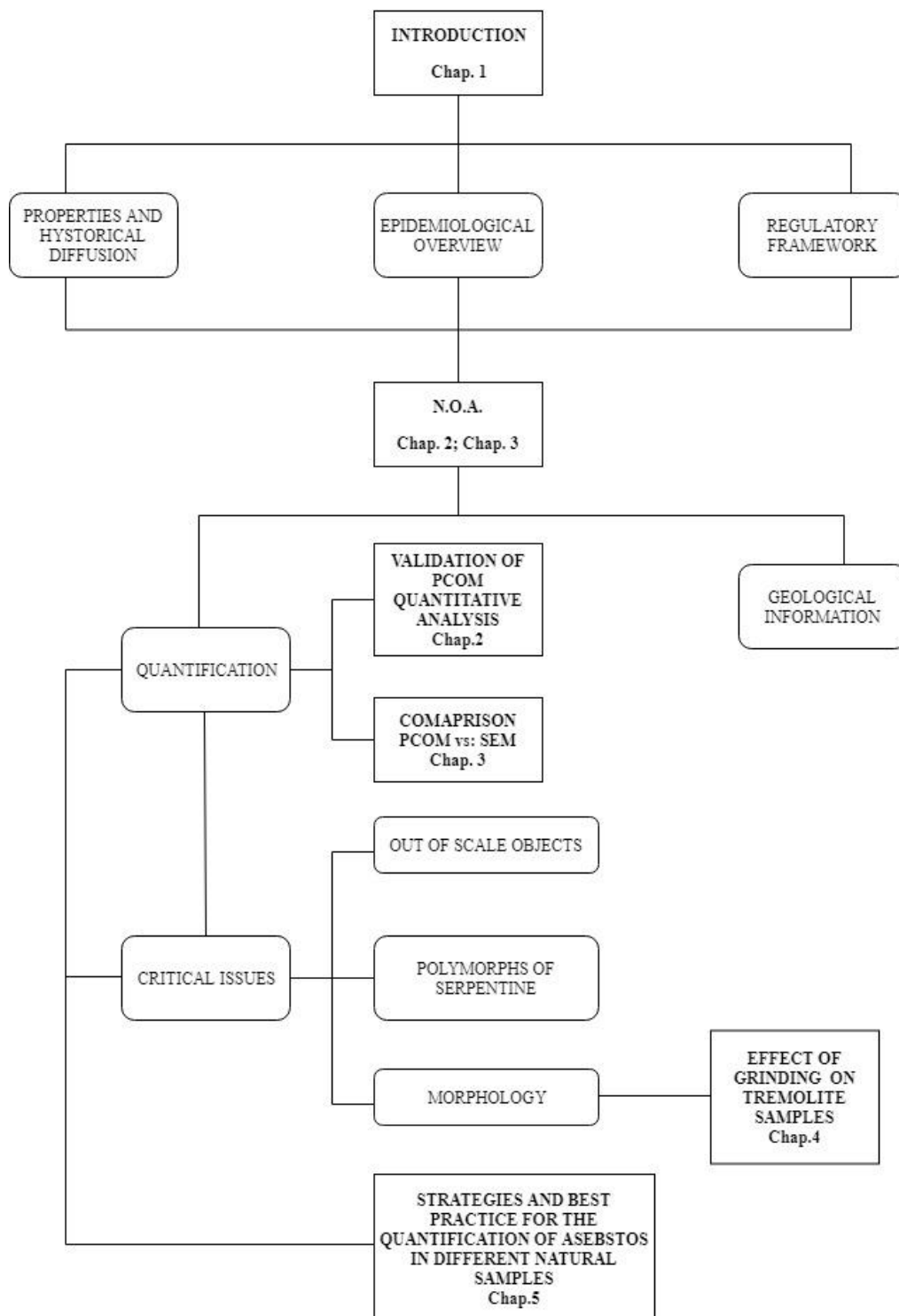
Figure 1: Thesis flowchart

1.1 ASBESTOS PROPERTIES AND PECULIARITIES

The term "asbestos" commonly refers to five minerals belonging to the inosilicate group and one to the phyllosilicates group. The common characteristic of these minerals is the crystalline habit that is called "asbestiform"; it describes a mineral that grows in a fibrous aggregate, with high tensile strength, flexible, long, and with thin crystals that readily separate. (National Academies Press, 2006). The United States Environmental Protection Agency (EPA), as the current Italian Legislation, defined six mineral as "asbestos" including those belonging to the serpentine group and to the amphibole group.

Asbestos of Serpentine:
- Chrysotile (white asbestos – $Mg_3Si_2O_5(OH)_4$ -)

Amphibole Asbestos:
- Amosite (brown asbestos- $(Mg, Fe)_7Si_8O_{22}(OH)_2$- trade name for the amphiboles belonging to the cummingtonite-grunerite solid solution series);
- Crocidolite (blu asbestos – $Na_2(Mg, Fe)_7Si_8O_{22}(OH)_2$ - fibrous variety of the amphibole riebeckite);
- Tremolite ($Ca_2Mg_5Si_8O_{22}(OH)_2$ – the name comes from Tremola valley (Switzerland));
- Anthophyllite ($(Mg, Fe)_2Si_8 O_{22}(OH)_2$);
- Actinolite ($Ca_2(Mg, Fe)_5Si_8O_8(OH)_2$)

Asbestos, which is an accessory or occasional fibrous mineral of secondary origin (USGS, 2001; HSE, 2005), can generally be found as a filling of micro or macro fractures of millimeter or sub-millimeter level dimensions. (Cavallo and Rimoldi, 2013). This mineral was extracted from open or underground mines; avery dangerous operation, because it causes the release of asbestos fibers into the atmosphere. Asbestos, if inhaled, is a known human carcinogen (IARC, 1973; Barrett et al., 1989; Bourdès et al., 2000; USGS, 2001; Niklinski et al., 2004; Pan et al., 2005; Baumann et al., 2015) but the fibers must meet certain requirements to be considered respirable.

Despite its dangerousness, asbestos continues to be used in many parts of the world. In Italy the law 27/03/1992 n.257 established "the cessation of the use of asbestos, and in particular the ban on its extraction, importation, exportation, marketing and production of asbestos products and products containing asbestos." Unfortunately, before that date, Italy was one of the countries with the greatest use of asbestos artefacts. This is due to its characteristics which have made it one of the most used materials at the beginning of the twentieth century in constructions of building. In particular it was used for the construction of pipes, fire panels, roofing sheets, thermal insulation and so on.

In these objects asbestos ensures the following characteristics:

- Resistance to fire and heat;
- Resistance to the action of chemical and biological agents;
- Resistance to abrasion and wear;
- Easily spinnable;
- Sound-absorbing and heat-insulating properties;
- Easy to bind with building materials and some polymers.

These excellent physic-chemical properties have favoured its massive use not only in construction industry but also in many familiar objects. In the following figure (*Figure 2*) is shown the cover of a 1967 marvel comic in which the Human Torch deals with Asbestos Man, this curious fact is an index of the diffusion that asbestos has had within the industry and society since the 1950s.

Figure 2: Strange Tales #111: The Asbestos Man debuts. Cover art by Jack Kirby and Dick Ayers. (August 1963)

Modern asbestos mining started in the 19th century, and Canada became one of the leading producers of asbestos early on. In the 1850s, significant deposits of chrysotile, the most commonly used form of asbestos, were found in Thetford, Quebec, south of Quebec City (*Figure 3*). At the height of asbestos mining, around the 1970s, there were dozens of asbestos mines in the world, but about half of the asbestos used around the world was coming from this one mine in Canada. In Asbest, Russia, mining is ongoing. "When I work in the garden, I notice asbestos dust on my raspberries," one resident told the New York Times in 2013. Regulators there consider it safe when properly handled, they told the Times. Most of the asbestos used in the U.S. in recent years has come from Brazil, but now those mines are shutting, too. Russia could become an asbestos supplier to the U.S., and the people of Asbest are looking forward to it (Laskow, 2018).

Figure 3: The Jeffrey Mine in the 1940s. LIBRARY AND ARCHIVES CANADA/CC

The largest asbestos mine in Europe was the asbestos quarry of Balangero, in the province of Turin, which was closed in 1990 to begin the reclamation work and which was never reopened in application of the law n.257/1992. After the safety measures, the site is now subjected to constant maintenance and monitoring of the airborne fibers. As for the other asbestos mines, we highlight the mines of Valmalenco (Lombardy) that were active until the mid-70s, Emarese (Valle d'Aosta) and the green stone quarries that are concentrated in few regions of Italy including Emilia-Romagna, Lombardy, Piedmont and Liguria. Considerable

asbestos presences are also found in Trentino Alto Adige, Veneto, Tuscany, Campania, Basilicata, Calabria, and finally Sicily with the quarry of Monte Calvario in Biancavilla. (Paglietti, et al., 2011)

Asbestos is present in large quantities all over the world, but the most important examples of natural asbestos are in Libby (Montana, USA), El Dorado Country (California, USA), Fairfax Country (Virginia, USA), Cappadocia (Turkey) , South Africa, China, Cyprus, Greece, Spain, Russia and many others (Paglietti, et al., 2011).

Human activities involving NOA (Naturally Occurring Asbestos) such as asbestos mines, green stone quarries, and tunnel excavations require special attention in order to limit NOA's harmful characteristic. The risk related to the presence of asbestos in nature (NOA), derives from the possible mobilization of asbestos fibers, both for natural processes but also and above all anthropogenic activities such as mining, drilling, excavation and demolition (Coraglia, et al. 2006).

The natural presence of asbestos in the various environmental matrices is an environmental and health problem subjected to close attention.

The Italian legislation for the definition of breathable fiber refers to the morphological parameters indicated by the WHO (Ministry of Health, World Health Organization) (Surace, et al. 2011) which are:

- Length exceeding 5μm;
- Diameter less than 3μm;
- Length / diameter ratio greater than 3.

The length of the fibers is essential for penetration and persistence in the lungs. The longer fibers appear to be more harmful, while the short fibers (below 5 microns) are purified and destroyed by the body's defense cells, the macrophages. (Righi, et al., 2003). Asbestos fibers, compared to other varieties of silicates, have extremely small diameters, in the micron range, thanks to the particular property of separating in a longitudinal direction which generate very fine and potentially inhalable fibers (Pisu, et al. 2008).

Table 1 shows a comparison between the typical diameters of asbestos minerals and some examples of fine fibers.

Fiber	Diameter μm
Chrysotile	0,75-1,50
Amphibole	1,50-4
Glass fiber	1-5
Rockwool	4-7
Cotton	10
Wool	20-28
Human hair	40

Table 1: Diameter comparison between different kinds of fibers

1.2 HISTORICAL REFERENCES

The etymology of the name asbestos, from the Greek asbestos, means inextinguishable, for its heat resistance property. Most likely, this characteristic was referred to the perpetual lamps of the temples whose wicks probably consisted of asbestos fibers cords. The Italian name "amianto" derived from the Greek amiantos, which means incorruptible, due to its particular properties, referring on one side to the resistance to corrosive agents, on the other to its use, since ancient times, to make clothes suitable for cremation.

Historical records of the use of asbestos can be found in the "Naturalis Historia" by Plinio il Vecchio (23-79 AD). Asbestos is defined as a rare and precious substance, used in the packaging of the funeral mantles of the Kings. In this regard it was intended to be immaculate, in fact, the fire made it white and pure, so the shrouds made with it were able to avoid the contamination of the real ashes. Again, Plinio il Vecchio reports a further use, which consisted in placing around the trunk of the trees to knock down an asbestos cloth to dull the noise during the fall. From these uses of asbestos as a tissue is evident, as in ancient times it was known the ability to weave fibers, as well as its properties of acoustic insulation.

There are confused reports of asbestos utilization throughout all the Middle Ages. The asbestos origin is also erroneously attributed to the animal world. Thanks to Marco Polo in his "Milione" it is possible to dispel this myth. In his book it is reported that "in the Chinese province of Chingitalas, by spinning this mineral it was obtained a fabric used to make tablecloths". Even if the use of asbestos was quite common during the last five centuries, it is during the 20th century that this mineral became fundamental in different industrial usages. (Benvenuti,2010)

In Italy the main deposits are associated with the alpine metamorphic formations of Val d'Aosta, Piedmont (Valle di Lanzo, Val di Susa, Balangero) and Lombardy (Val Malenco). In 1938, for example, the production of long fiber asbestos coming from Sondrio was 174 tons on a national production of 220 tons.

Other deposits can be found in the Mediterranean area on the island of Cyprus, Macedonia and Romania. In Italy, in particular between the two world wars, asbestos entered strongly in some types of buildings and therefore also in homes and in hundreds of everyday products, sometimes even for uses that today we call improper, ie for functions not related to the chemical and physical properties of asbestos they could also be carried out by other substances: toys, wine filters or through talc, whitening, are examples of asbestos uses.

In the second half of the 50s, the railway carriages, previously isolated with cork, were insulated with asbestos.

The most used asbestos were chrysotile, crocidolite and amosite. About 75% of the production has been absorbed by the fiber cement, while the remaining 25% almost exclusively by friction materials.

1.3 EPIDEMIOLOGY

The discovery of diseases related to asbestos is not very recent. The first reports of health effects caused by asbestos date back to 1906 (London), the year in which Dr. Montague Murray transmitted the notification of the first case of pulmonary fibrosis, found in a worker who reported a serious condition of respiratory failure. Later, thanks to Cook (1927), the term "pulmonary asbestosis" could be coined.

The correlation between exposure to fibers of asbestos and the onset of lung tumours was admitted by the scientific community only after the first systematic epidemiological sounding conducted by Doll in 1955 on English textiles. In the following thirty-year period, numerous studies on working populations in the various asbestos-user sectors confirmed the carcinogenic risk linked to inhalation of asbestos fibers and the causal nexus of the onset of pleural mesothelioma.

In 1977 and 1978 the International Agency for Research on Cancer (IARC, 1977) (IARC, 1987) came to obtain results that demonstrated the carcinogenicity of asbestos for humans (without any distinction between the various types) and therefore the belonging of this mineral to "Group 1 - Carcinogens for humans".

The danger of asbestos derives both from the capability to release fibers, especially in friable materials or minerals, and from phenomena (comminution) able to make the fiber small, fine and more dispersed in the environment. This situation can lead to human exposure, in work or life environments (exposure models classified by Peters & Peters (1988)), to fibers that if inhaled can penetrate deep into the respiratory system and bypass the mechanisms of natural defense of the lungs.

The definition of "breathable fiber", according to the Italian legislation, refers to the explanation dictated by the World Health Organization (WHO, 1986) and it is based on dimensional parameters:

- Length greater than 5 μm;
- Diameter less than 3 μm;
- Length/diameter ratio greater than 3: 1.

The danger related to the inhalation of the fibers depends on the degree of penetration into the respiratory tract, which in turn depends on the size of the fibers: those with a smaller aerodynamic diameter (function of the geometry and density of the fiber) are likely to penetrate more deeply the tree bronchial up to alveoli (*Figure 4*); the larger diameter particles, which are deposited in the upper airways (nasal and tracheo-bronchial), can be eliminated through the mucociliary transport of the epithelium.
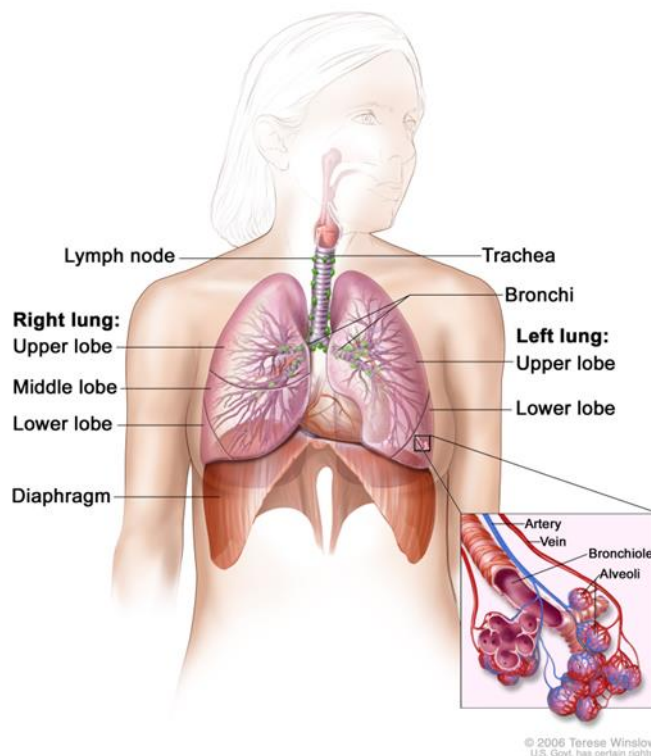
Figure 4: Respiratory system, (NIH - National Cancer Institute)

It therefore appears that the dangerous nature of the asbestos fiber (inhaled) is linked to interconnected factors, such as:

- The shape and size;
- Bio persistence;
- The chemical composition and reactivity of the surface.

The diseases that occur mainly affect the respiratory system or the lining membrane (pleura). Diseases are serious, irreversible and the effects are not immediate because the disease has high latency times (about 10-15 years for

asbestosis, about 20-40 years for lung carcinoma and mesothelioma). There are no threshold limits above which the disease can be contracted. Therefore, any exposure can cause cancer, but several studies indicate that the risk of lung cancer due to asbestos is related to the duration of exposure and cumulative dose (IARC, 1987; Luberto et al. 2004; Szeszenia-Dabrowska N. et al., 2002; Wilczynska U et al. 2005). They are therefore distinguished:

- Asbestosis;
- Pulmonary carcinoma;
- Mesothelioma (pleural, peritoneal or pericardial).

Although rarer, there are other possible diseases related to asbestos such as: laryngeal tumour, tumour of the gastrointestinal tract and uterus. See *Figure 5*.
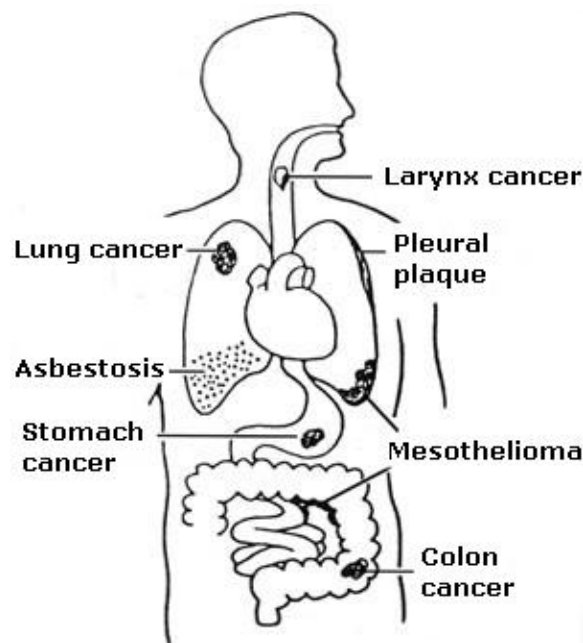
Figure 5: Asbestos related diseases: (Arpa Veneto, 2013).

The impact of asbestos on public health is a topic recently investigated both on subjects with occupational and non-occupational exposure. (Visonà et al., 2018)

1.3.1 ASBESTOSIS

Asbestosis, first found in textile workers (Cooke, W.E. 1928), is a chronic, diffuse respiratory disease characterized by the development of fibrous tissue nodules that cause scarring of lung tissue (pulmonary fibrosis) (Mossman B.T., 1998). The progression of the disease compromises the elasticity of the lungs which leads to serious difficulties in their normal activity of expansion and relaxation, ie

respiratory insufficiency with cardiocirculatory complications (difficulty in oxygenation of the blood).

The symptoms that are found are mainly: breathlessness, irritating cough, asthenia and sometimes weight loss; subsequently the symptoms of the altered lung function appear.

1.3.2 LUNG CARCINOMA

Lung carcinoma is a lung cancer characterized by the uncontrolled growth of cells in the lung tissues leading to the formation of the malignant tumor (Mossmann et al., 2011). The growth of the tumor causes the obstruction of the air passage. Lung cancer is also commonly caused by smoking, but cigarette smoking itself can amplify the carcinogenic effect of asbestos. The symptoms that are found are: breathlessness and cough; the pleura (serous membrane lining the lungs) may have different pathological manifestations of varying severity, starting from pleural thickening, to the appearance of pleural plaques, to malignant mesothelioma

1.3.3 MESOTHELIOMA

Mesothelioma is considered the most serious asbestos-related disease (Maule et al. 2007). It mainly affects the pleura (serous membrane lining the lungs) and the peritoneum (lining that surrounds the upper digestive tract), but can also develop in the pericardium (double-layer membrane lining the heart) or in the vaginal tunic (serous coating of the testis). Malignant mesothelioma, as opposed to lung cancer, identifies the asbestos as the only factor causing the pathology. For this reason it is considered a "sentinel event". The principal symptoms are shortness of breath and chest or abdominal pain (in the thorax may occur pleural effusions accompanied by shortness of breath, cough and persistent fever).

1.3.4 ASBESTOS-RELATED PLEUROPATHIES

The pathological manifestations commonly found in asbestos-related diseases (Gevenois et al. 1998) that occur due to the sensitivity of the membranes to this mineral are:

- Pleurisy: inflammation of the two pleural layers, dictated by roughening and subsequent rubbing between the two membranes.
- Pleural effusion: accumulation of excess fluid between the two pleural layers surrounding the lungs;
- Pleural plaques: fibrous thickenings or partial calcification;
- Hyalinises: hyaline degeneration;
- Diffuse pleural thickening: cicatrisation, calcification and/or thickening of the pleura;

- Atelectasis: collapse or closure of the lung with consequent reduction or absence of air exchanges.

1.4 ITALIAN EPIDEMIOLOGICAL SITUATION

In Italy, starting from the post-war period to 1992, a rapid increase in the production and importation of asbestos was observed, with trends and quantities shown in *Figure 6*.

This trend can be divided into three phases: the first associated with the initial stages of the mineral extraction process, lasted until the 1980s and with a predominantly increasing trend; the second one related to the diffusion and use of asbestos in many sectors up to 1992; and the last one, associated with the cessation and the subsequent necessity of reclamation and maintenance, corresponding to zero production.
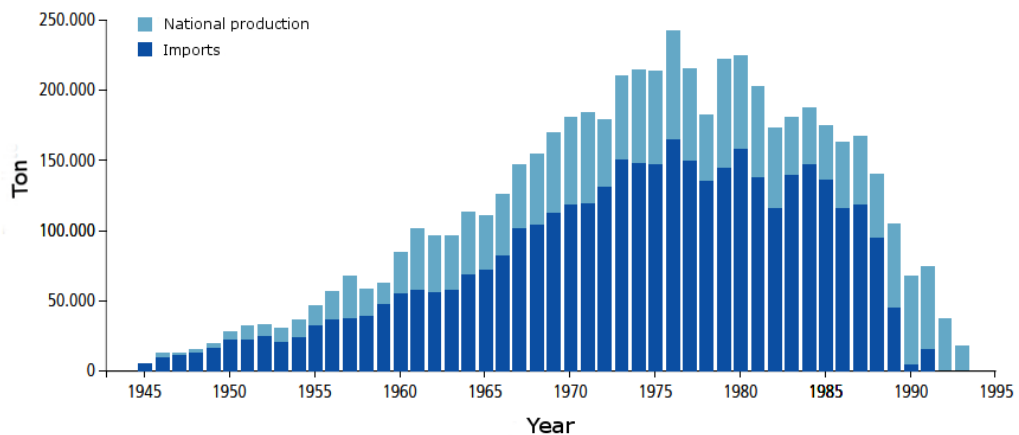
Figure 6: National production and importation of asbestos between the years 1946 and 1992 (Italian Ministry of Health, 2012).

Since 2003, in Italy, the National Registry of Mesotheliomas (ReNaM) has been established with the purpose of conducting detailed estimates on the effect of asbestos associated with the pathology of mesothelioma. As shown in the graph of *Figure 7*, taken from Borin et al. (2011), the curve that describes the trend of deaths for mesothelioma is parallel to that which describes the consumption of asbestos per capita, but follows it with about forty years of delay to confirm the long latency of the disease.
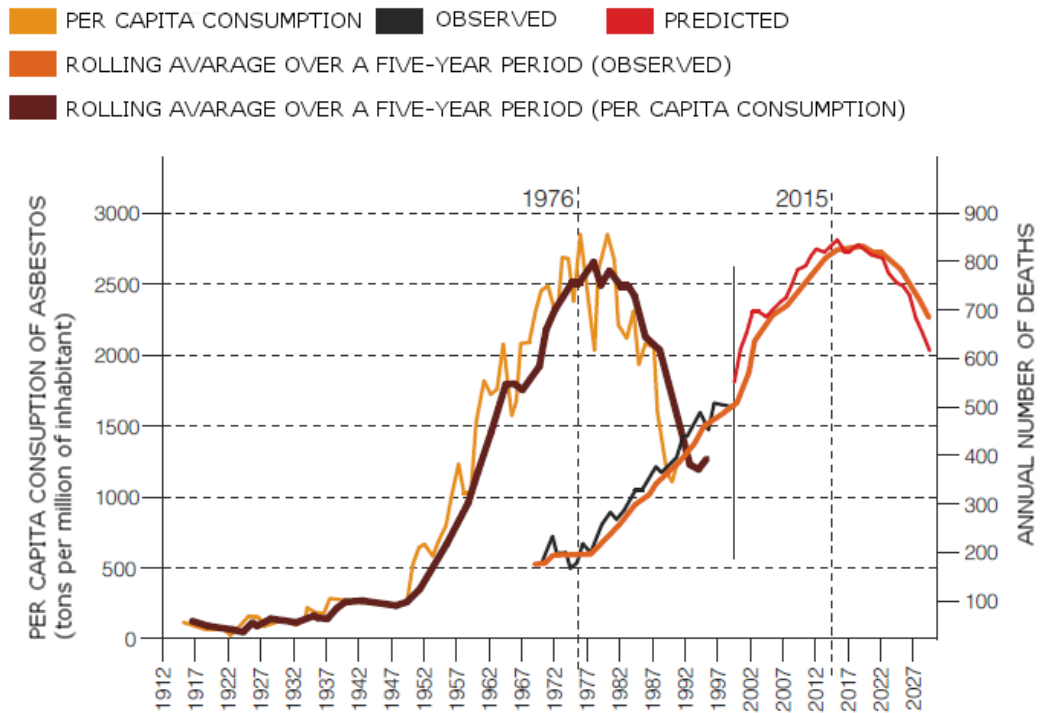
Figure 7: Correlation between per capita consumption of asbestos and annual number of death (Borin et. al, 2011)

The VI Report of the ReNaM refers to cases of mesothelioma with a diagnosis included between 1993 and 2015. Information regarding 27,356 cases of malignant mesothelioma (MM) with diagnosis up to 31/12/2015 is reported. Over 90% of registered mesothelioma cases are pleural, there are also 1,769 cases peritoneal (6.5%), 58 and 79 cases respectively of the pericardium and vaginal tunic of the testis. Up to 45 years the disease is very rare (only 2% of the total registered cases). The average age at diagnosis is 70 years without evident differences on gender. The gender relationship (male gender cases for each female case: M / F) is 2.5. 72% of the cases filed are male. The percentage of women goes from 27.4% for pleural mesotheliomas to 32.8% and 41.1% respectively for cases of pericardium and peritoneum.

Exposure modalities were detailed for 21,387 cases (78.2%) and, among these, 70.0% have occupational exposure (certain, probable, possible), 4.9% family, 4.4 % environmental, 1.5% for a leisure or hobby activity. For 20% of cases exposure is unlikely or unknown. Therefore, the percentage of mesothelioma cases for which exposure to occupational, environmental, family, asbestos or hobbies has been detected is equal to 80.1%. Considering the entire observation window (1993 - 2015) and the only subjects affected by the disease for professional reasons, the sectors of activity most involved are construction (15.5% of the total case series), heavy industry, and in particular metalworking (8.6%), metallurgy (4.0%) and manufacturing of metal products (5.7%), shipyards (6.1%), asbestos cement

industry (3.1%). The remaining picture is extremely varied and fragmented with the presence of numerous production areas in which the exposure took place due to the presence of the material in the workplace and not for direct use.

1.5 ASEBSTOS BANS

On the basis of the effects of asbestos on the health of the exposed subjects, in terms of employment and not, over the years there has been a growing need to stop the processes of extraction, use and marketing however these processes are still widespread in the world. Approximately 55 countries, including the 28 Member States of the European Union, have banned the use of asbestos (*Figure 8*). However, there are countries where part of the economy remains focused on the production and / or consumption of asbestos. Important changes in the marketing of this mineral date back to 1977, when many of the "industrialized countries" decided to move away from the market for the production, extraction and/or consumption of asbestos, but causing the approach of "developing countries" (Thailand, India, Indonesia, Ukraine and Vietnam) (Carnevale, 2011). The world health organization (WHO) affirms that currently about 125 million people in the world are exposed to asbestos at the workplace. In 2004, asbestos-related lung cancer, mesothelioma and asbestosis from occupational exposures resulted in 107,000 deaths and 1,523,000 Disability Adjusted Life Years (DALYs). In addition, several thousands of deaths can be attributed to other asbestos-related diseases, as well as to non-occupational exposures to asbestos.
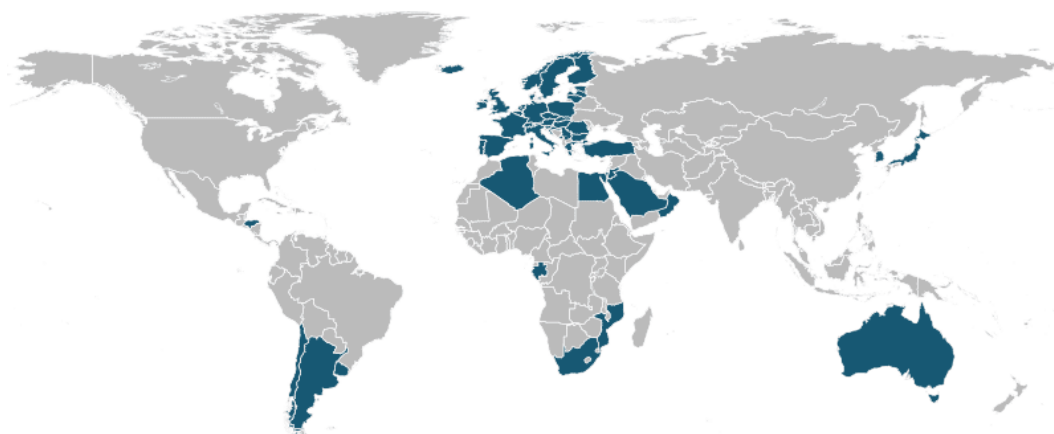
Figure 8: World map of countries with no use and production of asbestos (blue) (asbestosnation.org)

The main asbestos producers are Russia, which manufactured 1 million tons of asbestos in 2010, China that produced 400 thousand tons and Brazil with 270 thousand tons, followed by Kazakhstan, Canada, Zimbabwe and Colombia. These seven countries are responsible for about 96% of the world's production of asbestos; more than 85% of the world's asbestos production is used for manufacturing products in Asia and Eastern Europe. Among the major users is still China with over 613 thousand tons, followed by India with 426 thousand tons and Russia with 263 thousand tons of asbestos followed by Kazakhstan, Brazil, Indonesia, Thailand, Vietnam and Ukraine. In *Table 2* are listed all the National asbestos ban.

National Asbestos Bans				
Algeria	Czech Republic	Iceland	Malta	Serbia
Argentina	Denmark	Ireland	Mozambique	Seychelles
Australia	Egypt	Israel	Netherlands	Slovakia
Austria	Estonia	Italy	New Caledonia	Slovenia
Bahrain	Finland	Japan	Norway	South Africa
Belgium	France	Jordan	Oman	Spain
Brunei	Gabon	Korea (South)	Poland	Sweden
Bulgaria	Germany	Kuwait	Portugal	Switzerland
Chile	Greece	Latvia	Qatar	Turkey
Croatia	Honduras	Lithuania	Romania	United Kingdom
Cyprus	Hungary	Luxembourg	Saudi Arabia	Uruguay

Table 2: National Asbestos Bans (www.asbestosnation.org)

1.6 REGULATORY FRAMEWORK

The complexity of the asbestos problem and the technical difficulties related to the measurement and quantification of this mineral define a vast and complex regulatory framework.

The most relevant standards from the environmental point of view regarding asbestos both at the International, European, National and Regional levels will be listed below.

1.6.1 ASTM STANDARD PROCEDURE (USA)

The Environmental Protection Agency (EPA) has no general ban on the use of asbestos. However, asbestos was one of the first hazardous air pollutants regulated under Section 112 of the Clean Air Act of 1970, and many applications have been forbidden by the Toxic Substances Control Act (TSCA). The United States has extensive laws regulating the use of asbestos at the federal, state, and local level.

Moreover, ASTM (American Society for Testing and Materials) and EPA provide different procedures to define test method that must be carried out for asbestos concentration evaluation.

The standardization procedures regulated by the ASTM allow to define the test methods that must be used in presence of asbestos.

The methods are different, and the most important include:

- ASTM C136/C136M-14: "Standard Test Method for Sieve Analysis of Fine and Coarse Aggregates", this method includes the determination of particles with dimensions ranging from fine to coarse through the sieving method. This test is mainly used to determine the classification of materials that can be used as aggregates. The results of this method are used to determine the conformity of the particle size distribution;

- ASTM D6281-15: "Standard Method for Airborne Asbestos Concentration in Ambient and Indoor Atmospheres as Determined by Transmission Electron Microscopy Direct Transfer (TEM)", this test method is an analytical procedure that uses the transmission electron microscope (TEM) to determine the concentration of asbestos fibers in the atmospheric environment. The transmission electron microscopy has sufficient resolution to allow the identification of small thin fibers and allows the identification of most of the asbestos fibers;

- ASTM D7521-16: "Standard Method for Determination of Asbestos in Soil", this test method includes a procedure for: identifying the asbestos present in the soil, providing an estimate of the concentration of asbestos in the sampled soil and optionally providing the concentration of asbestos as a number of asbestos structures per gram of sample, using two types of methodologies, polarized light microscopy (PLM) and transmission electron microscopy (TEM). This test method has an analytical sensitivity of 0.25 % by weight with optional procedures to allow for an analytical sensitivity of 0.1 % by weight;

- ASTM E691- 15: "Standard Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method", this practice describes the techniques for the design, conduct, analysis and treatment of the results of an inter laboratory study of a test method. The statistical techniques described in this practice provide adequate information for the formulation of the precision declaration of a test method.

Another important method is provided by The California Air Resources Board with the:

- Test Method 435 - Determination of Asbestos Content of Serpentine Aggregate

1.6.2 EUROPEAN REGULATION

At present, the EU regulations for a safety work in asbestos-bearing materials are still not exhaustive because of the great complexity of the matter and uncertainties in geological risk definition (Turci et al. 2015). The most important regulation are listed below.

- Directive 1999/77/EC 26 July 1999; this law adapts for the sixth time to technical progress Annex I of Directive 76/769/EEC where Member States bring into force the laws, regulations and administrative provisions relating to restrictions on the marketing and use of certain substances and dangerous preparations, or the use of asbestos and the products containing it;

- Air quality guidelines - global update 2005 (WHO, 2005);

- Directive 2003/18 /EC of the European Parliament and of the Council of 27 March 2003 amending Council Directive 83/477/EEC on the protection of workers from the risks related to exposure to asbestos at work;

- Directive 2004/37/EC of the European Parliament and of the Council of 29 April 2004 on the protection of workers from the risks related to exposure to carcinogens or mutagens at work (sixth individual Directive within the meaning of Article 16 (1) of Council Directive 89/391/EEC). This directive is applied to asbestos and includes provisions that are more favorable to health and safety at work than those of Directive 83/477/EEC are;

- EC Regulation n. 1272/2008 of the European Parliament and of the Council of 16 December 2008 on the classification, labeling and

packaging of substances and mixtures which amends and repeals Directives 67/548/EEC and 1999/45/EC and which contains amendments to Regulation (EC) 1907/2006;

- Directive 2009/148/EC n.148 of 30 November 2009 on the protection of workers against the risks associated with exposure to asbestos at work.

1.6.3 ITALIAN REGULATION

Ban on Use of Asbestos:

- Law 27/03/1992 n.257: "**Rules regarding the termination of the use of asbestos**" and in particular the ban on the extraction, importation, exportation, marketing and production of asbestos, asbestos products and products containing asbestos. Furthermore, it introduces some articles for the protection of the environment and health (classification, packaging, labeling, and control of dispersions during processing, removal of asbestos and regional plans and of the autonomous provinces) and defines support measures for workers and companies.

- Ministerial Decree of 12/14/2004 bans the installation of materials containing intentionally added asbestos manufactured before Act 257/92 came into force and subsequently stocked by both public and private subjects.

Moreover, concerning the Italian regulation:

- Ministerial Decree D.M. 6/09/1994: contains regulations and technical methodologies relating to:
  - o Standards and technical methods for risk assessment, control, maintenance and reclamation of materials containing asbestos present in building structures;
  - o Quantitative determination of asbestos in bulk samples, general aspects of the analytical problem;
  - o Quantitative determination of airborne asbestos fiber concentrations in indoor environments;
  - o Qualitative identification of asbestos fibers by means of the chromatic dispersion technique in optical microscopy;

- o Criteria for a correct choice of personal respiratory protective equipment;
- o Card for the verification of the presence of materials containing asbestos in buildings.

- Health Ministerial Decree D.M. 26/10/1995: Standards and technical methods for risk assessment, control, maintenance and remediation of materials containing asbestos present in rolling transport.

- D.Lgs.17 March 1995 n.114: implementation of Directive 87/217/CEE on the prevention and reduction of environmental pollution caused by asbestos, which defines the limit value of emissions into the atmosphere, limit values in liquid effluents, demolition activities of manufactured goods and the removal of asbestos or materials containing asbestos and the collection and transmission of data.

- Health Ministerial Decree D.M. 14/5/1996, n.178: Regulations and technical methods for remediation interventions, including those to make asbestos harmless, provided by art. 5, paragraph 1, letter f), of the law of 27 March 1992, n. 257, containing: "Rules concerning the termination of the use of asbestos".

- Ministerial Decree D.M. 12/02/1997: Criteria for the approval of asbestos replacement products.

- Ministerial Decree D.M. 07.07.1997: approval of the application form for the quality control program for the suitability of the analysis laboratories operating in the "asbestos" sector.

- Ministerial Decree D.M. 20/08/1999: Standards and technical methods for the removal of materials containing asbestos present on board ships or similar units. Requirements for encapsulating coatings for the removal of asbestos cement products. Criteria for selection of personal protective equipment for the respiratory system.

- Ministerial Decree D.M. 25/10/1999, n.471: Regulation laying down criteria, procedures and methods for the safety, reclamation and environmental restoration of polluted sites, in accordance with Article 17 of Legislative Decree of 5 February 1997 n.22 and subsequent modifications and additions.

- Deliberation 1/02/2000: criteria for registration in the category 10 category of art. 8 of the D.M. 28/4/1998, n.406 for reclamation of goods containing asbestos.

- Ministerial Decree D.M. 25/7/2001: rectification to the decree of 20 August 1999, concerning "Extension of the regulations and technical methods for remediation interventions, including those to make asbestos harmless, as provided for by article 5, paragraph 1 letter f), of law March 27 n. 257, laying down rules on the cessation of the use of asbestos "

- Decree of the President of the Council of Ministers n. 308, December 10, 2002: regulates the determination of the model and modalities of keeping the register of mesothelioma asbestos-related cases pursuant to article 36, paragraph 3 of legislative decree n.277 of 1991.

- Decree of the Ministry of the Environment 18.03.2003 n.101: regulation for the mapping of the areas of the national territory affected by the presence of asbestos, in accordance with article 20 of the law of 23 March 2001, n.93.

- Ministerial Decree D.M. 29/7/2004, no. 248: the technical regulations on the methods for transporting and storing asbestos waste as well as the treatment, packaging and covering of the waste in landfills are approved by the commission for the evaluation of environmental problems and health risks related to the use of asbestos. The more complete disposal of the waste containing asbestos (RCA) and the reuse, or better, the use as raw material of materials deriving from the transformation of asbestos, are regulated in more complete matters.

- Circular National Register of Waste Managers 21.04.04 n. 2700 on the modalities and amounts of the financial guarantees to be provided to the state by the companies carrying out the reclamation of the assets containing asbestos.

- Health Ministerial Decree D.M. 14/12/04: establishes a ban on the use of materials containing intentionally added fibers of crocidolite, chrysotile, amosite, anthophyllite, actinolite and tremolite or from these constituted; the use of materials already installed or in use at the date of the decree until the elimination or end of their life; safe use for as long as necessary following appropriate risk assessment.

- Ministerial Decree D.M. 3/8/2005: establishes the criteria and procedures for the admissibility of waste in landfills, in accordance with the provisions of Legislative Decree 13 January 2003, n.36. The waste is allowed to landfill only if they comply with the eligibility criteria of the corresponding category of landfill as established by this decree. Landfills for hazardous waste have a level of environmental protection superior to those for inert waste, therefore the provision of waste that meets the criteria for admission to each category of landfill in landfills having a higher level of protection is allowed.

- Legislative Decree D.Lgs.257/2006: implementation of the EEC directive 2003/18 / EC on the protection of workers from risks related to exposure to asbestos at work. It establishes that from April 1994 the extraction, the importation, the exportation the commercialization and the production of asbestos and of products of asbestos or containing asbestos are forbidden.

- Legislative Decree D.Lgs. 9/4/2008 n.81: "Consolidated Law on Occupational Safety ", implementation of Article 1 of the Law of 3 August 2007, n.123, concerning the protection of health and safety in the workplace, through the reorganization and coordination of the same in a single normative text.

- Legislative Decree D.Lgs. 3/8/2009 n.106: supplementary and corrective provisions of the Legislative Decree 9/4/2008, n. 81, concerning the protection of health and safety in the workplace.

- Directive 30/11/2009 n.2009/148/EC: on the protection of workers from the risks associated with exposure to asbestos at work, for the remediation of asbestos and materials containing asbestos in public places or open to the public for the progressive replacement of asbestos materials with other comparable use products, as well as with regard to equality of access to social security benefits for workers exposed to asbestos.

- Circular 25.01.2011 of the Ministry of Labor: the Permanent Commission (CCP) for health and safety at work approved the practical guidelines for the determination of sporadic and low intensity exposures (ESEDI) to asbestos in the framework of the planned activities art. 249 paragraph 2 of Legislative Decree 9th of April 2008, n. 81 as amended and supplemented by Legislative Decree dated August 3rd, 2009, n.106.

- Legislative Decree D.Lgs 10/8/2012, n.161 "Terre e rocce da scavo" "Lands and rocks from excavation ": regulation on the use of excavated earth and rocks. In Annex 4, which lists the physical chemical characterization procedures and the assessment of environmental qualities, it is established that:

  o The samples to be analyzed in the laboratory or in the field must be free of the fraction greater than 2 cm (to be discarded in the field) and the analytical determinations in the laboratory must be conducted on the particle size of less than 2 mm. The concentration of the sample must be determined by referring to the totality of the dry materials, also including the skeleton sampled (fraction between 2cm and 2mm);
  o In the minimal set of analytical parameters to be considered, the asbestos parameter is included;
  o The results of the analysis on the samples should be compared with the Contamination Threshold Concentrations referred to in columns A and B table 1 in annex 5, in Title V part IV of Legislative Decree No. 152 of 2006;
  o Physical chemical analyzes will be carried out using officially recognized methods, such as to guarantee the achievement of values 10 times lower than the limit concentration values;
  o In the case of the impossibility of reaching such quantification limits should be used the best officially recognized analytical methodologies;
  o Compliance with the environmental quality requirements pursuant to art. 184 bis paragraph 1 letter d) of D.Lgs.152 / 2006 for the use of excavation materials as by-products, is guaranteed when the content of polluting substances in the excavation materials is lower than the Contamination Threshold Concentration (CSC);
  o The excavation materials can be used for filling, backfilling, interventions at sea, land improvements etc., if:
    ▪ If the concentration of pollutants within the limits set out in column A of Table 1 in Annex 5 of Title V of Part IV of Legislative Decree 152 of 2006, at any site regardless of its destination;
    ▪ If the concentration of pollutants is between the limits indicated in column A and B table 1 in annex 5, in Title V part IV of Legislative Decree No. 152 of 2006, in sites destined for production (commercial and industrial).
- Law of 9 August 2013, n. 98: Additional provisions on earth and rock excavation.

1.6.4 REGIONAL REGULATION (PIEDMONT)

- Deliberation of the Regional Council 07/04/1997, n. 71-18113: general authorizations for emissions into the atmosphere from construction sites for the demolition and removal of asbestos or materials containing asbestos from buildings, structures, equipment and plants

- Deliberation of the Regional Council n. 51-2180 of 05/02/2000: approval of the regional plan of protection, decontamination, disposal and reclamation of the environment for the purposes of defense against dangers arising from asbestos;

- Regional Law of 14 October 2008, n. 30 rules for the protection of health, environmental remediation, reclamation and disposal of asbestos;
- Deliberation of the Regional Council June 3, 2009, No. 30 -11520 - Article 4 of Law. 30/2008 definition of the criteria and procedures for the granting of grants for the reclamation of artifacts containing asbestos;

- Deliberation of the Regional Council 8 February 2010, n. 75-13258 - Art 4 of Law 30/2008 - Integration of the D.G.R. n. 30 - 11520 of 3 June 2009. Funding program for the reclamation of asbestos-filled buildings in public school buildings;

- Deliberation of the Regional Council 18 December 2012, n. 40-5094: approval of the regional protocol for the management of petition/warning related to the presence of asbestos-cement roofing in buildings. Internal procedure ARPA Piemonte URPT104: assessment of the state of conservation of asbestos cement roofing;

- Deliberation of the Regional Council 18 December 2013, No. 25-6899: approval of operational indications for the removal and collection of modest quantities of asbestos-containing materials in cement or resin matrix present in civil users by private citizens.

1.7 LAW LIMITS

The critical issues related to the asbestos problem are not few, on one hand the regulatory problems due to the lack of a single asbestos regulation must be considered, and, on the other hand, the technical problems due to the intrinsic limit of detection of asbestos fibers (Cavariani, 2015). The concentration of asbestos is evaluated in fiber/liter as regards the analysis of airborne samples or in mg/kg for what concerns the massive samples. The D.Lgs.152 / 2006 establishes that the CSC (concentration threshold of contamination in the soil and in the subsoil) for the asbestos parameter is indicated in 1000 mg/kg.

The current legislation that regulates the management of land and rocks is DM 161/2012, which repeals Article 186 of Legislative Decree 152/2006 (Consolidated Environmental Law). Ministerial Decree 161/2012 states that to use excavated materials as by-products, the content of asbestos inside excavated earth and rocks must be lower than the limit value of 1000 mg / kg.

The analyzes performed in order to verify that the concentration of asbestos in the earth and rocks from excavation are lower than 1000 mg/kg are conducted on samples that are purified in the sampling phase by their coarse fraction, ie the one above 2 cm. *Table 3* summarizes the different limit values for asbestos provided for by the Italian legislation for the various applications.

Application	Limit value	Analytical method	Legislative framework
WORKING ENVIRONMENT			
TLV-TWA (Threshold limit value – Time Weighted Average for a conventional 8-hour workday and a 40-hour workweek)	100 f/l	PCOM	T.U. della Sicurezza (D.Lgs. 81/2008), Art. 254, subparagraph 1
ASBESTOS INTO BUILDING – LIVING ENVIRONMENT			
Indicative value of pollution in a building (it mediates on 3 samplings)	20 f/l 2,0 f/l	PCOM SEM	D.M. 6/09/94, Point 2c

REMEDIATION MEASURES			
Early-warning threshold for the monitoring of areas surrounded the reclamation site		PCOM	D.M. 6/09/94 Point 11/1
Alert threshold for the monitoring of areas surrounded the reclamation site	50 f/l	PCOM	D.M. 6/09/94, Point 11/2
Internal monitoring in reclamation site to restore a clean-up environment	2,0 f/l	SEM	D.M. 6/09/94, Point 6/b
ASBESTOS CONTAMINATED SITES			
Limit value of concentration in the ground	1000 mg/kg	DRX-FTIR	CODICE AMBIENTE (D.Lgs. 152/2006) Part IV - Title V - Annex 5 - Table 1
EMISSION TO THE ATMOSPHERE			
Asbestos concentration in the discharges emitted into the atmosphere through exhaust ducts	$0,1$ mg/m^3 of extracted air	Gravimetric	D.Lgs. 114/95 Art. 1, subparagraph 1
	2000 f/l	PCOM	D.Lgs. 114/95 Art. A, II
LIQUID EFFLUENTS			
Asbestos concentration in the discharges emitted into the atmosphere through exhaust ducts	30 g of total material suspended in a discharge liquid effluent (m^3)	Membrane filtration	D.Lgs. 114/95 Art. 2, subparagraph 1
GREEN STONES EXTRACTION SITES			
Limit value to define the danger of the extracted material	0,1	Release index	D.M. 14/05/96 Annex 4

Table 3: Italian law limits

Chapter 2

2. (N.O.A.) NATURALLY OCCURRING ASBESTOS: VALIDATION OF PCOM QUANTITATIVE ANALYSIS

Part of the work described in this chapter has been previously published in a Resource Policy Journal article: "Naturally occurring asbestos: Validation of PCOM quantitative determination" Oliviero Baietto, Paola Marini (2018)

2.1 INTRODUCTION

The NOA "naturally occurring asbestos", is the asbestos as it surfaces, or sub-surfaces, in rocks and their products of alteration in the natural environment, with a variable content that can not be predicted quantitatively (Malinconico, Paglietti, Rimoldi, & Sala, 2011).

With the term NOA we generally refer not to the asbestos extracted and used for commercial purposes but rather to those same fibrous minerals that are released into the air from their own matrix especially due to mining, excavation or road grading works. Some recent studies (Bloise et al., 2016, Noonan, 2017, Wagner, 2015,) have investigated the presence of natural occurring asbestos pointing out the direct correlation between human activities and the dispersion of asbestos airborne fibers; while others (Bayram et al., 2013, Bloise et al., 2017, Cattaneo et al., 2012, Cavallo and Rimoldi, 2013, Dichicco et al., 2018, Gaggero et al., 2017, Gualtieri et al., 2014, Noonan, 2017, Vignaroli et al., 2014) refer to elevated mesothelioma incidence in people who live close to NOA deposits worldwide, including those of Italy.

A Risk Management Protocol (Protocollo Amianto, 2018) was adopted as part of the Resolution approving the Final Design of the work for which the "Politecnico di Torino is a consultant". The adoption of the Protocol for the excavations of the "Terzo Valico dei Giovi" Tunnels, as prescribed by the Ministry of the Environment, guarantees the correct management of the "Asbestos Risk" by providing the tools for the best execution of the works. The drafting of this

document was attended by all public and private bodies involved in the construction of the opera. The following description of the geological context in which the work is framed is extrapolated from the Protocol.

The assessment of the presence of asbestos in mineral matrix concerns the geological context of the territory and the presence in it of ophiolite rocks, or residual portions of an ancient oceanic crust incorporated and redistributed within the Alpine and Apennines chain in the phases of orogenesis that led to their formation.

From the petrographic point of view, ophiolites are essentially constituted by associations of basic and ultrabasic magmatic rocks (basalt, gabbro, peridotite), more or less serpentinized and / or metamorphosed, with typical dark-greenish colors sometimes associated with diaspri (siliceous sedimentary rocks). The presence of asbestos in the rocks is due to a natural process that, starting from basic and ultrabasic magmatic rocks (protoliths) in relation to the specific geological history that combines the tectonic deformation activity, the metamorphic/metasomatic processes and the interaction between fluids and rock, determines the formation of asbestos minerals. The possibility of encountering asbestos in rocks is therefore correlated to Ophiolitic geological units, basic and ultrabasic, which have been subjected to these geological processes; such rocks are commonly called "Green Stones".

In nature about 85% of the extracted asbestos is distributed along discrete tectonic bands (lenses and veins associated with faults-cutting zones) along which there has been circulation of fluids during the metamorphic processes of basic and ultrabasic rocks. Sometimes even diffused within the matrix of the rock mass according to a network of minor secondary fractures (Robinson et al., 1982; Wrucke, 1986; Deer et al., 1997), although it is not excluded the case of development of mineralized lattice of submillimeter size.

Normally these processes take place gradually in time and space and in certain thermobaric conditions (medium-low metamorphic grade; Ross 1981; Schreirer 1989), therefore the rocks can also be only partially affected by asbestos veins/mineralization (Ross 1981, Ross & Nolan, 2003, Schreirer H., 1989, Wruke, C.T., 1986).

In addition to veins and fractures, fibrous minerals can also develop into cataclastic or milonitic bands on contact between lithotypes with different chemical compositions, in particular between basic rocks and carbonate rocks, due to metasomatic reactions (Page 1967, Cerney, 1968, Van Gosen, 2007).

During the consulting activity, it was also noted the possibility of finding asbestos in tertiary clastic sedimentary rocks (sandstone, conglomerates and breccias, etc.) and in the quaternary deposits (fluvial, fluvial and glacial) whose sediments, which have been transported and stored from other geological environments, derive from the erosion of pre-existing rock masses containing asbestos.

In fact, during the progress of the work it was found that some marly-arenaceous sedimentary formations belonging to the Piedmont Tertiary Basin (BTP), contain a variable quantitative of mineral fragments of typically ophiolitic genesis. The carried out analyzes have highlighted the presence of asbestos minerals. The presence of asbestos in sedimentary formations derives from minerals of secondary origin, which occurred in a geological past, connected to the erosion phenomena of pre-existing lithologies in Green Stones and subsequent deposition and accumulation of these within the Piedmontese Tertiary Basin

2.2 THE PROBLEM OF THE QUANTIFICATION

The methodology for the assessment of the concentration of asbestos fibers in airborne samples is described in the regulations cited in the previous chapter. The analysis technique and the statistics on which it is based is sufficiently solid as opposed to the methodology for the evaluation of the concentration in massive samples.

During human activities such as excavation works and tunnelling there is a continuous monitoring of the airborne samples. These analysis are extremely important because they provide an alarm bell on the presence of asbestos in the worked (or moved) material. Unfortunately, it was not possible to perform a direct correlation between the concentration of airborne fibers in the working environment and the asbestos concentration present in the excavated rock. In fact the concentration of fibers in the airborne samples is strongly influenced by the place of work itself (air circulation, distance from the contaminating source etc.) and by the type of work in progress. It has to be underlined that the continuous airborne sampling is, however, the best methodology to have a fast information about the possible presence of asbestos in the excavated rock, even in extremely low quantity.

The managing of huge amounts of natural material containing asbestos is a complex problem to deal with, particularly during tunnelling opera or excavation works. In this context the quantitative determination of the content of asbestos in rock matrices became extremely important but is a complex operation that is susceptible to significant errors. The principal instruments used for the analysis are the Scanning Electron Microscope (SEM) and the Phase Contrast Optical Microscope (PCOM).

As previously seen the threshold for asbestos concentration in soils is 1000 mg/kg (0.1%), as defined by Italian law in force. (D. Lgs. 152, 2006). This value is extremely important because the D.M. 161/2012 establishes that if the quantity of asbestos is less than 1000 mg/kg the material can be reused as a by-product, otherwise it must be managed as waste.

The procedures contemplated by current Italian law concerning asbestos concentration in soils involve the use of three instruments: the X-ray Diffraction

(XRD) (D Lgs 152/2006), the Fourier-transform infrared spectroscopy (FTIR) (DM 14/5/96 and D Lgs 152/2006) and the Scanning Electron microscopy (SEM) (DM 6/9/94 and DM 14/5/96) while the Phase Contrast Optical Microscopy (PCOM) is considered only for qualitative analysis.

These analytical techniques are described in the first attachment of the previous cited DM 6/9/94, but not in an exhaustive and correct manner. There is a huge paradox, for example, in the XRD and FTIR technique. The quantification limit indicated for the X-ray Diffraction and for the Fourier-transform infrared spectroscopy is the 1% of the concentration which is a value ten times higher than legal limit. For this reason, these two technologies are not effective for this kind of analysis. Moreover, the decree never refers properly to rock samples but only to bulk samples namely asbestos cement or to powdery samples as talcum powder.

The development of our methodology comes from the desire of proposing a reliable procedure based on the use of PCOM in an inadequate and insufficient regulatory framework. PCOM is a smaller, cheaper and more dependable (in the correct characterization of the type of asbestos) instrument than the SEM. According to DM 6/9/94 the resolution of the PCOM is 0.25 μm while for the SEM is 0.01 μm but, even if the resolution of the PCOM can lead to errors in the determination of fibers measurements, a right methodology can make this error negligible. The DM 6/9/94 hypothesizes the use of optical contrast phase microscopy also for quantification but does not recommend its use due to three factors.

The first is the non-unique possibility of recognizing the various types of asbestos but, as we will see in the following paragraphs, PCOM is indeed extremely accurate in distinguishing the different minerals thanks to their unique optical properties.

The second is the poor depth of field of the optical microscope. Our methodology, however, provides the division into different granulometric classes, thus allowing to avoid this problem.

The third is the lower resolution, but, although the PCOM resolution is inferior to that of SEM, PCOM analysis has several advantages. These advantages include: more representativity of the analysed sample, more effective recognition of chrysotile and a lower cost. These advantages will be deeply investigated in the next section. The error evaluation, concerning asbestos quantification in rock matrices, generally provided by analysis laboratories varies between 50% and 150%. There are not, however, enough specific studies that discuss every error in addition to the instrumental error or that link them to the asbestos content in rock samples. Our work in this chapter aims to provide a validation of a methodology for the determination of the total content of asbestos using PCOM.

2.3 INTRODUCTION TO PHASE CONTRAST MICROSCOPY

Dutch physicist Frits Zernike in 1932 describes for the first time the Phase contrast microscopy (PCOM). This technique is a contrast-enhancing optical technique that can be utilized to produce high-contrast images of transparent specimens, such as living cells, microorganisms, thin tissue slices, lithographic patterns, fibers, latex dispersions, glass fragments, and subcellular particles. In *Figure 9* a PCOM configuration is described.
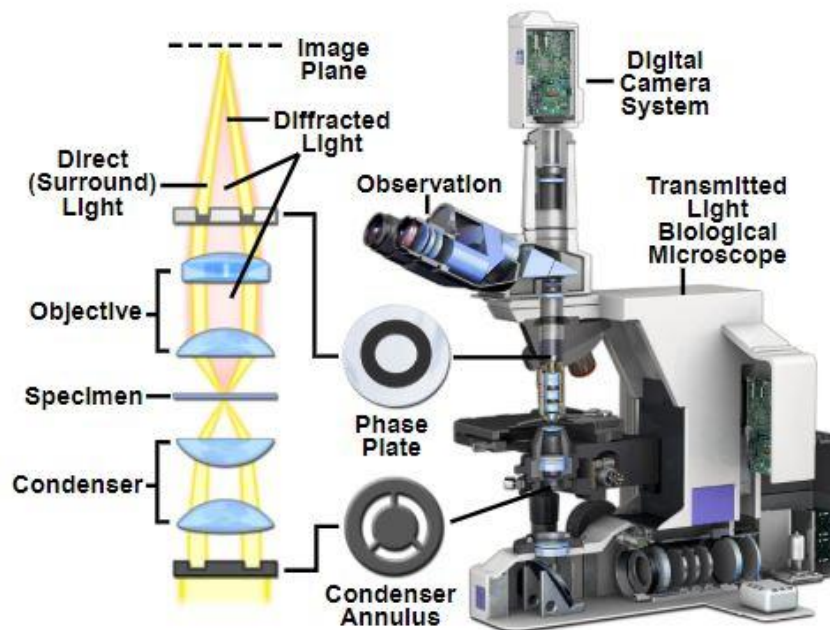
Figure 9: Phase Contrast Microscope Configuration (www.microscopyu.com)

Light is constituted by an oscillating phenomenon that varies over time and spreads in space, the so-called luminous excitation. By fixing on a point along the propagation axis, light is expressed by the simple equation of harmonic motion:

$$x = a*cos(\omega t)$$

x: luminous excitement

a: maximum amplitude of excitation

ωt: phase (varies over time t)

It is possible to represent the luminous excitation as a cosine function generated by a "virtual" rotating vector with angular velocity ω. It is therefore the definition of harmonic motion (*Figure 10*) where ω determines the period $T=2\pi/\omega$ and the frequency $\nu=1/T$.
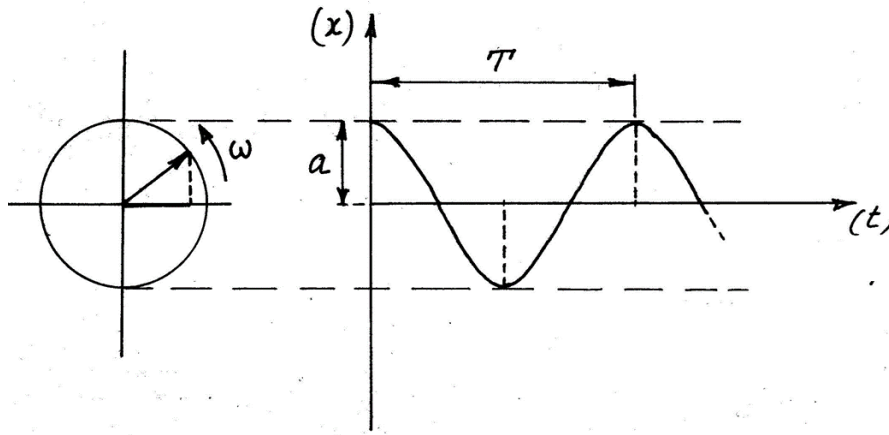
Figure 10: Harmonic motion (Clerici, 2006)

From the point of view of perception by the human eye:

- the amplitude (a) determines the luminous intensity

- the frequency (ν) determines the colour

- (ωt) determines the phase, as the light excitation goes from a max to a min passing through zero. The eye sees the light constant and not variable because the frequency of oscillation is enormous (trillions of oscillations per second) and there is the phenomenon of the persistence of images on the retina.

Imagining to block the oscillation along the z-axis at any instant, the situation is shown in the *Figure 11*.
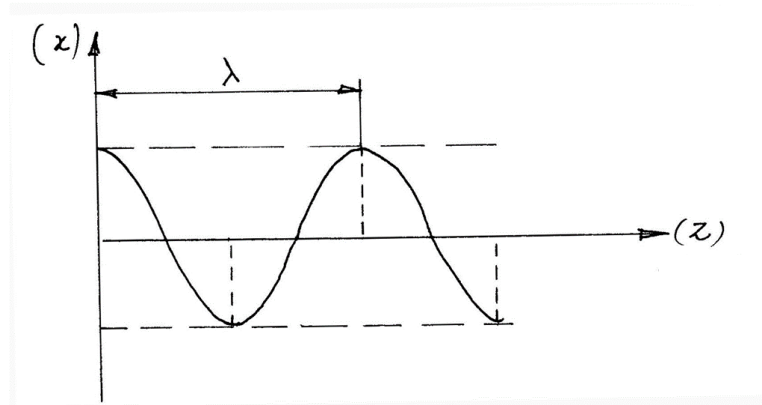
Figure 11: Wavelength (Clerici, 2000)

A new quantity is identified, the wavelength λ, which is the minimum distance between 2 points that oscillate in phase concordance (ie in the same oscillation situation: 2 maxima, or 2 minima, etc.).Since it is necessary to pass a time T (period) in order to have phase concordance, λ is also the space reaches by the oscillation in a period: $\lambda = VT$. The differences, usually called contrasts, between the light waves are:

- Amplitude contrast = different brightness for human eye (*Figure 12*)

- Frequency contrast = different colour for human eye (*Figure 13*)

- Phase contrast = different wave oscillation situation (maximum, minimum, zero, etc.) with no differences for human eye (*Figure 14*)
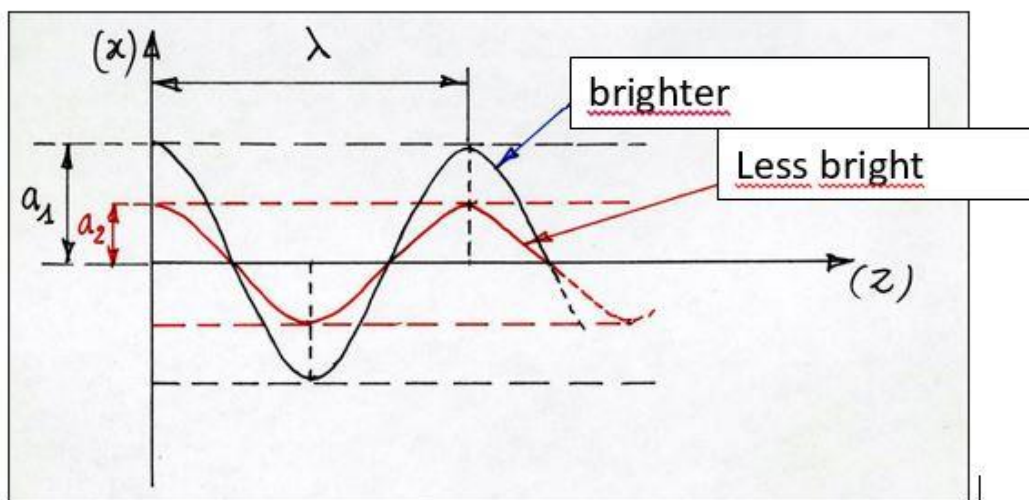
Figure 12: Amplitude contrast (Clerici, lessons 2012)

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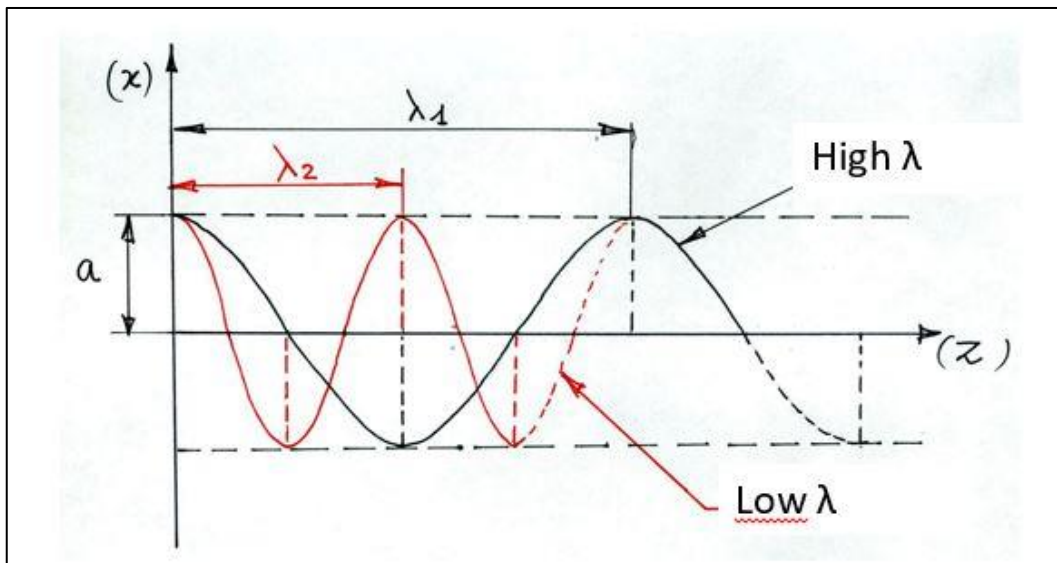
Figure 13: Frequency contrast (Clerici, lessons 2012)
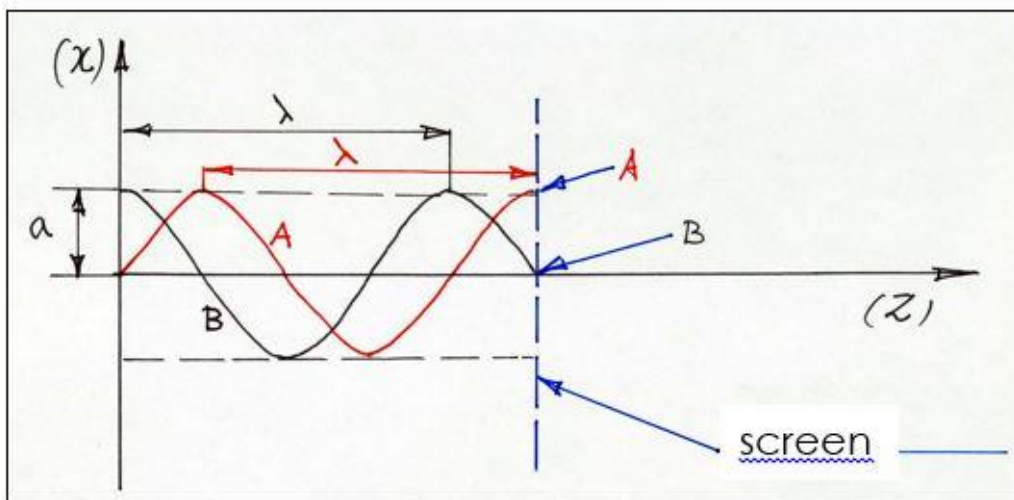
Figure 14: Phase Contrast (Clerici, lessons 2012)

In microscopic vision, objects are visible if there is a contrast between objects and the luminous background (the field of the microscope). So they are visible if darker (or lighter) than the background (amplitude contrast) or coloured (frequency contrast).

When small transparent objects are observed, the amplitude and frequency contrasts are attenuated until they disappear. All objects tend to become equally transparent and colourless. But colourless transparent objects can also be contrasted

in phase with respect to the background, when they have a different index of refraction (n) than the transparent medium that surrounds them. The phase contrast microscope using an artifice (phase plate) inserted along the path of the light rays inside the microscope obtains the transformation of the phase differences into differences in amplitude (= different luminosity) and therefore perfectly visible. A phase-contrasted particle is seen as a darker (or lighter) particle of the bottom.

2.4 MATERIAL AND METHODS

2.4.1 THE PCOM (PHASE CONTRAST OPTICAL MICROSCOPE)

The PCOM used is a Leica Phase Contrast DMLP Trinocular Polarizing Microscope equipped with Leica HC PL FLUOTAR objective with a magnification of 10X, 20X and 40X. The microscope has Leica HC PLAN eyepieces with a magnification of 12.5X. Phase Contrast Optical Microscopy analysis is based on an optical principle: using a microscope equipped with a polarizer and a device for phase contrast, it is possible to recognize asbestos fibers thanks to the variation of a refraction index which depends on the wavelength of the incident light. If the fibers are placed in specific high-dispersion liquids, they show typical chromatic effects that allow their identification. The optical resolution of the instrument allows the recognition of particles up to 0.5/1 μm.

The PCOM is more often considered as a qualitative methodology: after a macroscopic observation of the sample, if the operator finds some fibrous material that can be associated to asbestos, he must take a small part of the material and proceed with preparing one or more microscope slides for the identification. The resolution of the PCOM is 0.25 μm but is possible to measure with a good precision objects with dimension of 2-3 times higher than the potential resolution (DM 6/9/94, attachment 1). A correct methodology can make the PCOM a reliable instrument for quantitative determination too. Each kind of asbestos and non-asbestos fibrous material can be recognized by its own optical property, if the slide is prepared with a specific high-dispersion liquid with a known refraction index. The evaluation of the correct refraction index is based on three parameters: luminosity, colour and birefringence (Clerici et al. 1975; Niskanen et al. 2013).

Luminosity: the particle brightness must be defined in relation to the background consisting of a liquid with known refractive index (n). The refractive index (or index of refraction) of a material is a dimensionless number that describes the propagation of light through that material. It is defined as the ratio between the light speed in vacuum and the phase velocity of light in the medium. If the refractive index of the liquid is higher than the refractive index of the particle ($n_l > n_p$) the particle will become lighter than the background and vice versa.

Colour: if $\Delta\_n=n\_l- n\_p\approx0$ (between -0,020 e +0,020) different chromatic effects are obtained, with colorations both of the particle and the surrounding halo. In the following Table are described the colour effects for different Δ_n and different K (ratio between slopes of the chromatic dispersion curves of liquid and solid). In *Figure 15* the upper part of the colour pairs is referred to the colour of the particle while the lower to the colour of the surrounding halo.
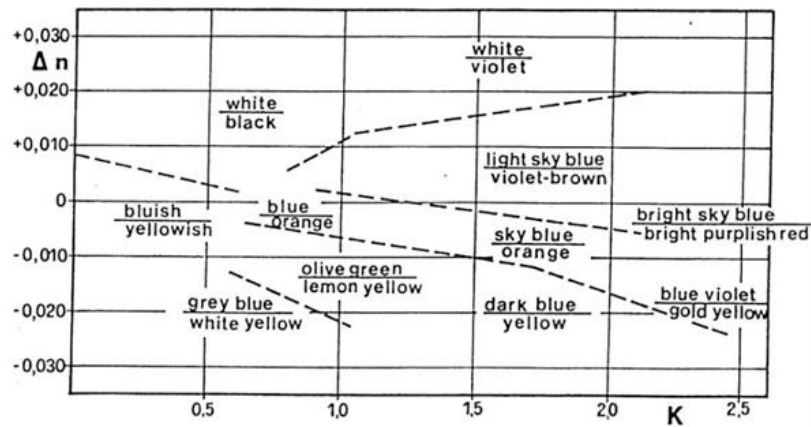
Figure 15: Table Of colour effects (Schmidt, 1955)

Birefringence: change of the colour of the fiber varying its orientation. Thanks to the evaluation of the colour alteration more informations may be obtained about the range of variation of the particle refractive index. An example of Chrysotile asbestos recognition in rock matrix is given in *Figure 16*.

Figure 16: Chrysotile asbestos (refractive index oil n=1.550)

2.4.2 ASBESTOS' OPTICAL CHARACTERISTICS

In the following figure (*Figure 17*) the six fibrous minerals regulated as asbestos are described:
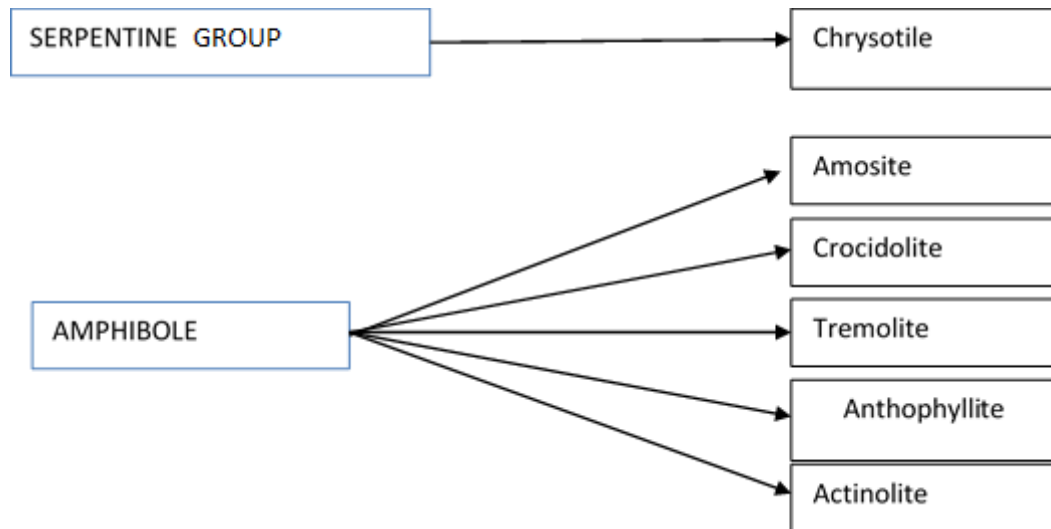
Figure 17: Regulated asbestos minerals

The HSE (Health & Safety Executive UK) defines the properties of the different asbestos type suggesting the correct refraction indices to be used. In our laboratory, 6 samples of pure asbestos were analyzed to detect the characteristics that, in most cases, were similar. It should be noted however that some variations may depend both on the type of asbestos analyzed (origin, morphology ...) and on the sensitivity of the different operators. In the following figure (*Figure 18*) the HSE asbestos properties and subsequently our description of the different kind of asbestos.

Asbestos type		Chrysotile	Amosite	Anthophyllite	Tremolite	Actinolite	Crocidolite
RI liquid Property		1.550	1.670	1.605	1.605	1.640	1.700
Morphology	Fibrous	Fibrous	Fibrous	Fibrous	Fibrous	Fibrous	Fibrous
Pleochroism	Fibre parallel	None	None	None	None	Green	Blue
	Fibre perpendicular	None	None	None	None	Grey	Grey
Birefringence		Low	Moderate	Moderate	Moderate	Moderate	Low/ anomalous
Extinction		Complete, or undulose with curved fibres; parallel	Complete; parallel	Complete; parallel	Complete; parallel or small angle	Complete; parallel or small angle	Complete; parallel
Sign of elongation		Usually positive (length slow)	Positive (length slow)	Positive (length slow)	Positive (length slow)	Positive (length slow)	Usually negative (length fast)
Dispersion staining	Fibre parallel	Purple	Yellow	Yellow-orange	Yellow	Yellow-brown	Blue
Objective colours	Fibre perpendicular	Blue	Purple-red	Blue-red	Blue	Blue-purple	Blue
Phase contrast	Fibre parallel Fibre colour Halo colour	Pale-blue Orange	Grey Yellow	Dark-grey Orange	Dark-grey Yellow	Dark-grey Yellow	Blue Red-brown
Objective colours	Fibre perpendicular Fibre colour Halo colour	Pale-blue Orange	Blue Orange	Blue Orange-yellow	Blue Orange	Blue Orange	Blue Red-brown
Refractive	n_α	1.537-1.554*	1.670-1.675*	1.596-1.654+	1.599-1.620+	1.619-1.658+	1.680-1.692*
Index ranges	n_γ	1.545-1.557	1.683-1.694*	1.625-1.667+	1.622-1.641+	1.641-1.677+	1.683-1.700*

(Note: Fibre parallel or fibre perpendicular describes orientation with respect to the polariser. Dispersion colours relate to the HSE reference standards.[58] Slight compositional variations will give rise to differences in the dispersion staining colours observed. RI ranges marked * were obtained from commercial asbestos fibre;[59] RI ranges marked + were obtained from non-commercial fibres).[03]

Figure 18: Asbestos properties (HSE, 2006)

In the following paragraph each asbestos observed with PCOM is described.

CPL = Cross Polrized Light
DL = Direct Light
n_α ; n_γ = refractive index of the fast and slow ray (Deer et al. 1966) ("the speed of light in vacuum divided by the speed of light in the medium")

2.4.2.1   CHRYSOTILE (Mg₃Si₂O₅(OH)₄)

Chrysotile is an hydrated Mg-phyllosilicate mineral part of the serpentine group. The principal minerals of this group, which have the approximate composition of $H_4Mg_3SI_2O_9$ (Deer et al., 1966) are chrysotile, a fibrous variety which was the most important source of commercial asbestos ; antigorite, a variety occurring in either plates or fibres; and lizardite, a very fine-grained, platy variety. The structure of all serpenitines is a Mg-rich trioctahedral equivalent of the kaolinite group with the same 1:1 layer structure but all the positions in the octahedral sheet are filled with Mg2. Lizardite shows an ideal layer topology owing to shifts of the octahedral and tetrahedral cations away from their ideal positions and to the limited Al3 + for Si4 + substitution in the tetrahedral sites (Rinaudo et al., 2003), whereas antigorite is modulated and chrysotile is bent (Brigatti et al., 2013). (J.T. Kloprogge, 2017). Chrysotile's structure consists of layers of concentric cylinders, which is the typical asbestiform mineral habit (Wicks and Whittaker 1975). It is a magnesium phyllosilicate, monoclinic.

On microscopic examination this mineral is recognized to be made up of thin, long, curved or wavy fibers. With crossed nicols (CN), it presents very light interference colors and right extintion.

Table 4 and *Figure 19* show the features and optical properties of this mineral.

FEATURE	VIEW	DESCRIPTION		
Morphology		Thin, long, curved or wavy fiber		
Extintion angle	CPL	Right		
Selected refractive Index	–	$n_\gamma \geq 1{,}545$ $n_\alpha \leq 1{,}550$		**1,550**
	PCOM	$n_l = 1{,}550$	Blu orange halo	Light blu pink halo

Table 4: Chrysotile features
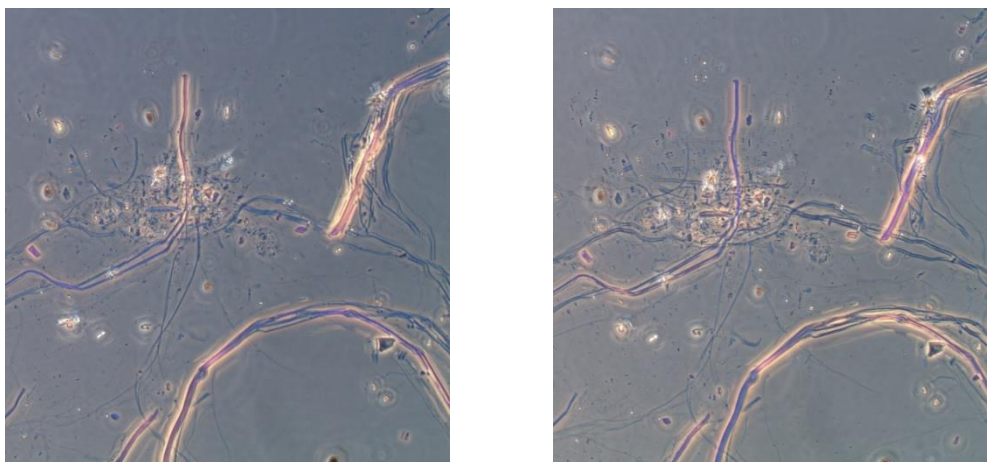
Figure 19: CHRYSOTILE , 10x, PCOM, OIL 1,550

2.4.2.2 AMPHIBOLE GROUP

Amphibole group, according to the definition given by the Dictionary of Geology, is a family of silicate minerals forming prism or needlelike crystals composed of double chain of SiO_4 tetrahedra. Amphiboles crystallize into two crystal systems, monoclinic and orthorhombic. The minerals belonging to this group defined as asbestos are five. Tremolite, amosite, actinolite and crocidolite that crystallize in the monoclinic system while antophillyte crystallize in the orthorhombic system. Generally amphiboles occur in a wide range of thermal and pressure environment and are common constituent of both igneous and metamorphic rocks.

2.4.2.3 TREMOLITE ($Ca_2Mg_5Si_8O_{22}(OH)_2$)

Tremolite forms a continuous series with the other minerals in the tremolite-actinolite series. It crystallizes in the monoclinic system and occurs naturally in three distinct morphological forms or mineral habits. It may occur as asbestos when in fibrous habit or with a predominantly prismatic habit. It crystallizes in the monoclinic system. This mineral has low/medium birefringence and an extinction angle of approximately 14°. *Table 5* and *Figure 20* show the features and optical properties of this mineral.

FEATURE	VIEW	DESCRIPTION		
Morphology		Straight prismatic fiber, with pointed end		
Extintion angle	CPL	Oblique		
Selected refractive Index	–	$n_\gamma \geq 1,640$ $n_\alpha \leq 1,600$		**1,610** **1.615**
	PCOM	$n_l = 1,615$	Dark Blu Yellow halo	Light blu Pink halo

Table 5: Tremolite features
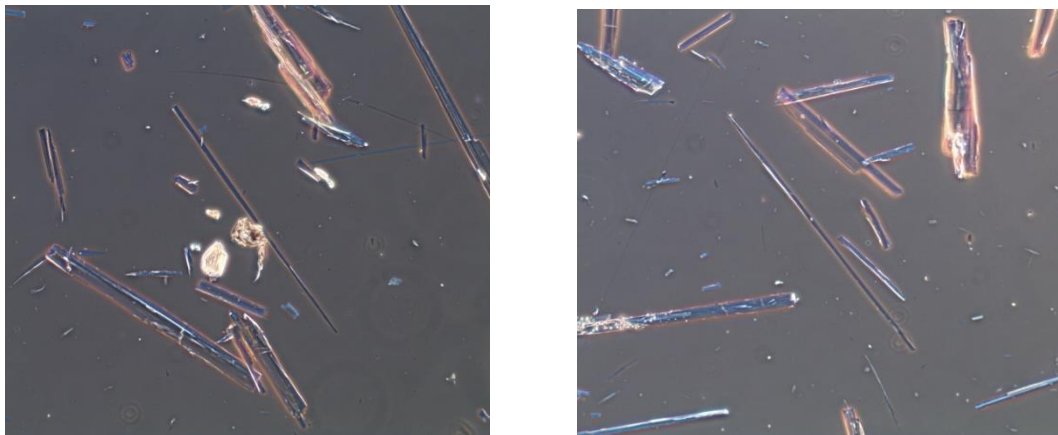
Figure 20: TREMOLITE, 10x, PCOM, OIL 1,615

2.4.2.4 ACTINOLITE ($Ca_2(Mg_{<4,5}Fe^{2+}_{>0,5})Si_8O_{22}(OH)_2$)

Actinolite is an intermediate member in a solid-solution series between tremolite (magnesium-rich) and ferro-actinolite (iron-rich). Mg and Fe ions can be freely exchanged in the crystal structure. It crystallizes in the monoclinic system and shows oblique extinction and low birefringence. (*Table 6* and *Figure 21*)

FEATURE	VIEW	DESCRIPTION		
Morphology		Straight acicular fiber, with pointed end		
Extintion angle	CPL	Oblique		
Selected refractive Index	–	$n_\gamma = 1,62 \div 1,66$ $n_\alpha = 1,64 \div 1,68$		**1,640**
	PCOM	$n_l = 1,640$	Blu Yellow halo	Light blu Orange halo

Table 6: Actinolite features
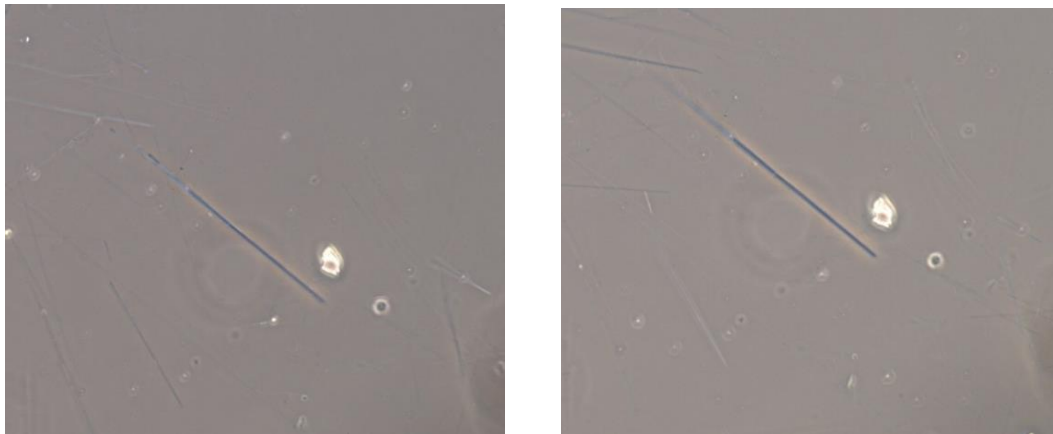
Figure 21: ACTINOLITE, 10x, PCOM, OIL 1,640

2.4.2.5 ANTHOPHYILLITE ($(MgFe)_7Si_6O_{22}(OH)_2$)

The anthophyllite is an amphibole with magnesium-iron-manganese. Usually the anthophyllite is found in short fibrous and acicular aggregates or columnar gray-green to light brown, rarely in prismatic crystals. It crystallizes in the rhombic system. It shows right extinction and medium birefringence. *Table 7* and *Figure 22* show the features and optical properties of this mineral

FEATURE	VIEW	DESCRIPTION		
Morphology		Short fiber, acicular or columnar, rarely prismatic.		
Extintion angle	CPL	Right		
Selected refractive Index	–	$n_\gamma = 1,60 \div 1,65$ $n_\alpha = 1,63 \div 1,67$		**1,615** **1.620**
	PCOM	$n_l = 1,620$	Dark Blu Yellow halo	Light blu Orange halo

Table 6: Antophyllite features
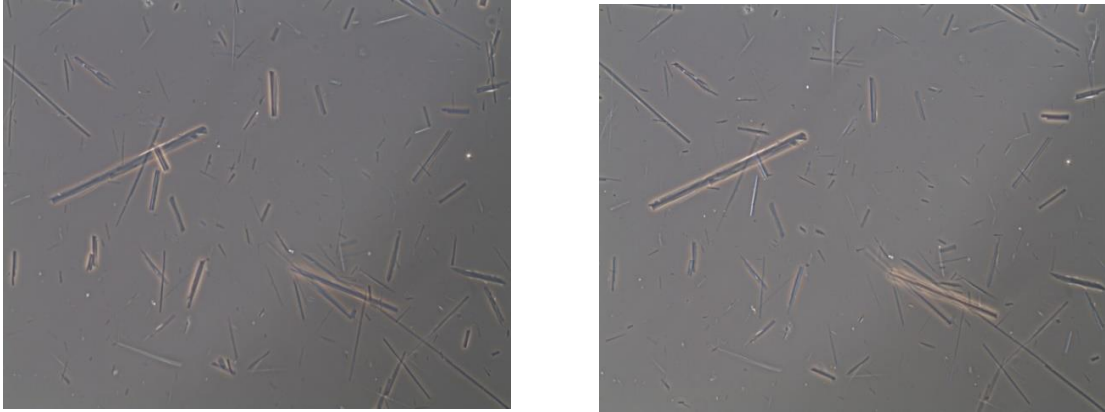
Figure 21: ANTHOPHYLLITE, 10x, PCOM, OIL 1,620

2.4.2.6 AMOSITE (Mg,Fe)$_7$Si$_8$O$_{22}$(OH)$_2$)

Amosite, called brown asbesto, is the fibrous form of the cummingtonite-grunerite solid solution series of the amphibole group. Its name derives from the acronym AMOSA (Asbestos Mines of South Africa). This mineral crystallizes in the monoclinic system and its optical properties are: right extinction, not present pleochroism and medium birefringence (*Table 8, Figure 23*).

FEATURE	VIEW	DESCRIPTION		
Morphology		Straight acicular fiber		
Extintion angle	CPL	Right		
Selected Refractive Index	−	$n_\gamma = 1{,}67 \div 1{,}68$ $n_\alpha = 1{,}68 \div 1{,}69$		**1,670**
	PCOM	$n_l = 1{,}670$	Dark Blu Yellow halo	Light blu Pink halo

Table 7: Amosite features
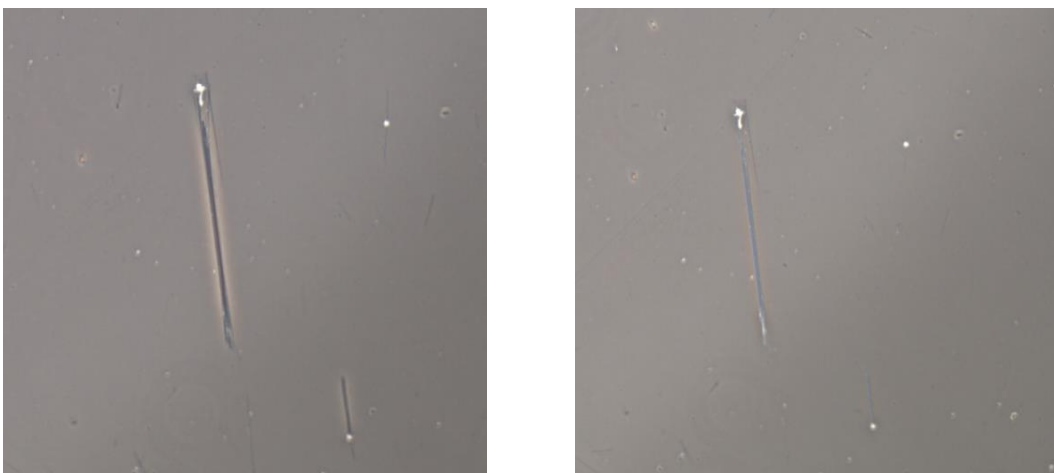
Figure 22: AMOSITE, 10x, PCOM, OIL 1,670

2.4.2.7 CROCIDOLITE ($Na_2(Fe^{2+},Fe^{3+})_2Si_8O_{22}(OH)_2$)

Crocidolite (blue asbestos) is the fibrous form of Riebeckite amphibole. It comes in the form of long, linear and flexible blue fibers. It is the most dangerous type of asbestos for humans. It presents a strong pleochroism; the n_α direction is unusually parallel to the fiber; this is the reason of the pleochroism (dark blue in direct light -DL-). Crocidolite crystallizes in the monoclinic system and it presents extinction straight, weak birefringence. *Table 9* and *Figure 24* show the features and optical properties of this mineral

FEATURE	VIEW	DESCRIPTION		
Morphology		Long fiber		
Pleochroism	DL	Fiber from dark blu / Smoked brown		
Extintion angle	CPL	Right		
Refractive Index	–	$n_\gamma = 1,68 \div 1,69$ $n_\alpha = 1,68 \div 1,70$		**1,700**
	PCOM	$n_l =1,670$	Dark Blu Orange halo	Light blu White halo

Table 8: Crocidolite features
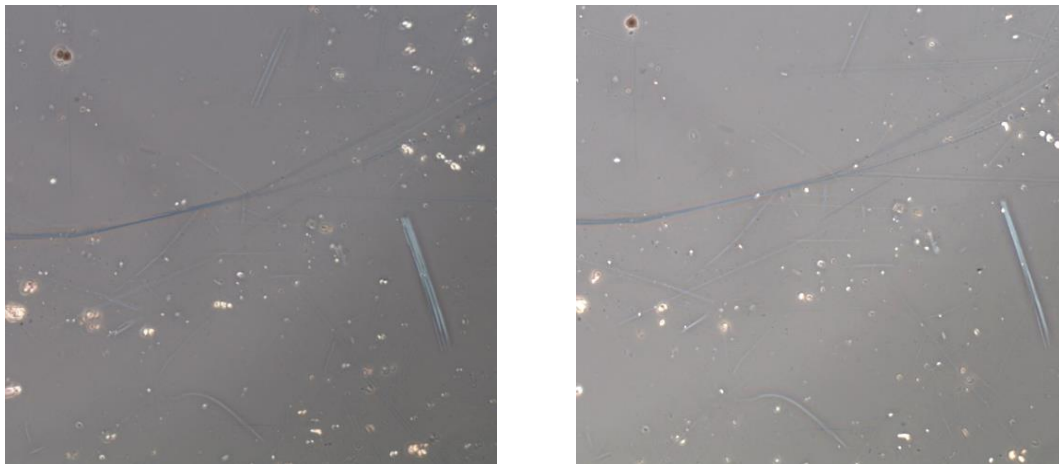
Figure 23: CROCIDOLITE, 10x, PCOM, OIL 1,700

2.4.3 METHODOLOGY

For this research a core sample of serpentinite rock is chosen, which, on the base of previous analysis, contains only chrysotile in quantities close to 0.05%, corresponding to half of the legal limit. The sample had previously been analyzed according to the following methodology and had provided information on the asbestos content.

The sample, is comminuted in a crushing chamber and quartered under a fume hood. A portion of approximately 120 gr is placed in a corundum jar in a planetary mill equipped with a sealing gasket. The jar is inserted in the mill and the material is milled for a time ranging between 15 and 20 seconds depending on the material and the instrument used. The process continues with several successive grindings to obtain a particle size smaller than 0.6 mm and well distributed granulometrically.

Subsequently, the sample separation is carried out in 5 grain size classes by wet sifting under light water jet and with the recovery of the finest material. Sieves with the following apertures are used: 0.6 mm; 0.3 mm; 0.15 mm; 0.075 mm. Once dry, every grain size class is weighed. On the coarser class (> 0.6 mm), if present, a wet sorting of the fiber is performed: the class in question is placed in a becker, added with water, under mechanical agitation and again filtered, dividing the fraction floating from the one that tends to settle on the bottom of the container.

The fiber suspension procedure has to be repeated until no more fibers can be seen by the analyst (Bellopede et al. 2009). The two fractions obtained for each class are weighed. The PCOM occurs that the fibers are of asbestos after have put them on a slide with the appropriate refractive index oil (n=1.55). From each dried filter of the remaining portions, sample rates of known weight are deposited on a slide and added with suitable refractive index oil to be subjected to phase-contrast optical microscopy analysis.

After the classification of the sample, a known amount of material for each grain size class is observed with PCOM (0.6–0.3mm =80 mg; 0.3-0.15mm =40 mg; 0.15 - 0.075mm =16 mg; <0.075 mm =0.5mg). The combination of four slides of different granulometric classes constitutes a set representative of the whole sample. This kind of preparation allows the operator to better understand each characteristic of the sample. Moreover the granulometric homogeneity allows to overcome the problem of the lower depth of field of the optical microscope compared to the electronic one.

Ten sets of slides are prepared. Each set represents a different analysis carried out on the quartered sample.
The goal is to simulate 10 independent procedures for each sample. Each operator proceeds with the analysis of a fixed number of observation fields. In our

case we chose to observe 25 fields for each slide for the classes lower than 0.3mm and the entire slide for the coarser class. The magnifications chosen are function of the dimensions of the analyzed material, we recommend the 10X objective for all classes except for the finest where the 20x and 40x objectives are more effective.

Furthermore, the use of the right refraction oil is the most unequivocal way to recognize which kind of fibrous mineral is being investigated. In this case the only kind of asbestos contained in the sample is Chrysotile, so the correct oil is the 1.550 refraction index oil. The flowsheets of this experiment are shown in the following *Figure 25* and *Figure 26*
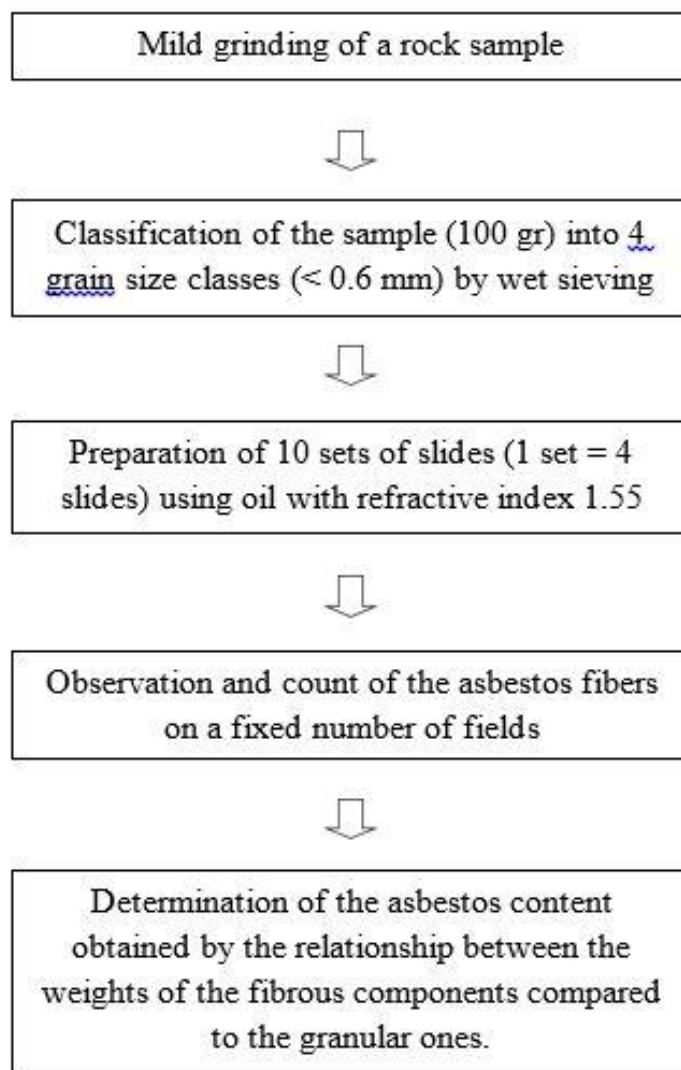
Figure 24: Experimental flowsheet

Crushing chamber

Sieve column

Fume hood

PCOM

Figure 25: Instrumental flowsheet

The errors to which this methodology is subjected to are similar to those of every other microscopic analysis. In order to perform a validation of this methodology laboratory tests have been carried out. Several statistical tests were performed to assess the reliability of the methodology. The analysis is discussed in the next section.

2.5 ANALYSIS AND DATA PROCESS

2.5.1 VARIABLES

In the following table *(Table 10)* are indicated all the variables that contribute to the evaluation of the size of the fibers and the consequent assessment of the concentration of asbestos in the sample.

Analyzed sample variables	
Mass of the particle size 0,6-0,3mm: 28,2 (g)	c_1
Mass of the particle size 0,3-0,15mm: 24,7 (g)	c_2
Mass of the particle size 0,15-0,075mm:11,2 (g)	c_3
Mass of the particle size <0,075mm: 29,1 (g)	c_4
Mass on the slide (mg)	M_v
Asbestos mass on the slide(mg)	$M_{asbestos\ slide}$
Mass of the fibers on the slide (mg)	M_f
Asbestos mass for each particle size (g)	M_{ic}
Asbestos Content (mg/kg)	Ca_{tot}
Mass of the fiber (mg)	m_i
Volume of the fiber (m³)	V_i
Length of fiber (μm)	l_i
Diameter of the fiber (μm)	ϕ_i
Chrysotile Density (2,6 kg/dm³)	ρ
Dusted Area (mm²)	A
Field Area (mm²)	a
Number of Fields	N
Lens	2,5X-10X-20X-40X

Table 9: variables for the calculation of the total asbestos content

2.5.2 HOW TO REACH TO THE CONCENTRATION

With the observation at the PCOM it is possible to individuate the asbestos fibers and, thanks to the graduated grids present in the objectives, measure theirs length and diameter.

Knowing the length and diameter of each fiber, it is possible to calculate the mass of the single counted fibers on the slide (M_f), approximating each fiber to a cylinder.

$$V_{tot} = \pi \sum l_i \left(\frac{\phi_i}{2}\right)^2$$

$$M_f = V_{tot}\rho \text{ (mg)}$$

Once obtained M_f, considering the dusty area (slide area), the number of fields and the field area the asbestos mass actually present on the slide is calculated.

$$M_{asbestos\ slide} = \frac{M_f(mg)A\ (mm^2)}{N\ a\ (mm^2)}$$

Subsequently, the mass of asbestos for each grain size class is calculated (M_{ic})

$$M_{ic} = \frac{M_{asbestos\ slide}\ (mg)c_i(g)}{M_v\ (mg)}$$

Finally, the total content of asbestos in mg/kg is calculated

$$Ca_{tot} = \frac{M_{ic1}c_1 + M_{ic2}\ c_2 + \cdots + M_{ic4}c_4}{M_{sample\ tot}}$$

The total asbestos content (Ca_{tot}) of the ten set of slides, considered as different samples, obtained by both operators following the methodology described above is reported in the following Table (*Table 11*).

	Operator 1	Operator 2
	Ca_{tot} (mg/kg)	Ca_{tot} (mg/kg)
Sample 1	546	393
Sample 2	575	406
Sample 3	449	514
Sample 4	423	515
Sample 5	398	370
Sample 6	567	485
Sample 7	228	370
Sample 8	326	380
Sample 9	405	294
Sample 10	414	404

Table 10: total content of asbestos in mg/kg

In a sample of natural material, there is no certainty of the exact asbestos content. In order to validate the methodology of analysis, the distribution of the results obtained by each operator and their comparison will be evaluated in the next paragraph.

2.5.3 STATISTICAL VALIDATION

It is necessary to evaluate the statistical reliability of the data from the pattern obtained by the two operators.

The statistical model chosen to perform this verification is Student's test because it allows to compare the means of two distributions in order to verify whether their difference is significant or is due only to the chance.

The test allows evaluating if the difference between the means is due to chance or if, instead, there is a difference, due to systematic or human factors, between the means of the two populations.

The Student's t-test starts from the distribution analysis and can be applied in two ways:

- In order to assess if the means of the two distributions has a significant difference; in this case, the Student test is used in the bilateral form (also called two-tier);

- In order to assess if an average is greater than the other; in this case the student test is used in the unilateral form (also called one-tier);

For this study, wanting to evaluate whether the difference between the mean of the two distributions is statistically significant, the student t test is used in the bilateral form. However, to use this type of test, two different sample checks must be performed:

- Verify that the sample distribution is Gaussian;

- Verify the variance equality by Fisher test.

After the two tests, the two means are compared with the Student's t-test. The first verification is made using the Minitab statistical software. Minitab is a statistical software developed in 1972 at the Pennsylvania State University. As other statistical software automates calculations and the creation of graphs, allowing the user to focus more on the analysis of data and the interpretation of results. The verification that proves that the two data divisions are distributed in a normal manner can be graphically performed through the Q-Q plot functions. This check is performed with a 95% probability. For both samples, the verification is positive because the points fall within the expected range, as can be seen from *Figure 27 and Figure 28.* It must be underlined that this kind of test has a greater and more real statistical significance for a larger number of samples. It is however necessary to perform it, and, in view of the complexity of the analysis, a set of 20 analyzes can be considered significant.
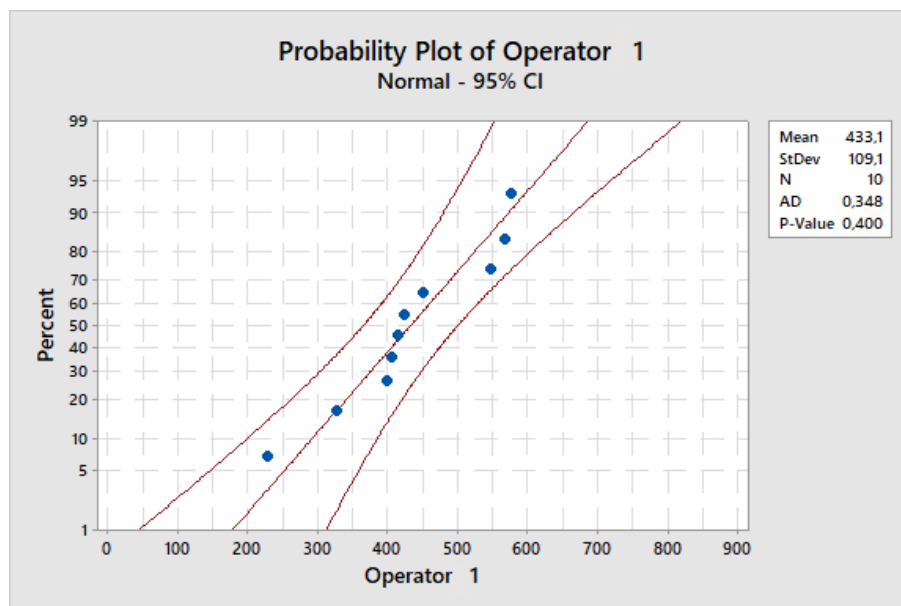
Figure 26: Verification of the Gaussian distribution of data – operator1

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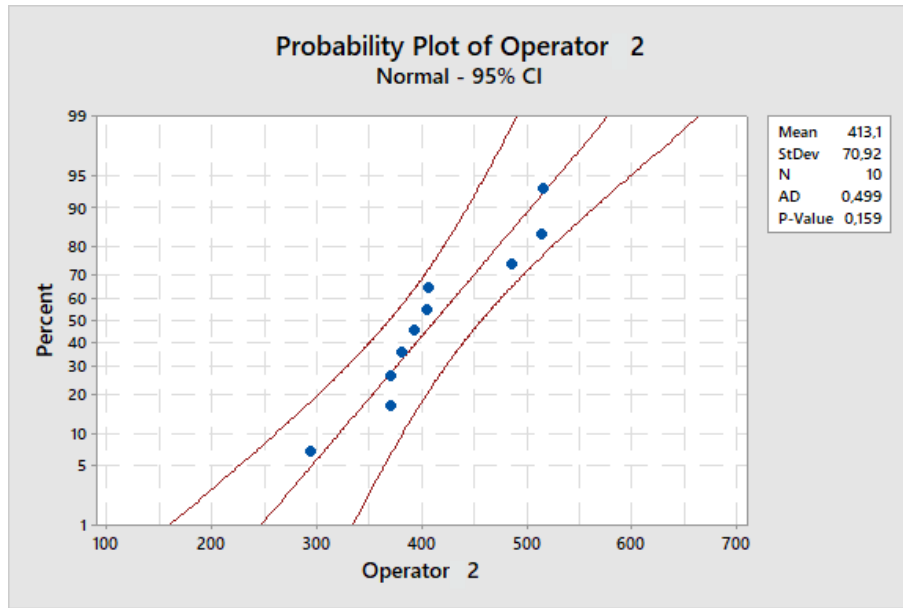
Figure 27: Verification of the Gaussian distribution of data – operator2

After checking that the two samples are Gaussian-type specimens, it has been possible to control the homogeneity of the variances of the two samples through Fisher's test (Fisher, 1958) on Excel.

Fisher's test is a statistical test aimed at verifying the hypothesis that two populations that follow both normal distributions have the same variance. Fisher's test is based on two key concepts, the acceptance or rejection of the zero hypothesis. In this case, the zero hypothesis predicts that the variances of the two populations are comparable (there are no significant differences), while the alternative hypothesis says the opposite, that the variances of the two peoples are not comparable (there are significant differences):

$$H_0: \sigma_1{}^2 = \sigma_2{}^2$$

$$H_1: \sigma_1{}^2 \neq \sigma_2{}^2$$

If the null hypothesis is true and since the variable follows a normal distribution in both populations, the relationship between two sample variances follows the null distribution of Fisher, also known as F distribution (*Table 12 and Table 13*). To perform the test it is necessary to evaluate if the F-value obtained from the ratio between the two variances is minor than the F-value deriving from the Fisher distribution. The analysis was performed on Excel with a 95% significance level and such analysis, as seen in *Table 12* confirmed the zero hypothesis.

Fisher's Test	*Operator 1*	*Operator 2*
Mean ($\bar{X}$) [mg/kg]	433,1	413,1
Variance (S^2)	11898,7	5029,6
Tests (n)	10	10
Degrees of freedom	9	9

Table 11: Variables of Fisher's Test

$F = S^2_1/S^2_2$	2,3
P(F<=f) one-tier	0,11
F critic one-tier	3,17

Table 12: Two-sided Fisher test for variance

Since (F) < (F critical to one queue) it can be stated that the variances of the two groups are comparable.

After evaluating that the two samples are of Gaussian type and that the variances of the two distributions are not significantly different, the last thing to do is to compare the means, using Student's t-test (Student, 1908). To assess correctly the student test, the t-value must be calculated (see following formula). Subsequently the value obtained must be compared with the table t-value obtained with a level of confidence that, in our case, was chosen at 95%.

$$t = \frac{\bar{X}_i - \bar{X}_j}{\sqrt{\frac{s_i^2 \, (n_i - 1) + s_j^2 \, (n_j - 1)}{n_i + n_j - 2} \left(\frac{1}{n_i} + \frac{1}{n_j} \right)}}$$

Table 13 shows the values obtained by the Student's t-test.

Student's t-Test	Operator 1	Operator 2
Mean ($\bar{X}$) [mg/kg]	433,1	413,1
Variance (s^2)	11898,7	5029,6
Tests (n)	10	10
Degree of freedom (df)	18	
Obtaened t (Stat t)	0,486	
P(T<=t) one-tier	0,316	
t critic one-tier	1,73	
P(T<=t) two-tier	0,632	
t critic two-tier	2,10	

Table 13: Student t-Test

Comparing the t obtained by the critical value for 18 df, is possible to affirm that:

$$Stat\ t < t_{critic\ two-tier}$$

Consequently it can be stated that the mean of the two distributions are not significantly different and with a probability of 95% the two samples are part of the same population. This conclusion provides a good validation of the methodology.

Each measurement is affected by a random error that can affect the accuracy of the result. Casual errors are due to the operator and random and non-homogeneous distribution of asbestos fibers within the sample.

To consider these errors we can express the measurements with two values: the mean (μ) and the standard deviation (σ).

$$mean \pm\ standard\ deviation = \mu \pm \sigma$$

$$Operator\ 1 = 433{,}1 \pm 109{,}19$$

$$Operator\ 2 = 413{,}1 \pm 71$$

It is also possible to calculate the relative uncertainty of the measurements performed by operator 1 and operator 2

$$relative\ uncertainty\ of\ operator\ 1 = \frac{\sigma_1}{\mu_1} * 100 = 25.2\ \%$$

$$relative\ uncertainty\ of\ operator\ 2 = \frac{\sigma_2}{\mu_2} * 100 = 17.2\ \%$$

This means that there is a 25.2% probability that operator 1 measurements do not fall into the confidence interval, while for operator 2 this probability drops to 17.2%. The measurements obtained by Operator 2 seem more accurate than those obtained by Operator 1 but are, however, comparable. It should be noted that operator 1 of this experiment has less experience than Operator 2. However, the good results obtained confirm the relative ease and reliability of the procedure.

2.5.4 CONCLUSIONS

The results obtained with this validity test are extremely positive.

Although for the Italian legislation PCOM is considered a tool for qualitative analysis and for the identification of asbestos, we believe that with the appropriate procedures, as previously described, it can bring excellent results.

Referring to the American ASTM regulation, where the use of the optical microscope is expected, some aspects of this procedure must be underlined. The ASTM D7521 standard method for the determination of asbestos in soil could be synthetized with the following figure (*Figure 29*).
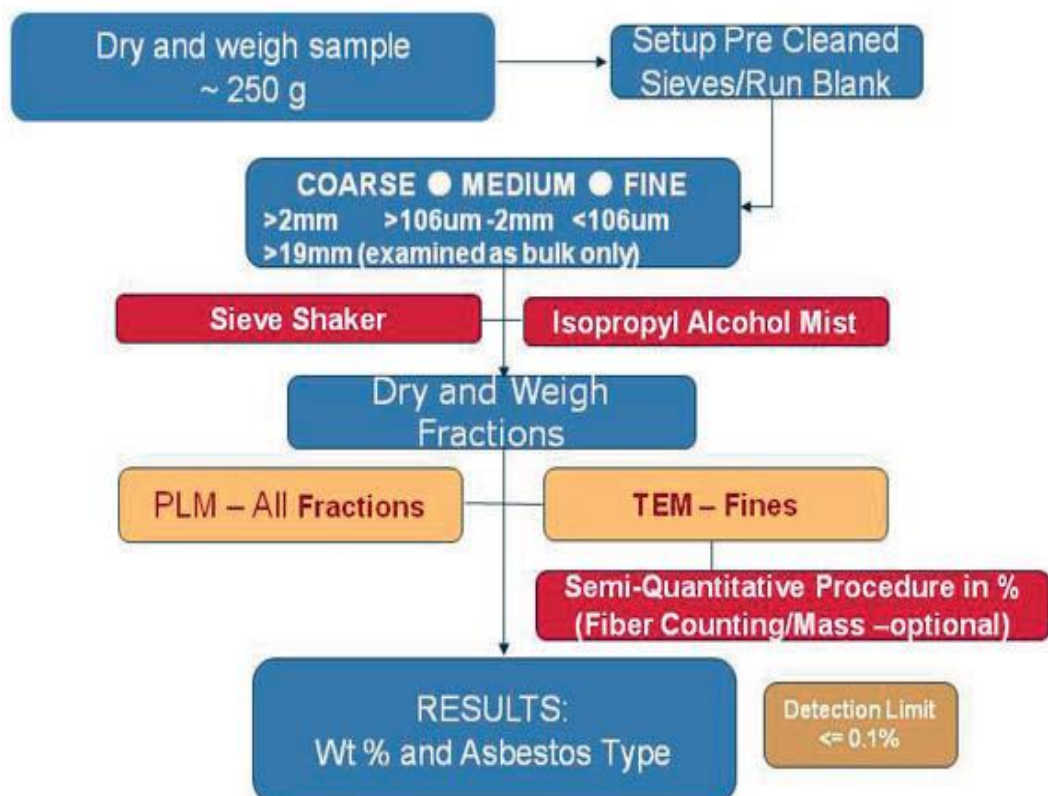
Figure 28: Analysis procedure for D7521 method. From Fitzgerald, S. M., "Chapter 3 Naturally Occurring Asbestos,"

In this procedure ASTM D7521, a sample of soil is collected from the surface or subsurface according to procedures in the appendix. A 250-cm^3 quantity is passed through 19 mm, 2 mm, and 106-µm sieves into three fractions on which analysis by PLM is performed. Pieces of material larger than 19 mm are removed during sample collection or before sieving for separate analysis by PLM and are not considered part of the sample analyzed by this method.

If no asbestos is observed by PLM in the two coarsest fractions (2 mm–19 mm and 106 µm–2 mm) and on the finest fraction (<106 µm), point counting may be used to quantify the amount present. If PLM does not detect any asbestos in any fraction, then TEM is used after gravimetrically reducing the sample.

The method does not grind the soil, and through the sieving process the soil is fractionated into three size ranges where subsequent microscopical analysis and detection of asbestos is enhanced.

With this methodology the analytical results are expressed as weight percent of the original soil sample or as the number of asbestos fibers per gram of soil, but the sensitivity given by this standard is between 0.25% and 0,1%. All the inter laboratory test used for the calibration contained amounts of asbestos as low as 0.1 % chrysotile by weight as well as two asbestos types (2 % chrysotile and 0.1 % crocidolite).

In case of presence of rocks probably another test method is more useful: the CARB METHOD 435 which provides the milling of the sample. In this case samples of serpentine aggregate taken for asbestos identification are first examined for homogeneity and preliminary fiber identification at low magnification. Positive identification of suspect fibers is made by analysis of subsamples through the use of PLM. For quantitative analysis, the procedure grinds the sample using a plate grinder or equivalent until the nominal particle size is less than 3/8 in. The sample is ground further using a Braun mill or equivalent until the majority passes through a 200 Tyler (<75 µm) mesh sieve. Also in this case the samples are analyzed by PLM using a 400-point count to determine asbestos content with a sensibility of 0,25% which is a value more than two times higher than Italian concentration threshold.

Fitzgerald reminds that, while this technique is useful for rock, there are problems if the grinding process is performed for too long. Chrysotile fibers may separate longitudinally to create multiple fibers due to grinding, creating artificially high values. It must be underlined that the production of a higher number of fibers could create an overestimation of the value with the point count method because the dimensions of the fibers are not considered: only their presence in a given field affect the determination of the concentration.

Chapter 3

3. PCOM vs. SEM: COMPARISON AT LOW ASBESTOS CONCENTRATION

3.1 INTRODUCTION

The use of SEM for the determination of asbestos concentration is regulated in Italy D.M. 6/9/1994: *"The method is applicable for asbestos concentrations between 100 ppm (0.01% -100 mg/kg) and 10000 ppm (1% -10000 mg/kg) or higher; the interval in which the method provides quantitative results is between 1000 ppm and 10000 ppm or higher. For asbestos concentrations below 1000 ppm the method provides semi-quantitative results. "*

In the previous sentence it is evident an inconsistency with the defined limits of the law, in fact it is stated that the results obtained for the concentrations lower than the contamination threshold of 0.1% are to be considered only as semi-quantitative and not as quantitative.

In the decree is possible to find some technical information about the method: *"... If the sample to be analyzed is a mass sample, it is obtained, by means of a suitable grinding, its comminution until the granulometric spectrum of the produced particulate is in the range between 10 and 100 μm."*

Moreover, there are some indications about the sensitivity of the procedure: *..."The sensitivity of the method depends on various factors: working conditions of the microscope, area of deposition of the sample on the working filter, number of reading fields fixed on the filter; in any case it is possible to estimate a sensitivity of about 100 ppm when the sample consists of about 0.1 mg of material deposited on an area of about 300 mm^2 (circular surface of about 1 cm of radius) and 400read fields at 2000 x of magnification".*

It is evident that the previous considerations must be "calibrated" on the instrument used for the analysis, considering the real area of the investigated field, the total investigated area and the deposited material.

In the first part of this chapter the methodology to be used for SEM analysis is described, then the results obtained on the sample previously used for the validation of the PCOM method are described.

In the second part, in order to evaluate the effectiveness of both methods in samples containing low concentrations of asbestos (from 0 to 300 mg/kg), double analyzes (PCOM and SEM) on 150 natural samples from were carried out.

3.2 METHODOLOGY

The Scanning Electron Microscope (SEM) used was a FEI operating at 5 and 20 keV. A SEM is a type of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that contain information about the surface topography and composition of the sample. The electron beam is scanned in a raster scan pattern, and the position of the beam is combined with the intensity of the detected signal to produce an image. Usually the SEM is equipped with an EDX probe in order to perform an analytical technique used for the elemental analysis and chemical characterization of a sample called Energy-dispersive X-ray spectroscopy.

In order to examine a sample that is as representative as possible, the SEM analysis procedure used brings some minor changes to that contained in the decree and provides the following steps:

- Obtain, by a suitable grinding, the comminution of the sample until the granulometric spectrum of the produced particulate is in the range between 10 and 100 μm;

- Disperse 10 mg of material (instead of 5 mg provided for by Decree DM 06/09/94) in 200 ml of solution; the use of an anionic surfactant facilitates the correct distribution of the solution on the membrane ensuring a homogeneous dispersion;

- Using an ultrasonic bath, homogenize the solution as much as possible;

- Considering that the effective area of the membranes used is three times the one provided by the DM (about 900 mm^2 instead of 300 mm^2), filter an aliquot of solution at a known concentration containing at least three times the 0.1 mg provided by DM. Specifically, 7.5 ml of solution is taken by means of a calibrated pipette (taking care to withdraw a third of aliquot from the bottom,

equally from half and the final third from the top) containing (theoretically) 0.375 mg of dispersed material. In this way the ratio between the membrane size and the mass of material dispersed in it is maintained, but the possibility that the sample is representative is increased. The content is placed on membranes having an effective diameter of 34 mm (instead of 20 mm);

- Place a portion of the membrane on the stub for SEM analysis

- Coat the stub with Au using a Sputter Coater
- Analyze 4 mm^2 of membrane (the decree indicates at least 1 mm^2).

- Evaluate the mass of the fibers using a density of 2.6 g/cm^3 for the chrysotile and 3.0 g/cm^3 for the amphiboles.

- Calculate the asbestos concentration C in the sample using the following formula:

$$C \text{ [mg/kg]} = \{[A \cdot (pc + pa)]/(n \cdot a \cdot P)\} \cdot 10^{-6}$$

Where:

A = effective area of the membrane [mm^2];
a = area of the observed field [mm^2];
n = number of observed fields;
P = total weight of the sample deposited on the filter [mg]
pc = dc*$\Sigma_j v_i$ = total weight of chrysotile fibers [mg];

Where:
dc = chrysotile's density [gr/cm^3 = mg/ mm^3];
v_i = volume of the i-th chrysotile fiber [mm^3]
pa = da·$\Sigma_j v_i$ = total weight of amphibole fibers [mg];
where:
da = amphibole's density [gr/cm^3 = mg/ mm^3];
v_j = volume of the j-th amphibole fibers [mm^3]

For the analysis of the sample used to validate the PCOM methodology seen in the previous chapter, two types of preparation were provided. The first sample was made starting from the original sample and following the procedure described above; the other, instead, has been realized by the two operators, taking half of the content in grams for the 4 granulometric classes obtained with the PCOM methodology. This sample was subsequently analyzed following the same procedure.

3.3 ANALYSIS

The decree plans to investigate at least 1 mm^2 of membrane, but we choose to analyse 4 mm^2 in order to have a greater representativeness of the data. The calculation of the n of fields is made by knowing the base and the height of the field, which are provided by the microscopist using the SEM; with which it's easy calculate the area of the field and then the number of fields simply by dividing the surface to be investigated for the field area, as shown in table (*Table 15*).

Calculation of the number of fields	
base (b)	0,370 mm
height (h)	0,230 mm
Field area: (b*h)	0,0851 mm^2
Investigated area (S)	4 mm^2
Number of fields : S/(b*h)	47

Table 14: Calculation of the number of fields

Therefore, for both samples, 47 fields were analyzed and for each field the asbestos fibers were searched, their length and diameter measured and the spectrum evaluated. The evaluation of the spectrum, associated with the morphological information and the previous information given by the PCOM analysis, allows to understand with which asbestos fibers you are dealing with.

In order to understand the nature of the different fibers, reference is made to the characteristic spectra of each fiber. These spectra are regulated by (ISO14966: 2002 (E), 2002). The spectrum visible in the following figure (*Figure 30)* represents the spectrum of a chrysotile fiber. Unlike the amphiboles, the chrysotile, as well as the other polymorphs of the serpentine, has a magnesium peak greater than silicon peak.
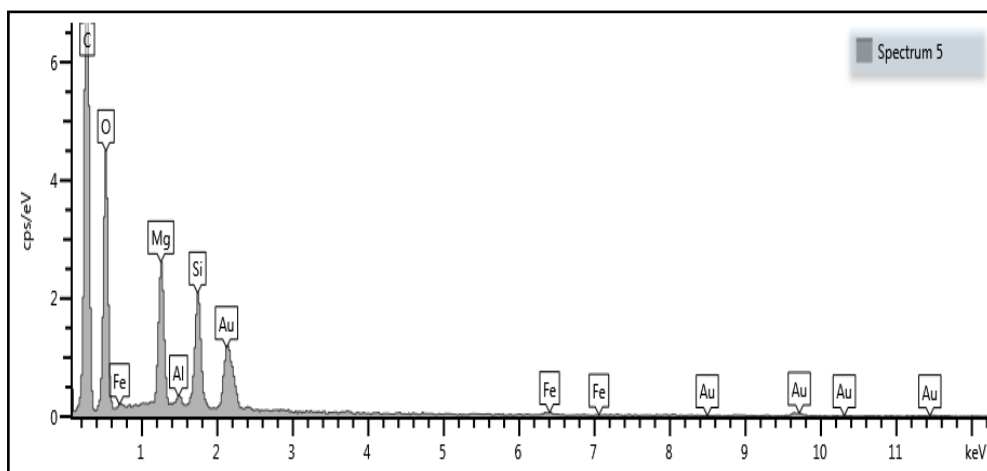
Figure 29: EDXA Chrysotile spectrum

For each analyzed fiber, the SEM returns a detailed image useful for calculating the length and diameter, as shown in *Figure 31* and potential detailed evaluations of the morphology, increasing the magnification (*Figure 32*).
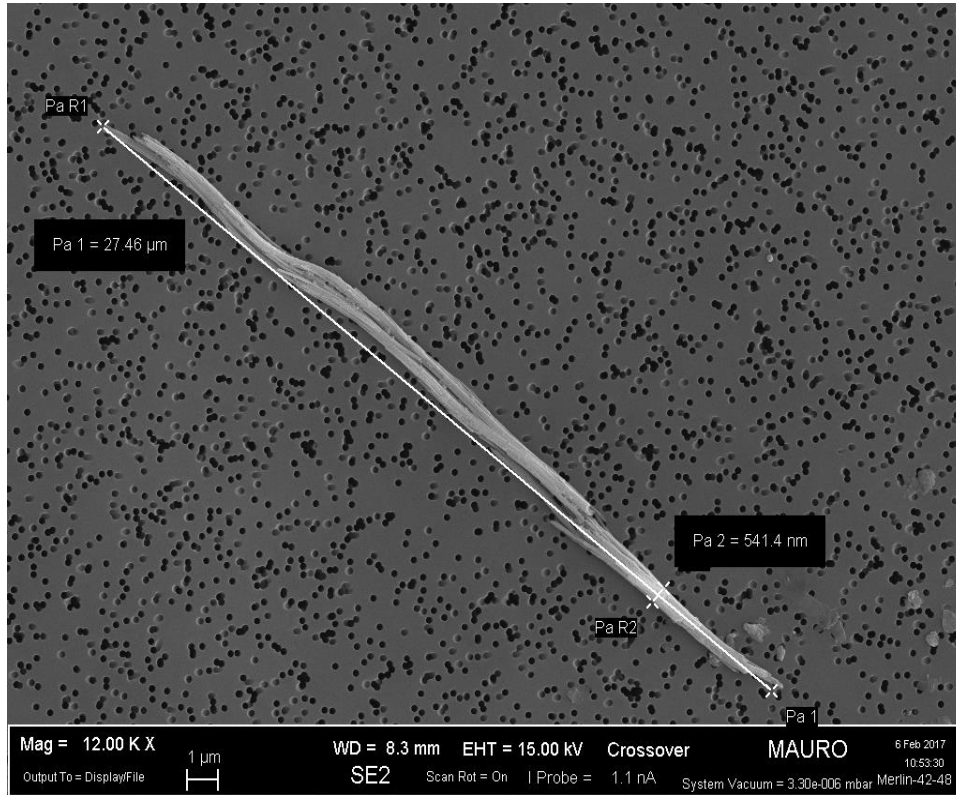
Figure 30: Chrysotile fiber, with the measurement of the length and diameter
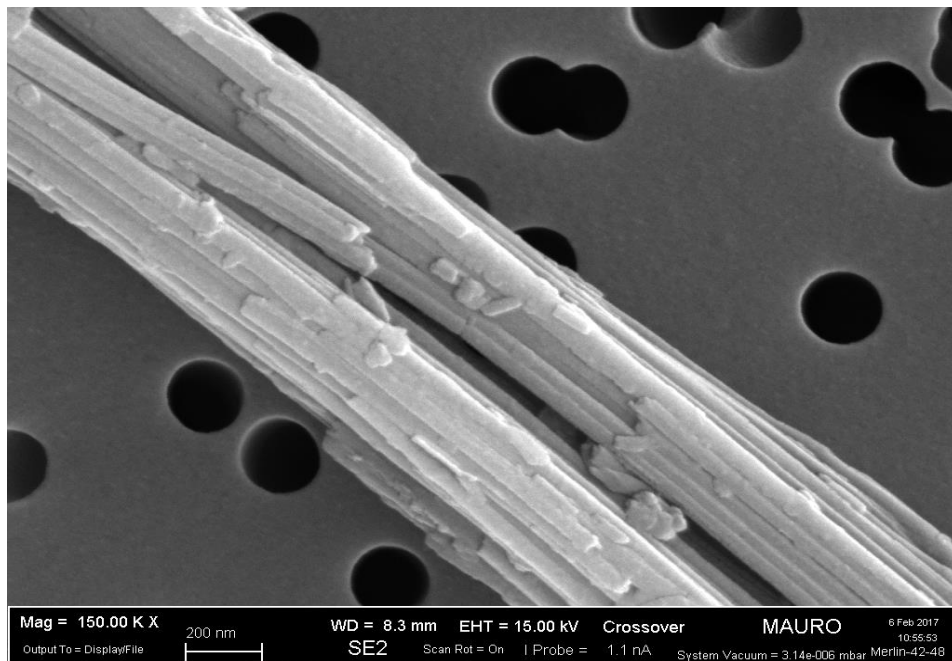
Figure 31: Detail of the chrysotile fiber

To compare the results obtained with the PCOM and the results obtained with the SEM it is necessary to calculate the asbestos content in mg/kg. First, however, it is important to define the variables to be taken into account when calculating the asbestos content, visible in the following table (*Table 16*).

Sample 1 (original sample)	
Dispersant solution	200 ml
Taken solution	7,5 ml
Mass dispersed in the solution	10 mg
Mass on the membrane(m)	0,375 mg
Effective diameter of the membrane (ϕ)	37 mm
Effective area (A): $(\frac{\phi}{2})^2 * \pi$	1074,6 mm^2
Investigated Area (s)	4 mm^2

Table 15: Variables for the calculation of asbestos concentration in Sample 1

To calculate the mass present in 4 mm^2 it is sufficient to make a proportion:

$$s : A = x : m$$

$$4 \; mm^2 : 1074,6 \; mm^2 = x : 0,375 \text{ mg}$$

$$x = m_1 = 0,0014 \; mg$$

Once the mass present in 4 mm^2 has been calculated, the values of length (l) and diameter (ϕ) of the fibers identified during the SEM analysis are inserted in an excel table previously created (*Table 17*). For the calculation of the fiber mass a chrysotile density (ρ) value equal to 2.6 kg/dm^3 is used, with the following formula:

$$M_f = \pi \sum (l_i * \left(\frac{\phi_i}{2}\right)^2) * \rho$$

Sample 1		
Length (l) (μm)	Diameter (φ) (μm)	Fiber mass M_f (mg)
Chrysotile density (ρ) 2,6 (kg/dm³)		
9,028	1,01	1,8806E-08
21,57	2,24	2,21009E-07
13,96	1,4	5,58734E-08
10,07	0,78	1,25107E-08
13,55	0,36	3,58598E-09
12,26	1,07	2,8663E-08
28,33	0,35	7,08673E-09
18,41	1,01	3,83495E-08
8,6	0,83	1,20981E-08
13,66	0,56	8,74762E-09
15,31	0,47	6,90612E-09
9,98	0,92	1,72492E-08
9,6	0,5	4,90088E-09
21,71	0,5	1,10831E-08
14,63	0,6	1,0755E-08
11,17	0,73	1,21552E-08
8,53	0,55	5,26911E-09
12,83	0,54	7,63972E-09
8,02	0,81	1,0745E-08
10,89	0,54	6,48453E-09
9,64	0,6	7,08668E-09
11,58	0,71	1,19203E-08
10,77	0,99	2,15551E-08
16,31	0,73	1,77486E-08
21,7	0,93	3,83256E-08
10,91	0,58	7,49452E-09
22,98	0,35	5,74843E-09
36,33	0,92	6,2792E-08
19,87	1,004	4,09005E-08
	Total mass m_{tot}	7,13489E-07

Table 16: Sample 1 asbestos mass calculation

Once calculated the mass of the fibers present in the first sample analyzed, is possible to calculate the asbestos content in percentage, obtained by dividing the value of the mass of fibers in mg views to the SEM for the value of the mass of

fibers theoretically present in 4 mm^2 equal to $1,4*10^{-3}$ mg, multiplying everything by 100 .

$$M_f : m_1 = x : 100$$

$$7,13 * 10^{-7} mg : 1,4 * 10^{-3}\ mg\ =\ x :\ 100$$

$$x = asbestos\ concentration\ (\%) = 0,0509\%$$

In order to obtain the asbestos content in mg/kg it is enough to multiply for 10^6.

$$asbstos\ content = \frac{7,13 * 10^{-7}}{1,4 * 10^{-3}} * 10^6 = 509\frac{mg}{kg}$$

Summarized in table (*Table 18*):

Σ mass of the fiber M_f(mg)	Fibers mass in 4mm^2 m_1(mg)
7,13E-07	$1,4 * 10^{-3}$
Asbestos concentration%	**Asbestos content mg/kg**
0,0509%	509

Table 17: Asbestos content in mg/kg for Sample 1

The same calculation is carried out on the sample obtained by taking half of the contents of each particle size class.

Also for this second sample the variables are the same, unlike the effective diameter of the membrane and the effective area (*Table 19*).

Sample 2 (created by operators)	
Dispersant solution	200 ml
Taken solution	7,5 ml
Mass dispersed in the solution	10 mg
Mass on the membrane(m)	0,375 mg
Effective diameter of the membrane (ɸ)	34 mm
Effective area (A): $(\frac{ɸ}{2})^2 * \pi$	907,46 mm^2
Investigated Area (s)	4 mm^2

Table 18: Variables for the calculation of asbestos concentration in Sample2

To calculate the mass present in 4 mm² it is enough to carry out, as previously done, a proportion:

$$s : A = x : m$$

$$4 \, mm^2 : 907,46 \, mm^2 = x : 0,375 \text{ mg}$$

$$x = m_2 = 1,6 * 10^{-3} mg$$

Once the mass present in 4 mm² has been calculated, the values of length (l) and diameter (ϕ) of the fibers identified during the SEM analysis are inserted in the excel table (*Table 20*). As previously done, for the calculation of the fiber mass a chrysotile density (ρ) value equal to 2.6 kg/dm3 is used, with the following formula:

$$M_f = \pi \sum \left(l_i * \left(\frac{\phi_i}{2} \right)^2 \right) * \rho$$

Sample 2 created by operators		
Length (l) (μm)	Diameter (ϕ) (μm)	Fiber mass M_f (mg)
Chrysotile density (ρ) 2,6 (kg/dm³)		
16,9	2,36	1,92209E-07
6,15	0,71	6,33075E-09
27,46	0,55	1,69625E-08
14,27	0,4	4,66237E-09
10,17	0,35	2,54402E-09
7,1	0,57	4,71055E-09
7,5	0,1	1,53153E-10
15,68	0,86	2,36813E-08
12,17	1,072	2,8559E-08
10,07	0,87	1,55644E-08
11,49	0,49	5,63346E-09
18,03	1,38	7,0116E-08
16,84	2,06	1,45928E-07
19,82	0,77	2,39965E-08
12,72	0,48	5,98457E-09
	Total mass (m_tot)	5,47036E-07

Table 19: Sample 2 asbestos mass calculation

For the second sample, once the mass of the fibers has been calculated, the asbestos content is calculated in mg/kg. As previously done, the asbestos content % is calculated, dividing the value of the mass of fibers in mg observed at SEM

by the value of the mass of fibers theoretically present in 4 mm² equal to 0.0016 mg, multiplying all by 10^6 to obtain the value in mg/kg.

$$M_f : m_2 = x : 100$$

$$5,47 * 10^{-7} mg : 1,6 * 10^{-3} mg = x : 100$$

$$x = asbestos\ concentration\ (\%) = 0,0342\%$$

$$asbestos\ content = \frac{5,47 * 10^{-7}}{1,6 * 10^{-3}} * 10^6 = 342\ mg/kg$$

Summarized in *Table 20*:

Σ mass of the fiber Mf (mg)	Fibers mass in 4mm² M₁(mg)
5,47E-07	$1,6 * 10^{-3}$
Asbestos concentration %	**Asbestos content mg/kg**
0,0342	342

Table 20: Asbestos content in mg/kg for Sample 2

3.3.1 RESULTS INTERPRETATION

After validation of the PCOM technique obtained in the previous chapter, the natural consequence was that of verifying the results by adopting another technique: that SEM required by current legislation. The results obtained by the two methods of investigation are summarized in *Table 22*

Comparison PCOM vs SEM			
SEM sample 1 (mg/kg)	SEM sample 2 (mg/kg)	PCOM Operator 1 μ_1 (mg/kg)	PCOM Operator 2 μ_2 (mg/kg)
509	342	433	413
SEM average (mg/kg)		PCOM average (mg/kg)	
425		423	

Table 21: Comparison between PCOM and SEM results

As is evident from the table, the data obtained by SEM analysis are of the same order of magnitude as the values obtained at the PCOM and fall within the acceptable range of the measurements. Although only two analyzes have been performed on the sample following the SEM methodology the comparison between the averages makes it possible to state that the values obtained through the use of the two analysis techniques are very close. This data confirms the fact that PCOM is a reliable procedure for the analysis of massive samples.

Scanning electron microscopy presents advantages related to the resolving power and the magnification capacity but certainly disadvantages concerning the representativeness of the sample and the univocal characterization of the identified fibers. Both these aspects will be discussed later. Regarding the representativity of the analyzed sample it is important to underline how in the procedure using the SEM, the quantity of material analyzed is equal to about 0.0014 mg.

This value is four orders of magnitude lower than the amount of material that is placed on the slides for PCOM analysis, which is about 136.4 mg of which about 85 mg are observed.

It should also be noted that the approximations related to the SEM procedure are difficult to assess. The first process involves the dispersion of 10 mg of material, previously ground into special micro powder grinding mill or by a jar in mill with eccentric mass, in 200 ml of distilled solution, which is further homogenized by using an ultrasound bath.

The evaluation of the weight of material deposited on the membrane is based on the assumption that the solution created is perfectly homogeneous, which is not verifiable experimentally in routine operations.

Then, to try to make the sample more representative, three aliquots of the solution from three different heights are taken from the beaker in which the solution is contained: 2.5 ml from the bottom, 2.5 ml from the center and 2.5 ml from above but even in this case it is difficult to verify the goodness of the operations.

Given the small quantities of material analyzed even small variations can create considerable variations on the assessment of the concentration of asbestos.

Certainly, the SEM has the PCOM, the advantage of high resolution, 0.2 μm of PCOM versus 5 nm of SEM, but has the disadvantage of fiber recognition. In fact, while at PCOM the identification of asbestos fibers is based on the unique optical characteristics of each mineral linked to the phenomenon of color dispersion, at SEM the identification of the various types of asbestos becomes much more complex, because all the fibers are visible in gray scales and the only way to recognize them is based on the recognition of their spectrum (not uniquely especially in natural samples) and on their morphology. This problem becomes especially important when it has to be distinguished a chrysotile fiber from an antigorite fiber or generally from one of the serpentine polymorphs not classified as asbestos.

3.4 SAMPLES WITH LOW ASBESTOS CONCENTRATION: COMPARISON BETWEEN PCOM AND SEM

The major criticism that directed to Optical Microscopy is relative to its resolving power, in fact, very thin asbestos fibers may not be identified except with the SEM.

Starting from this consideration, 150 samples have been analyzed by both techniques. The selected samples are samples with low concentrations of asbestos or in which asbestos was not detected with one of the two methods.

3.4.1 ZERO ASBESTOS AND < 120 mg/kg CONCENTRATION

In this subparagraph all samples for which both procedures give results of concentration minor than 120 mg/kg or at least one of the two methodologies gave null result will be taken into consideration.

A part of the analyzed samples will have conforms results with the two methods and both less than 120 mg/kg, which is the limits of determination indicated by the decree in force. Otherwise, for a part of the samples the absence of asbestos will be confirmed by both methods while, for a smaller number of samples, just one of the two techniques will find asbestos' presence.

After the summary tables we will analyze the probable reasons for these discrepancies.

A total 150 samples were analyzed. In 80 samples at least one of the two techniques did not detect asbestos. In 57 cases the presence of fibers was not found by both techniques. In the following figure (*Figure 33*) and table (*Table 23*) are summarized the results of these 150 analysis

ASBESTOS CONTENT

- NOT DETECTED (57)
- < 120 mg/Kg (42)
- NOT DETECTED BY PCOM (6)
- NOT DETECTED BY SEM (17)
- OTHERS (28)
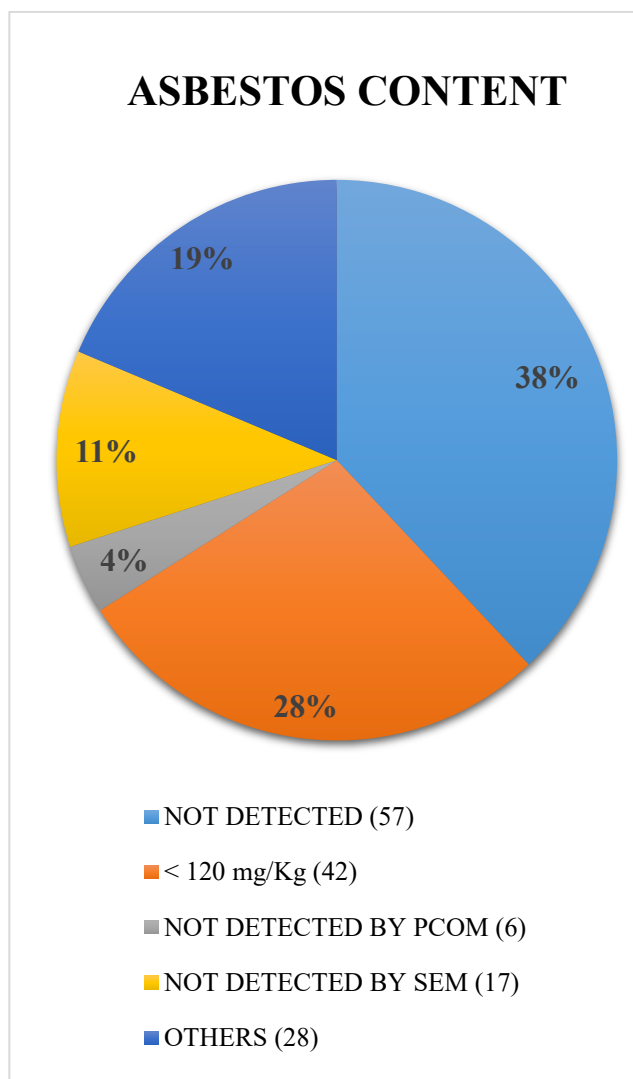
Figure 32: Asbestos content distribution

From the previous graph it is evident how in most cases the absence of asbestos is confirmed by both techniques. However, there is a 14% corresponding to 23 samples out of 80 in which one of the two techniques did n0t detect the presence of asbestos. More precisely in 7 cases the presence of asbestos was not detected by the PCOM procedure; in the remaining 16, the SEM methodology has not identified the presence of asbestos.

The concentration relatives to the sample in which there is a discordance between the two techniques in the identification of asbestos presence are reported in the following tables.

	PCOM (mg/kg)	SEM (mg/kg)
SAMPLE 1	0	1,0
SAMPLE 2	0	3,0
SAMPLE 3	0	9,7
SAMPLE 4	0	11,6
SAMPLE 5	0	27,0
SAMPLE 6	0	41,0
SAMPLE 7	0	117,4
SAMPLE 8	9,5	0
SAMPLE 9	10,0	0
SAMPLE 10	12,3	0
SAMPLE 11	14,1	0
SAMPLE 12	19,2	0
SAMPLE 13	26,3	0
SAMPLE 14	27,0	0
SAMPLE 15	32,3	0
SAMPLE 16	35,3	0
SAMPLE 17	35,6	0
SAMPLE 18	38,2	0
SAMPLE 19	40,4	0
SAMPLE 20	75,5	0
SAMPLE 21	77,6	0
SAMPLE 22	91,8	0
SAMPLE 23	99,8	0

Table 22: Discordant samples with concentration minor than 120 mg/kg

The table above lists all the asbestos contents found by a methodology when the outcome of the other was of absence of asbestos. It should be emphasized that no value exceeds 120 mg / kg, a value that the decree in force refers to as a

quantification limit. Therefore it is permissible to give more than one possible explanation to these data. Firstly it is assumed that there have been no contamination of the samples, certainly it is not to be sure of this fact since, despite cleaning the hood between one analysis and another, some fibers could have escaped the cleaning procedure and then deposited on membranes or slides.

However, the most plausible explanations are the following. In case the PCOM methodology (7 cases) did not find traces of asbestos, the most probable explanation is that the detected fibers were extremely thin and therefore difficult to detect with an optical microscope.

If the SEM methodology has not detected the presence of asbestos, reference is made to the already mentioned representativeness of the analyzed sample. In fact, at low concentrations of asbestos, observing a reduced sample aliquot, as in electron microscopy procedure, might not be sufficient to detect the presence of fibers given the difficult of a perfect homogenization of the sample.

In 42 cases out of the total both PCOM and SEM have encounted the presence of asbestos fiber with a concentration minor than 120 mg/kg.

	SEM	SEM
SAMPLE 1	14,51	13,7
SAMPLE 2	55,4	13,7
SAMPLE 3	3,6	14,6
SAMPLE 4	16,6	14,6
SAMPLE 5	49,4	15,3
SAMPLE 6	16,17	16,41
SAMPLE 7	107,68	16,41
SAMPLE 8	0,44	18,4
SAMPLE 9	0,78	18,4
SAMPLE 10	14,51	21,2
SAMPLE 11	55,4	21,2
SAMPLE 12	40	23,9
SAMPLE 13	26,58	24,39
SAMPLE 14	38	25,5
SAMPLE 15	57,31	27,5
SAMPLE 16	2,3	27,7

SAMPLE 17	35,4	27,7
SAMPLE 18	68,04	34,8
SAMPLE 19	111,5	36,1
SAMPLE 20	72,1	36,4
SAMPLE 21	111,8	36,4
SAMPLE 22	25,36	38,1
SAMPLE 23	20,7	40,1
SAMPLE 24	20,7	40,1
SAMPLE 25	35,02	41,03
SAMPLE 26	4,3	42,5
SAMPLE 27	111,8	47,5
SAMPLE 28	20,21	49,9
SAMPLE 29	102,1	56,3
SAMPLE 30	102,1	56,3
SAMPLE 31	94,38	61,3
SAMPLE 32	118,3	61,3
SAMPLE 33	359	64
SAMPLE 34	4,22	70
SAMPLE 35	94,38	70,4
SAMPLE 36	118,3	70,4
SAMPLE 37	38	71
SAMPLE 38	33	77,19
SAMPLE 39	23	77,5
SAMPLE 40	2,3	80,7
SAMPLE 41	35,4	80,7
SAMPLE 42	101,6	88,3

Table 23: Low concentration samples

In the previous 42 samples low concentration of asbestos were detected by PCOM and SEM.

This data combined with the previous ones is of fundamental importance. In fact, it shows that in natural samples, where the fibers are generally of various sizes and often also present in bundles, the optical microscopy also proves effective in recognition and quantification. The standardized SEM methodology indicates the analysis carried out between the values 100 and 1000 mg / kg as semi-quantitative. However, the techniques at our disposal allow to obtain quantitative results, which can be considered semi-quantitative for values less than 120 mg / kg.

It should be emphasized that in environmental monitoring activities it is of fundamental importance to: identify the presence even at low concentrations in order to guarantee the correct treatment of potentially dangerous materials and provide a sufficiently reliable concentration evaluation in values lower than the legal limit of 0,1%. For both techniques it is obviously easy to quantify high asbestos contents even if the relative error could be greater. Strongly contaminated samples are often identified by preliminary macroscopic analysis by an experienced operator.

3.4.2 OTHER SAMPLES

After the discussion about the first 122 samples the remaining 28 must be analyzed and discussed. The results obtained will be discussed and shown in the following *Figure 34 and Table 25.*

	PCOM (mg/kg)	SEM (mg/kg)
SAMPLE 1	24,01	380
SAMPLE 2	57	180
SAMPLE 3	60,3	220
SAMPLE 4	76,21	220
SAMPLE 5	81,42	236,4
SAMPLE 6	83,4	150
SAMPLE 7	130,4	28,1
SAMPLE 8	137,4	426,1
SAMPLE 9	150,6	421,8
SAMPLE 10	154,34	77,5
SAMPLE 11	170,2	398,7
SAMPLE 12	209,9	13,6
SAMPLE 13	210,4	551,7

SAMPLE 14	226,8	587,5
SAMPLE 15	237	550
SAMPLE 16	244	120
SAMPLE 17	266	159,5
SAMPLE 18	270,1	80
SAMPLE 19	278,3	600,3
SAMPLE 20	312,2	750,2
SAMPLE 21	317,27	118,06
SAMPLE 22	317,27	362,5
SAMPLE 23	335	100
SAMPLE 24	337,6	430
SAMPLE 25	359	64
SAMPLE 26	378,67	120
SAMPLE 27	444,7	180
SAMPLE 28	451,2	567,2

Table 24: Other samples
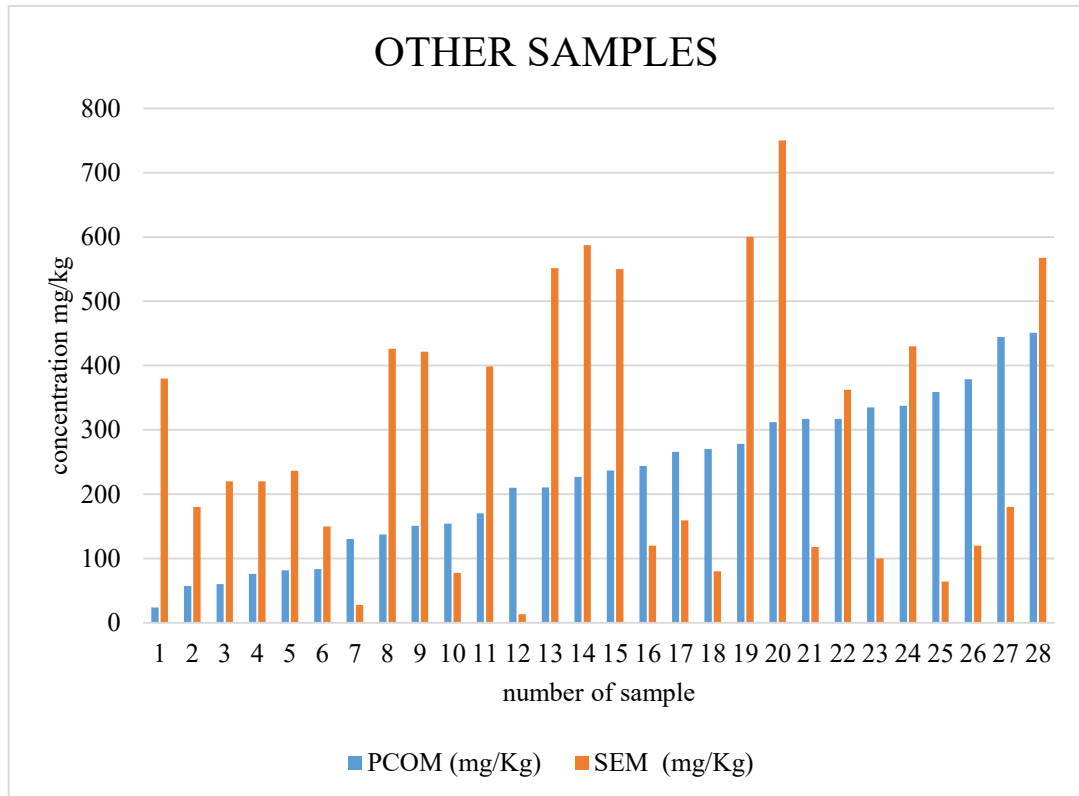
Figure 33: Other sample distribution

In the preceding table are listed all the samples in which both methodologies have found the presence of asbestos and at least one with a value exceeding 120 mg/kg. several cases can be observed:

- Values agreed or with a non-significant difference (Sample 6, 7, 10, 16, 17, 22, 24, and 28). In this cases the value obtained are similar or near to the value of 120 mg/kg

- Samples in which the value obtained with the PCOM are higher than the value obtained with the SEM (Sample 12, 18, 21, 23, 25, 26 and 27)

- Samples in which the value obtained with the SEM are higher than the value obtained with the PCOM (Sample 1, 2, 3, 4, 5, 8, 9, 11, 13, 14 15, 19 and 20)

3.4.2.1 PCOM VALUES HIGHER THAN SEM

As widely discussed above, the representativeness of the sample analyzed by the PCOM methodology is greater than the ones analyzed by SEM. In the event that the values obtained are higher it is legitimate to maintain that the data is more reliable. In fact, the major criticisms are in the least resolutive power that involves, if decisive, a reduction in the level of asbestos. It should be stressed once again how, however, it continues to be assumed that the homogenization of the sample is perfect, something which, however, it is impossible to have a feedback.

3.4.2.2 SEM VALUES HIGHER THAN PCOM, OUT OF SCALE OBJECTS AND ANTIGORITE CHARACTERIZATION

In the case of SEM value higher than PCOM is possible to find three different causes. The first one is once again relative to the resolution power, but in our experience is more determinant in the case of fibers' low quantities detection.

The SEM analysis present two big issues. The first one is the presence during the analysis of out-of-scale objects. The milling process has the function of grinding and homogenizing the whole sample. Asbestos fibers, however, due to their tendency to aggregate, to be often nested in bundles and thanks to the properties of tensile and flexural strength do not always undergo the desired commutation.

In the context of such a selective microscopic analysis, the possible presence of fiber bundles, therefore with a considerably greater volume of the fibers themselves, can provide very high concentration results. This problem during the analysis in optical microscopy is much less important having regard to the previous particle size distribution.

In fact the presence of just one bundle of fibers with dimension of 50μm*5 μm leads a presence of asbestos of more than 450 mg/kg. The experience of the operator and the consciousness of all the procedure are extremely important in order to avoid important errors. In the following figures are reported some examples of out of scale objects. A solution for this problem could be to carry out a further analysis at a lower magnification (for example 400 x). In this way the thinner fibers are neglected but with a similar number of investigated fields an extremely wider portion of membrane is analyzed. The contribution in determining the concentration of asbestos given by any objects out of scale can therefore be calculated by weighing their contribution over a larger area. Moreover with a further analysis at lower magnification, and therefore on a larger area, others out of scale objects, if presents, could be detected in order to evaluate if are isolated cases (contamination) or representative of the sample. Generally, it can be stated that the magnification level to be used during the analysis must be appropriate to the size of the particles investigated. The training of the operators is therefore of great importance and, at the same time, it is not easy to establish univocal rules for an analysis of this type.
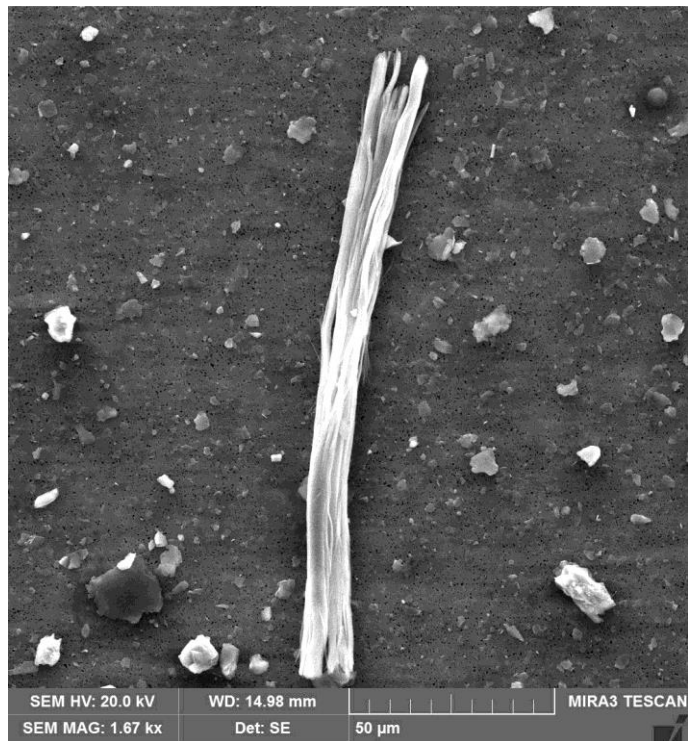
Figure 34: Chrysotile bundle

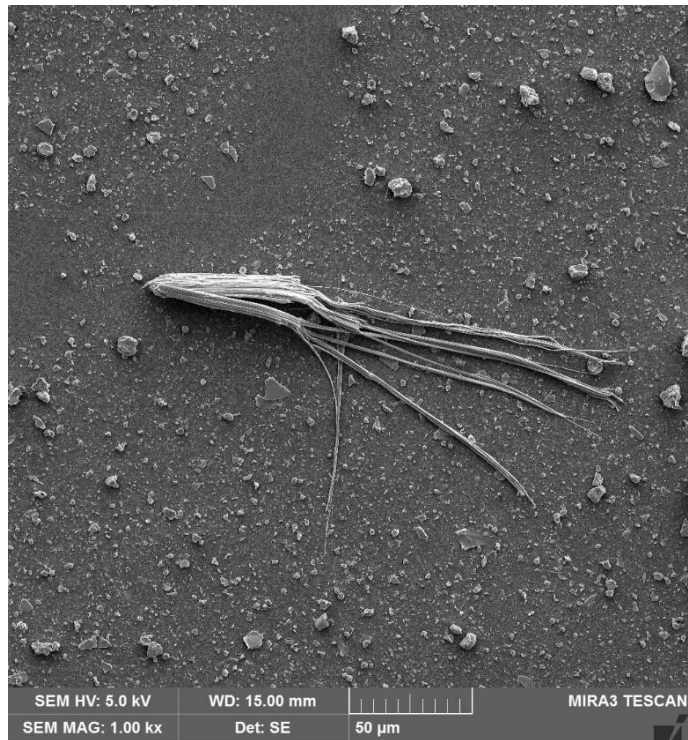*Figure 35: Chrysotile bundle (2)*

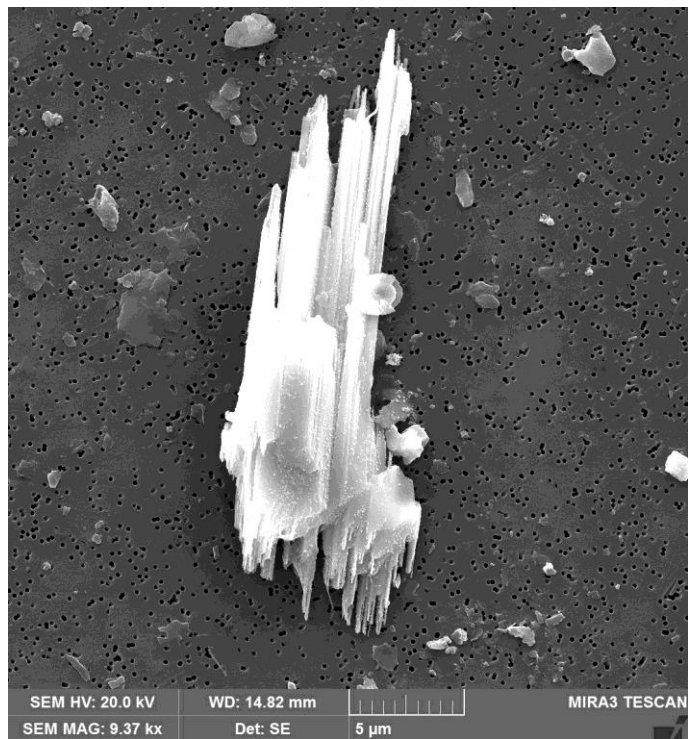*Figure 36: Chrysotile bundle (3)*

Figure 37: Tremolite bundle

The question is: how to face with this objects? There are different possibilities but, certainly, one of the most important thing is the experience of the operator who could easily understand if the concentration value obtained is too high thanks to the previous macroscopic observation. Moreover from the analytical point of view one opportunity is to observe the sample at low magnification in order to verify if the presence of a big bundle of fiber is an isolated case or if on the portion of membrane under analysis there are other big bundles. If the presence is isolated the best thing is to prepare again the sample and verify the outcome of the new analysis. Another possibility is to eliminate from the calculation an out-of-scale object that is not considered representative of the sample. In this case, however, even serious mistakes can be made.

The last chapter will analyze the different types of natural samples that can arrive at an analysis laboratory. Often the samples come from sites of infrastructural works and will underline the importance of the data coming from the monitoring of the airborne fibers in the workplace. In fact, although the asbestos concentrations in the excavated material and the concentration of fibers in the air are not correlated, the presence in the air is the first and most immediate alarm bell of an eventual presence in the rock.

Another important issue that have to be discussed is the ability, using SEM, to differentiate chrysotile from other polymorphs of serpentine. The most difficult case is the recognition of fibrous Antigorite. Antigorite spectrum is extremely similar to that of the chrysotile and the only way, using SEM, to discriminate between the two minerals is the observation of the morphology. The aspect of Antigorite should be more rigid, acicular and less supple compared with chrysotile but, in nature, when the fibers are found in bundle this distinction could be almost impossible. Using the optical properties and the PCOM instead it is easier to discriminate thanks to the different refractive index oil in which the two minerals show their typical color. As previously explained the refractive index oil useful for chrysotile recognition is 1.550, while for Antigorite is commonly used from 1,560 to 1,570 (Petriglieri et al. 2019). In the following figures are presented some different examples.
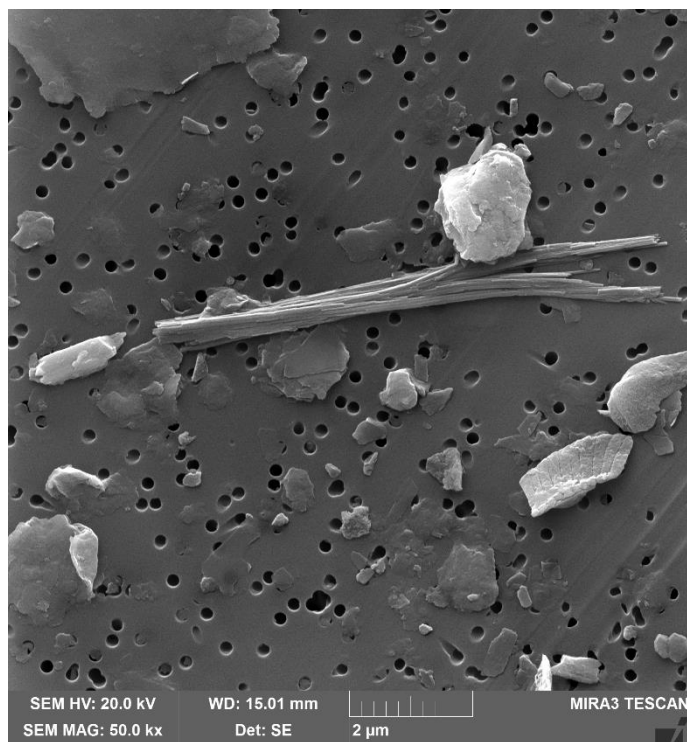
Figure 38: Antigorite

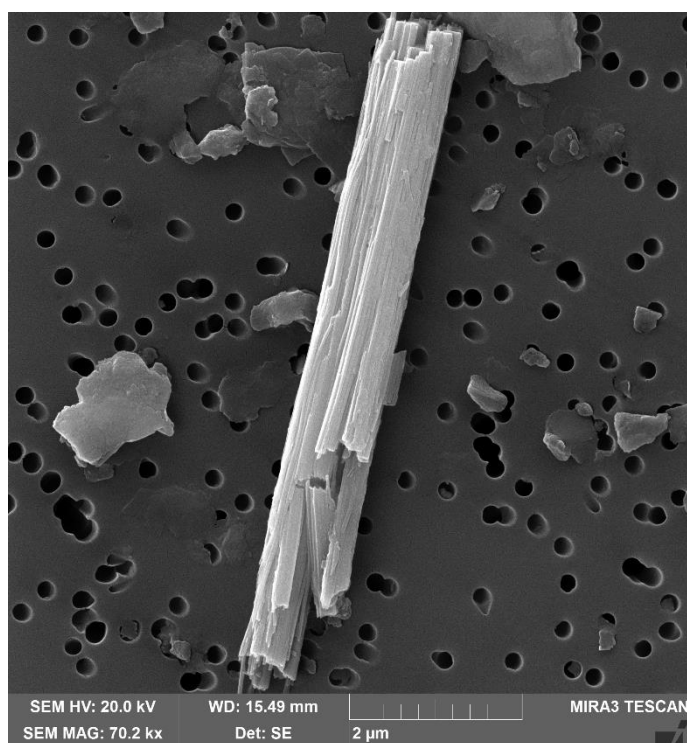*Figure 39: Antigorite morphology*

Figure 40: Antigorite

Figure 41: Chrysotile
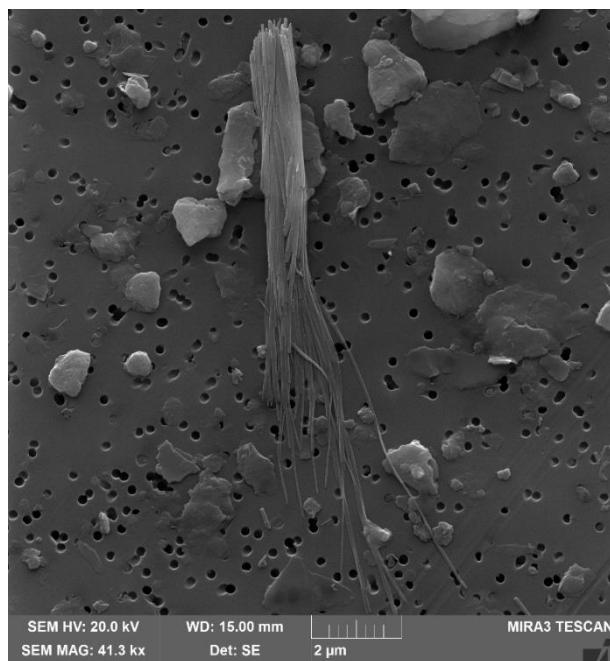
Figure 42: Chrysotile
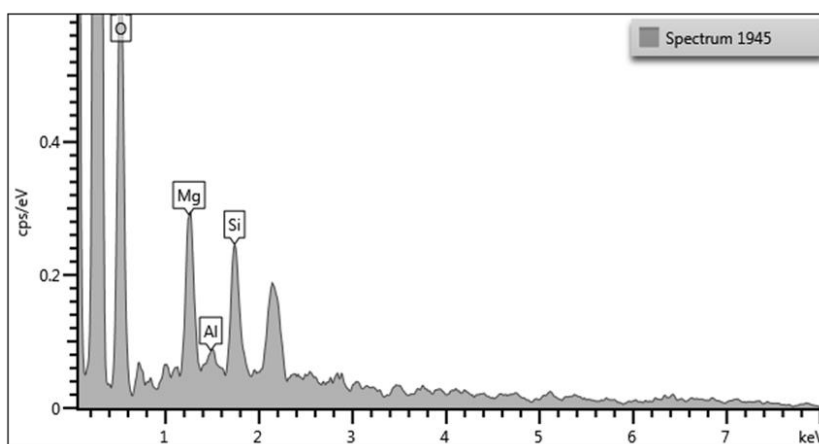
Figure 43: Antigorite EDX spectrum
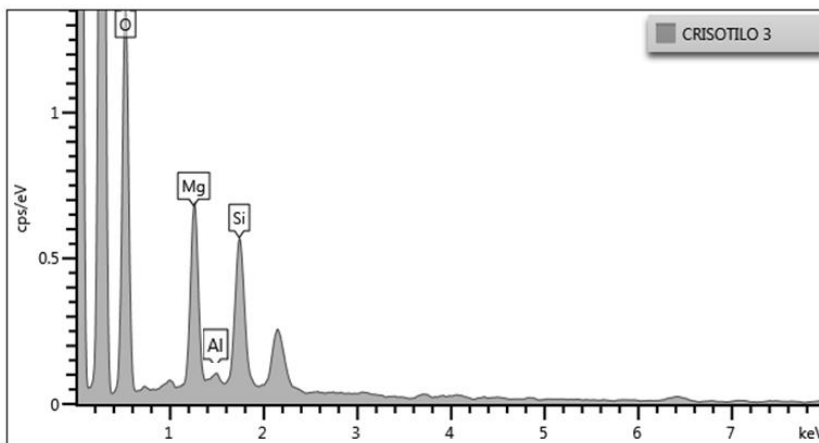
Figure 44: Chrysotile EDX spectrum
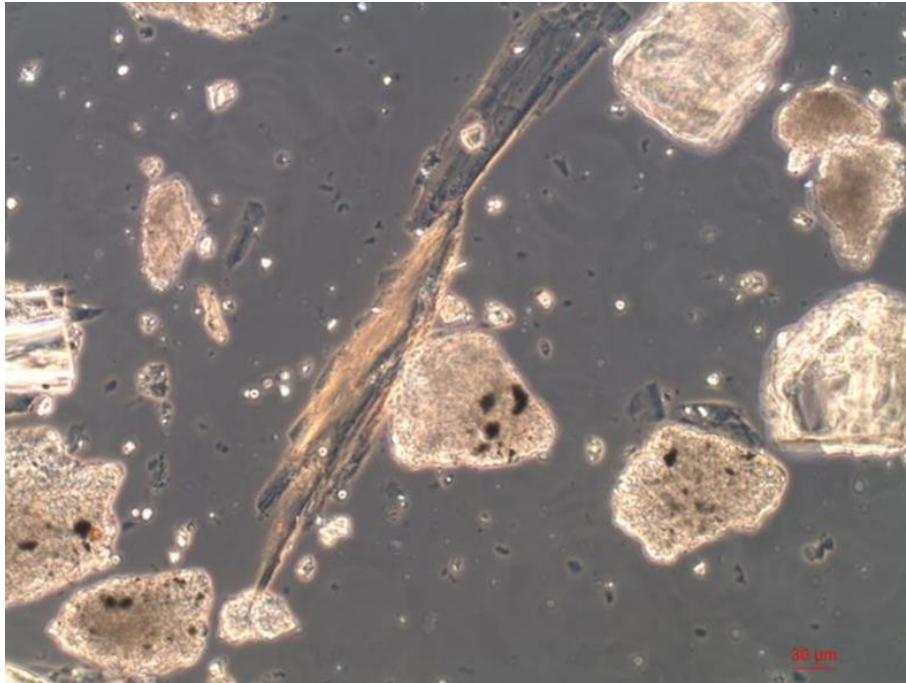
Figure 45: Antigorite, refractive index oil 1.550

Figure 46: Antigorite refractive index oil 1.560
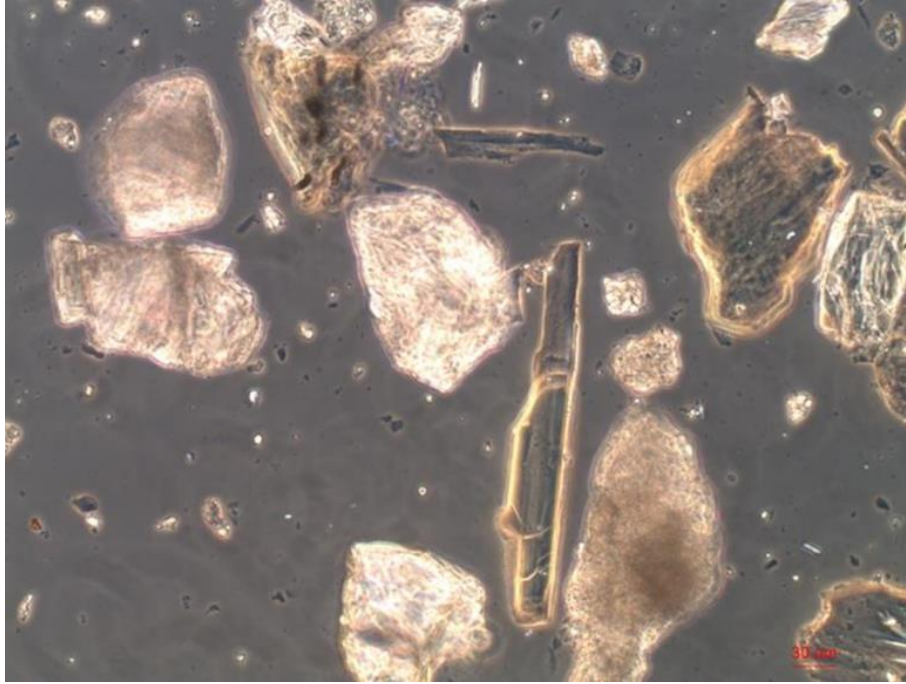
Figure 47: Antigorite, refractive index oil 1.550
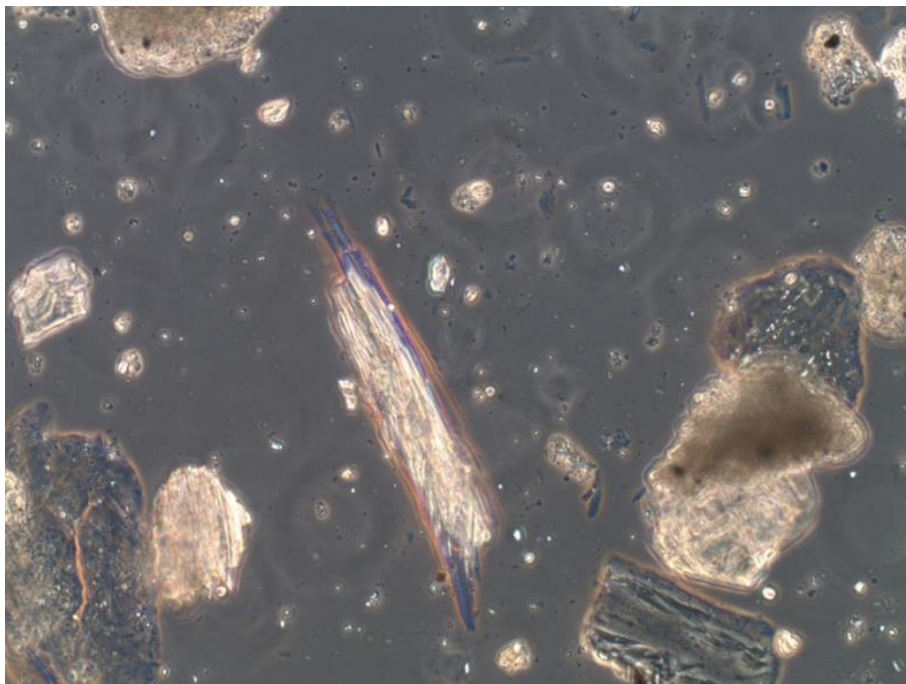
Figure 48: Antigorite refractive index oil 1.560

From the previous images it is clear how PCOM guarantees greater security in the unambiguous identification of the type of mineral observed. Although the study of morphology through the use of SEM is more accurate, the habit of fibrous minerals present in rock samples may be not easy to interpret. In fact the typical suppleness of chrysotile is characteristic of the material widely used in the creation of artifacts but coming from extraction sites in which the abundance and purity of the material was enormous. It is not uncommon to find the mineral in much smaller quantities and with less typical crystalline habits; in this case the choice of the correct refractive index oil provides unique information useful for the correct identification of the mineral.

In the figure 39, 40 and 41 are presented examples of antigorite. As already explained, it is impossible the univocal determination of antigorite using SEM. The sample, however, was previously observed using PCOM and the presence of antigorite was confirmed by using the 1.560 refractive index oil. It is possible to assume that the mineral observed with SEM is the same.

Chapter 4

4. EFFECTS OF GRINDING ON TREMOLITE SAMPLES

Part of the work described in this chapter has been previously published in "fibers" journal with the name: "GRINDING TEST ON TREMOLITE WITH FIBROUS AND PRISMATIC HABIT" by Oliviero Baietto, Mariangela Diano, Giovanna Zanetti and Paola Marini

4.1 INTRODUCTION

Even if the most worldwide extracted asbestos was chrysotile, Tremolite asbestos in the past have been mined in many parts of the world (Medici et al.1972; Geyer et al 1976; Bloise et al. 2014; Van Gosen et al. 2004; Bernstein et al. 1980).

The main objective of this chapter is the evaluation of the morphology change in tremolite particles before and after a grinding process. The crushing action, in addition to being part of the analytic process, simulates anthropic alteration of the rock, such as excavation in rocks containing tremolite during a tunnelling operation. The crystallization habit of these amphibolic minerals can exert hazardous effects on humans. The investigated amphibolic minerals are four tremolite samples, from the Piedmont and Aosta Valley regions, with different crystallization habits. The habits can be described as asbestiform (fibrous) for longer and thinner fibers and non-asbestiform (prismatic) for prismatic fragments, also known as 'cleavage' fragments. In order to identify the morphological variation before and after the grinding, both a phase contrast optical microscope and a scanning electron microscope have been used. The identification procedure for fibrous and prismatic elements is related to a dimensional parameter (length-diameter ratio) defined by the Health and Safety Executive. The results highlight how mineral comminution leads to a rise of prismatic fragments and, therefore, to a potentially safer situation for worker and inhabitants.

Tremolite is a hydrated calcium magnesium silicate ($Ca_2Mg_5Si_8O_{22}(OH)_2$), belonging to the tremolite-ferro-actinolite series (Deer W.A et al. 1966 , Gamble J.F et al. 2008), with an amphibolic structure characterized by long and parallel double chains of silica tetrahedral (SiO_4) with a strip of cations located between the double chains (Roggli V.L et.al 2002; Addison J et al. 2008; Ilgren E.B et al. 2014; Ross M et al. 2008)

Tremolite and actinolite can crystallize with two different crystalline habits and it is common to find them in fibrous habit, known as asbestiform, or in prismatic habit, non-asbestiform. This distinction can be described as (National Research Council 1984):

- 'Asbestiform habit' associated with a crystalline structure characterized by thin crystals similar to the morphology of organic fibres (hair; the resemblance is not in the width, 10-6 m for asbestos fibers vs 10-5 m for hair) or as a crystalline aggregation consisting of parallel fibers (bundles with indented extremities). The fibers are thin, long and similar to needle-shaped elements with a unidirectional growth (Dana S.D., et al. 1934).
- 'Non-asbestiform' refers to a structure characterized by tiny or "elongate prisms with a lozenge-shaped cross-section" (Addison J et al. 2008). The crystalline growth is not unidirectional.

A crushing event acting on tremolite crystals could have different effects on crystalline habits, as illustrated by Illgren et al. (2014). In fact, amphiboles with asbestiform habits have a significant propensity to be longitudinally split. The longitudinal separation produces 'fibrils' that are thinner and thinner without any perpendicular breakup to the elongation; therefore, fibers maintain their flexibility and tensile strength (Addison J et al. 2008, Illgren E.B et al. 2014, Dorling M. et al. 1987). Non-asbestiform habits, however, consist of amphibolic minerals with internal cleavage. This term relates to the fact that breakage occurs along preferential planes, especially planes of relative weakness, and mainly perpendicular to the length (Ilgren E.B et al. 2014, National Research Council 1984). The prismatic elements have, therefore, a tendency to fracture along these preferential planes, producing stocky prisms or acicular fragments with a reduction in their flexibility.

The particle morphology, size, physical-chemical properties and bio persistence correlated to the crystalline habits can involve relevant consequences to the human respiratory system. The Italian Minister of Health highlighted the difficult removal process for asbestiform amphiboles, caused by the length and persistence of the fiber. These fibers are more strong and flexible than cleavage fragments, so they tend to bend without breaking and also have negative repercussions on the defense mechanism operated by macrophages. Otherwise, Illgren et al. (2014) specified that non-asbestiform (or prismatic) amphiboles have weaknesses and fragile behaviour. Therefore, the prismatic component can be reduced into fragments that can progressively be cleared from the body by the macrophages phagocytosis.

Asbestos is included in Group 1 referring to "substances carcinogenic to humans" by the International Agency for Research on Cancer (IARC). It is important that the concept of regulated asbestos fibers, which correspond to fibers

defined as 'respirable' by the World Health Organization (WHO) (fibers having length > 5 μm, diameter ≤ 3 μm and length/diameter (aspect) ratio ≥ 3:1) (IARC 1973; Gualtieri A.F. 2018; W.H.O. 1986). For this reason, many trials were focused on the evaluation of the carcinogenic effect, such as the potential for mesothelioma induction, for a non-asbestiform tremolite by means of in vivo (animals or human) and in vitro tests. These studies, seen in *Table 25*, demonstrate a negative or lower rate of carcinogenicity for non-asbestiform tremolite compared to asbestiform one.

Test subjects	Procedure		Authors
Workers	Exposure to non-asbestiform tremolite		Gamble et al. (2008)
In vitro	Test by a variety of cellular endpoints		Timbrell et al. (1971) Wylie & Mossman (1997) Wagner et al. (1982)
In vivo (such as rat)	Experiment:	Inhalation	Davis et al. (1985) Lescano et. al. (2015)
		Intraperitoneal or intrapleural injection	Wagner et al. (1960) Smith et al(1979) Davis et al. (1991)
		Intrapleural implant	Stanton et al. (1981)

Table 25: Studies on exposure to non-asbestiform tremolite

This study is aimed to analyse the morphological changes of both the fibrous and the prismatic states in order to simulate the external process of rock deterioration. The grinding effect strictly depends, both on the characteristics of asbestos mineral (rocks cohesion and friability) and the rocks interaction with external 'phenomena.

In the last year the effect of grinding on tremolite asbestos was well investigated by Bloise et al (2018). In this chapter the study is focused not only on asbestiform samples but on prismatic tremolite too. This decision arises from the need to verify if the tremolite with prismatic habit, if subjected to a grinding process, can produce potentially harmful fibers.

The four samples of tremolite have been collected in four sites from the north of Italy, three in the Piedmont region (Bracchiello, Monastero di Lanzo and Caprie)

and the last in Aosta Valley (Verrayes). These tremolites showed different initial habits: prismatic for Bracchiello, Caprie and Verrayes; fibrous for Monastero. For each sample, the particle morphology was investigated before and after grinding, with both a phase contrast optical microscope (PCOM) and a scanning electron microscope (SEM). A counting strategy to differentiate the asbestiform fibers from the non-asbestiform 'particles' was chosen. This approach is focused on a dimensionless parameter, mainly the ratio between length and diameter (L/D), acquired during the PCOM observation, as according to the asbestiform definition of the Health and Safety Executive where an L/D > 20 identified a particle as fibrous (H.S.E., 2005).

The results showed increases in prismatic elements for all tremolite samples after grinding. Regarding the carcinogenetic studies previously proposed, the increase of prismatic particles could lead to a safer situation in term of less impact both on the health of exposed humans (workers, inhabitants, and health professionals) and on the economy and organization for companies mining operating in deposits where a non-asbestos tremolite is present (Gamble et al. 2008).

4.2 MATERIAL AND METHODS

4.2.1 MATERIAL

The four samples of tremolite analysed were from Piedmont and Aosta Valley. More precisely, three of four came from Piedmont: Bracchiello (TO), Monastero di Lanzo (TO) and Caprie (TO). The other one, from Aosta Valley, is Verrayes (AO). The samples were collected by Prof. C. Clerici and are part of the mineralogic museum of DIATI.

The sample characteristics are shown in *Table 26*

Sample nomenclature	Characteristic	Aspect
Bracchiello Bracchiello (TO)	Particles have variable length in the 1–10 mm range and present a rectilinear and rigid aspect (not flexible). They also appear translucent and not aggregate.	
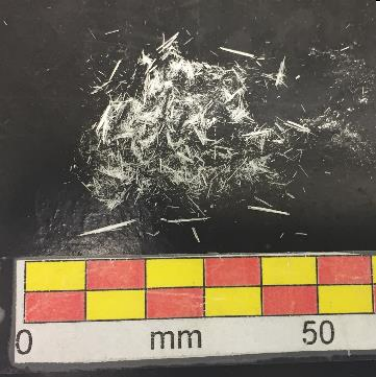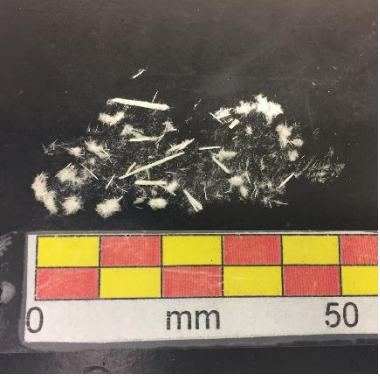
Monastero Monastero di Lanzo (TO)	Particles appear aggregated in bundles. This is the only sample that presents a fibrous aspect. Thus, particles have an apparent flexibility and the bundles have frayed extremities.	
Caprie Caprie (TO)	Particles have variable length in the 1–10 mm range. These are prismatic with a rigid and scattered aspect. There is a clear, high quantity of fine components.	
Verrayes Verrayes (AO)	Particles have extreme variability in length and thickness. Prismatic fragments and fine components are noticeable; only the latter are scattered or aggregated in a small mass. There is a clear, high quantity of fine components.	

Table 26: Aspect and characteristics of the samples

4.2.2 METHODOLOGY

A schematic description of the analysis methodology is reported in *Figure 49*. For each original sample, two microscopic analysis were carried out: the first on the original (natural) samples and the second on the ground samples. The main action of this analysis is to submit both original and ground samples to counting

and measuring using the PCOM. SEM was used for a visual comparison to PCOM images.

All the steps will be illustrated in the following subsection.
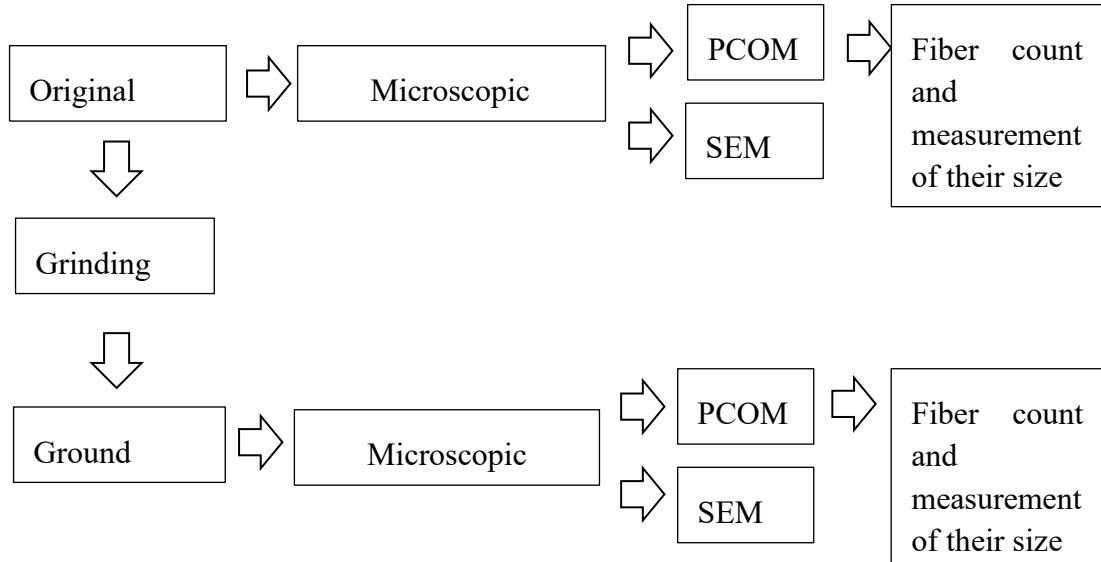
Figure 49: Analysis procedure flowsheet

4.2.3 SAMPLES PREPARATIONS FOR MICROSCOPIC OBSERVATION

The first analysis step consists of the preparation of samples for observation with PCOM and SEM. This action was carried out both for the natural samples and the ground samples.

For the PCOM observation, a portion of the sample was placed on a microscopic slide and immersed in a refractive index oil. To recognize a tremolite asbestos, a liquid with a refraction index equal to 1.615 is suggested (Bozzola J.J et al., 1992). In this study, a liquid with a refraction index of 1.600 has been used. This value, slightly lower than the refraction index of the investigated material, allows a maximum contrast between tremolite crystals and the background, reducing the chromatic effect and the halo surrounding the particles. This optical condition is useful for observing the particles with image software programs.

The sample weight must only be considered indicative of a good PCOM observation and particle measurement. In
Table 27, the quantity, in milligrams, of material located on the slide is shown.

Sample nomenclature	Natural sample (mg)	Ground sample (mg)
Bracchiello	0.6	0.3
Monastero	0.2	0.3
Caprie	0.3	0.3
Verrayes	0.3	0.3

Table 27: Amount of material on the microscopic slide for each sample

For SEM observation, the sample must be attached to a stub; each sample was prepared by mixing 10 mg of material in 200 ml of deionised water. Subsequently, 7.5 ml of this mixture was filtered on a polycarbonate membrane (0.4 µm porosity) and fixed on a metallic stub. After that, the specimen must be coated with a thin layer of gold, essentially to abate the increase of high-voltage charges on the specimen and dangerous heat (D.M.6/9/1994).

4.2.4 GRINDING

The tremolite behaviour after a grinding process was studied by subjecting an amount of original sample, approximately of 70 mg, to grinding for 1 minute in a jar with agate closed with a sealing gasket lid (model number 952/2 from Humboldt-Wedag).

All the steps of the analysis procedure, such as the selection of the sample, the extraction of the grinding material out of the jar and the preparation of the sample for observation, may lead to a potential exposure to tremolite fibres. Therefore, all steps were carried out using adequate protection devices and under a fume hood (Black Activa Plus from Aquaria).

4.2.5 DECISION-MAKING PROCESSES FOR COUNTING AND MEASUREMENT OF PARTICLES

The counting and measurement of each particle were carried out on PCOM images. Eight sample slides were analysed, four belonging to the original samples and the other four to the grinding samples. For each slide, the number of fields must be selected, as shown in

Table 28, which depends on the object dimensions.

The number of fields investigated differs considerably among the original samples, mainly for the different dimension of particles. A 10x objective has been used for original samples, while a 40x objective for the ground samples. Length and width of particles were measured for each field. The decision-making process is illustrated in *Figure 50*.

Sample nomenclature	Original sample		Ground sample	
	Number of fields examined	Number of particles counted	Number of fields examined	Number of particles counted
Bracchiello	100	240	36	331
Monastero	10	2929	25	358
Caprie	100	669	25	288
Verrayes	25	562	25	458

Table 28: Number of fields examined and particles counted for each sample
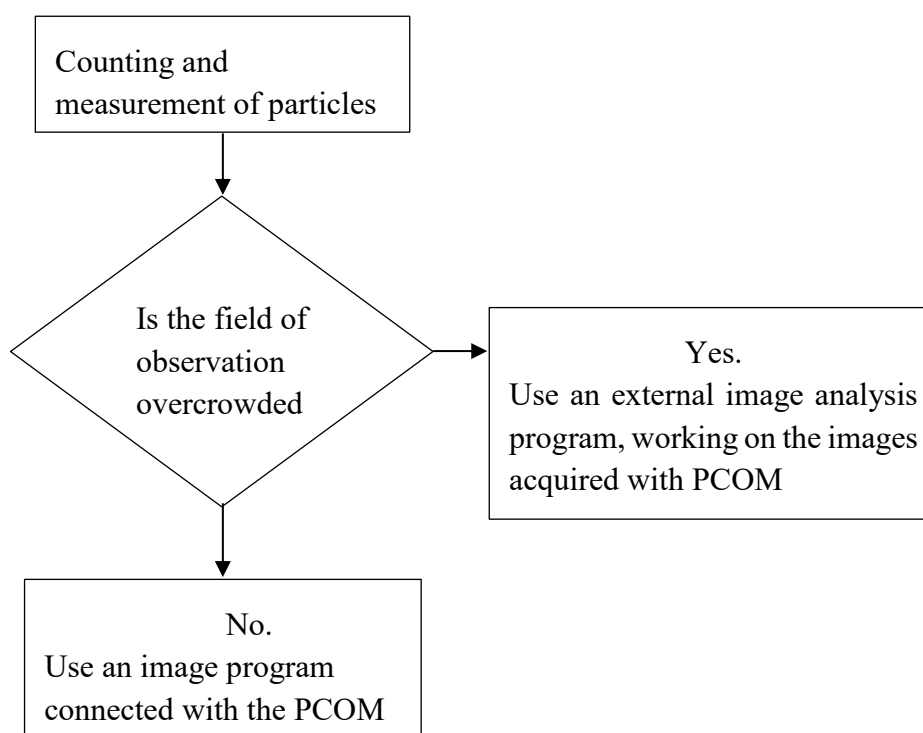
Figure 50: Decision-making process for particle counting and measurement on PCOM. Relationship with the field of observation overcrowding.

More precisely, in the case of readable fields of observation (not crowded), real-time measurements directly on the image coming from the microscope camera to a monitor were made. In crowded fields, all the acquired images were instead studied offline with a free image-processing software, ImageJ, and both length and thickness were measured. In *Figure 51*, two images are shown to illustrate field overcrowding, which affected the decisional-making process.
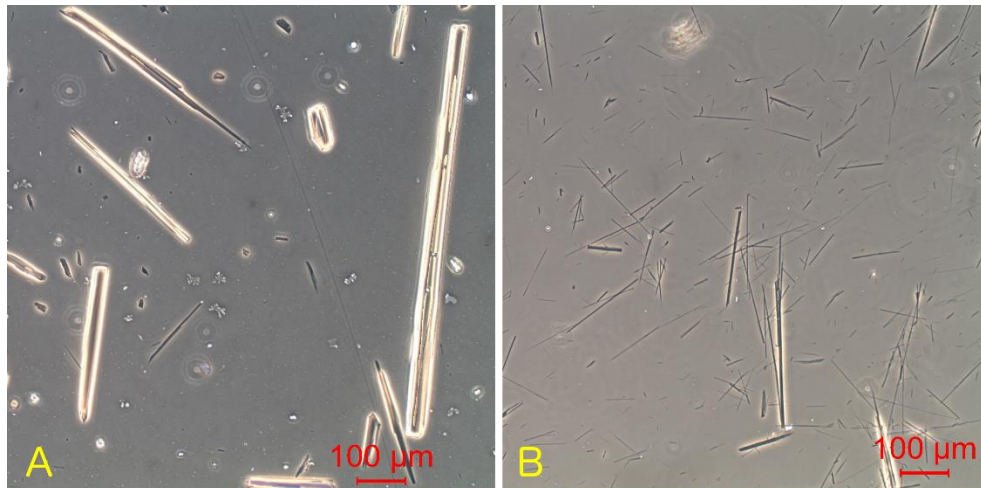
Figure 51: Examples of field crowding for counting and measuring elements: a) readable field, b) crowded field

Crystalline particles can be categorised as:

- fibrous: long and thin fibers;

- prismatic: elements with a significant thickness and flatness (resulting from planar rupture) or acicular extremities;

- acicular: long and thin fibres with at least one needle-shaped end;

- Bundle of fibres: indistinguishable elements inside a bundle, where one exists.

The dimensional distinction between fibrous (asbestiform) and prismatic (non-asbestiform) components was made according to the Health and Safety Executive. For HSE "the asbestiform habit is recognised by the following characteristics:

- a range of aspect ratios ranging from 20:1 to 100:1 or higher for fibres longer than 5 μm;

- The capability of splitting into very thin fibrils.

- Two or more of the following:
    – Parallel fibres occurring in bundles;
    – Fibre bundles displaying frayed ends;
    – Fibres in the form of thin needles;
    – Matted masses of individual fibres; and/or
    – Fibers showing curvature."

4.2.6 CLOSING REMARKS ON THE COUNTING AND MEASUREMENT OF PARTICLES

The counting and measurement of particles in each field were realized based on the following considerations:

- Only crystalline elements falling within the outline of the microscopic grid, and not the particles exceeding this area, have been considered, as shown in *Figure 52*;

- The fields of observation are casually selected inside the coverslip, more precisely following a grid and each field is not repeatable;

- During the measurement, fibre and/or fragment elements having length > 5 μm, without any restriction in the diameter, have been included; 5 μm is a threshold coming from the 'regulated' fibres definition (> 5 μm in length, ≤ 3 μm in diameter width, length/diameter (aspect) ratio ≥ 3:1) according to the World Health Organization and adopted in the Italian Minister Decree 06.09.94. It was chosen to not use the diameter threshold because we needed to measure the width of prismatic components, which are much greater than 3 μm;

- Particles are individually counted but, in the case of a bundle of crystalline elements, where these touch or cross each other it was counted as one fibre;

- In a prismatic fragment, which appears acicular or irregular at one or more point of its length, the diameter is measured along the section. This chosen section must not be influenced by breakage;

- A sufficient number of fields to reach the hundreds of elements were observed.
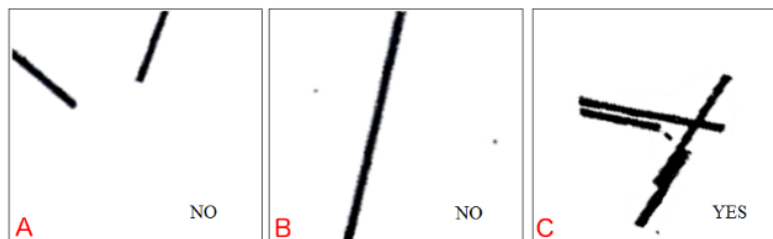
Figure 52: Particle counting: (a, b) if the elements are cut by the outline of the microscopic reticle, these will be discarded and (c) if the elements fall within the outline of the microscopic reticle, these will be considered (H.S.E. 2005)

4.3 RESULTS

Table 29 summarises all the information about the preparation and observation of samples, ground or not, using PCOM.

Original samples	Sample nomenclature	Bracchiello	Monastero	Caprie	Verrayes
	Weight on slide (mg)	0.6	0.2	0.3	0.3
	PCOM Objective	10x	10x	10x	10x
	Refractive index of oil	1,600	1,600	1,600	1,600
	Fields of observation	100 fields (20x5strips)	20	100 fields (20x5strips)	25
	Coverslip area (mm^2)	25x25	34x40	25x25	25x25
	Number of particles analyzed	240	2929	669	526
Ground samples	Sample nomenclature	Bracchiello	Monastero	Caprie	Verrayes
	Weight on slide (mg)	0.3	0.3	0.3	0.3
	PCOM Objective	40x	40x	40x	40x
	Refractive index of oil	1,600	1,600	1,600	1,600
	Fields of observation	25	25	25	25
	Coverslip area (mm^2)	25x25	25x25	25x25	25x25
	Number of particles analyzed	331	358	288	458

Table 29: Summarized information about original and ground samples

Table 30, PCOM, and *Table 31*, SEM, contain the most significant images for both the original and ground samples to give a visual comparison.

Sample	Pre-grinding (10x)	Post-grinding (40x)
Bacchiello	100 µm	25 µm

Monastero		
Caprie		
Verrayes		
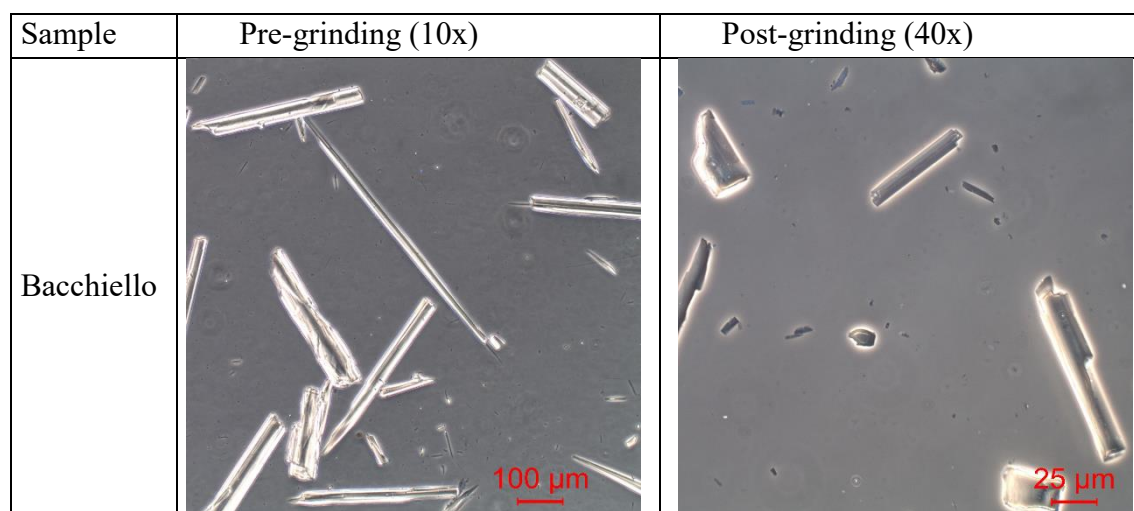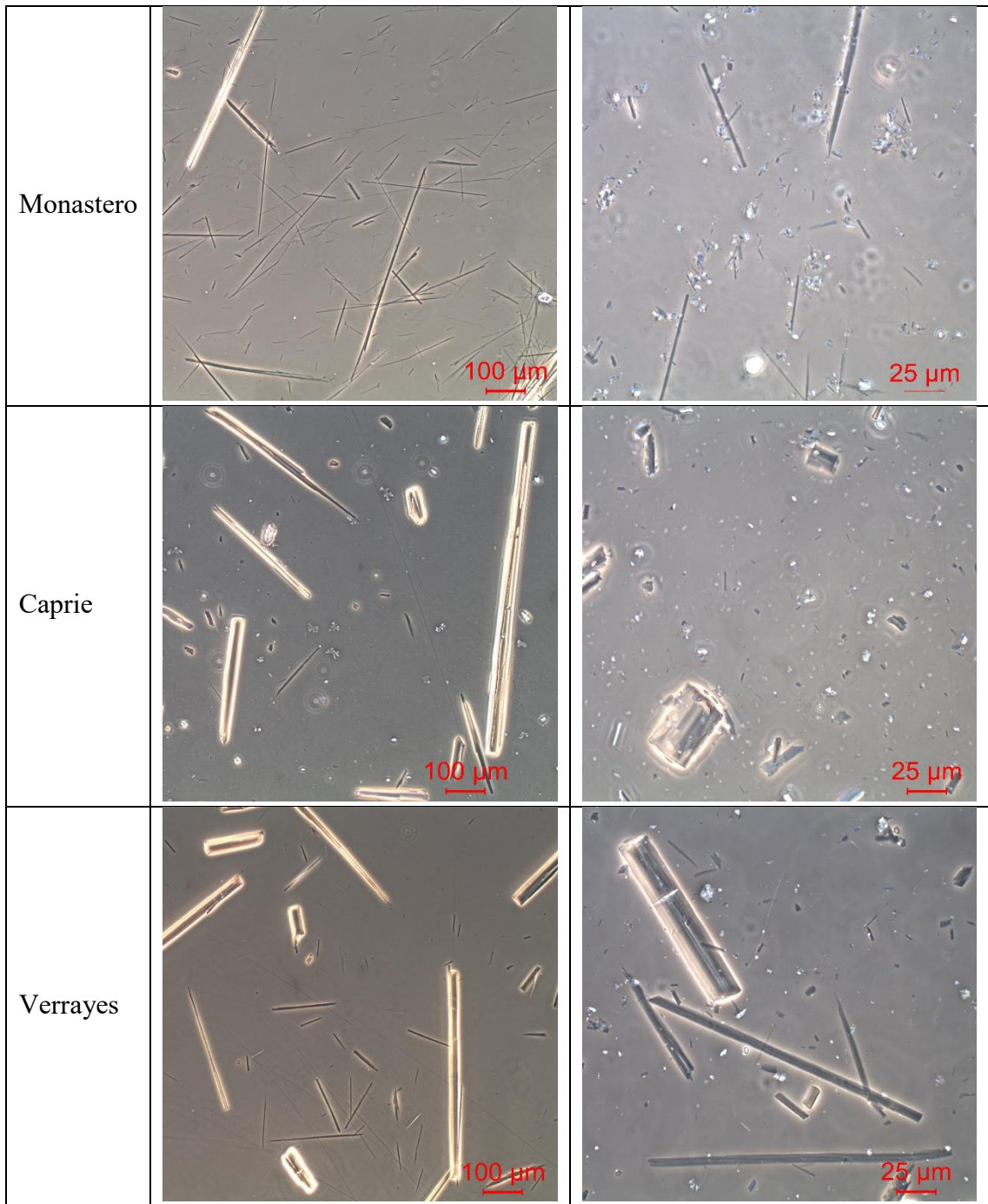
Table 30: Tremolite PCOM images of pre and post grinding
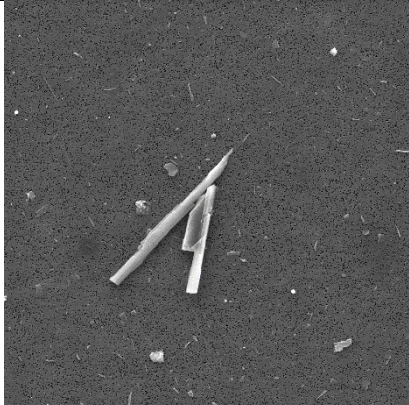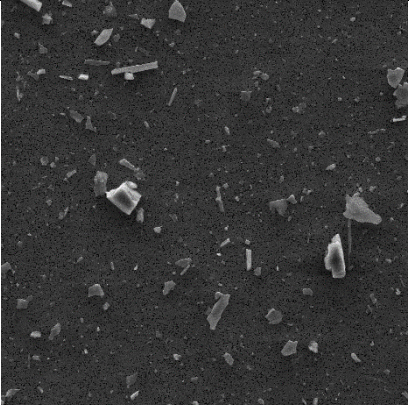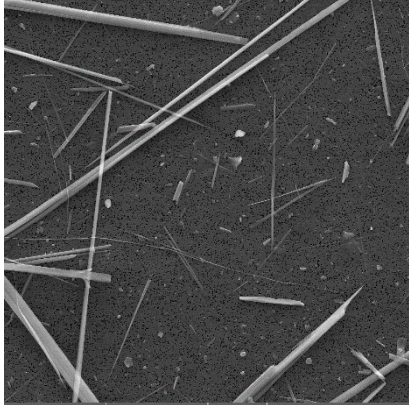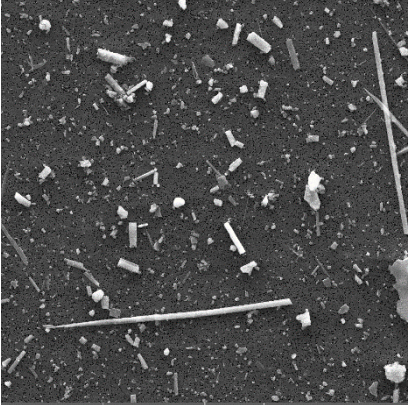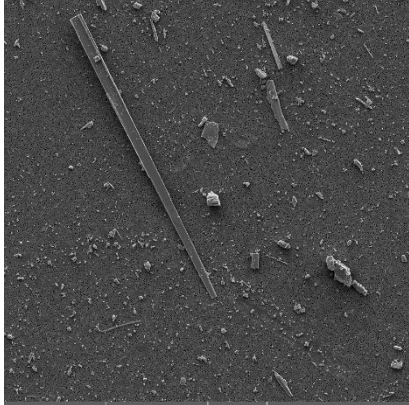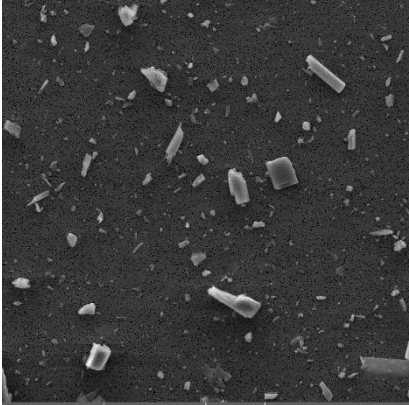
Sample	Pre-grinding	Post-grinding
Bacchiello		
Monastero		
Caprie		

Table 31: Tremolite SEM images of pre and post grinding

The charts in *Figure 54* show the results of the granulometric analysis, more precisely the trend of particle length before and after the grinding process. These graphs are frequency histograms, where each class of length is related to its frequency (%). A 5 μm minimum value for length has been chosen according to the minimum value in the 'respirable' fibre definition from WHO. For each class of length, the peak height is defined by the percentage frequency (%) as:

$$\%_i = \frac{(n.\,of\,fibers)_i}{total\,number\,of\,fibres\,observed} \cdot 100$$

where *i* is the ith class of length.

The difference in colours adopted in the graphs shown in *Figure 53* helps to recognize the two steps of analysis: yellow for the original samples and green for the ground samples.

The bar charts in *Figure 54* illustrate the difference between fibrous and prismatic components before and after grinding based on the Health and Safety Executive definition. A fibre is a component with a length-diameter ratio higher than 20, otherwise, it can be considered a prism.

Therefore, the number of fibrous (L/D > 20) and prismatic (L/D < 20) components were determined for the two-step of analysis and their percentage frequency was defined as follows:

$$\%_i fibrous = \frac{(n.\,of\,fibers\,with\,\frac{l}{D} > 20)_i}{(total\,number\,of\,fibres\,observed)_i} \cdot 100$$

$$\%_i prismatic = \frac{\left(n.\,of\,fibers\,with\,\frac{l}{D} < 20\right)_i}{(total\,number\,of\,fibres\,observed)_i} \cdot 100$$

where i is the ith status of the sample (original or ground).
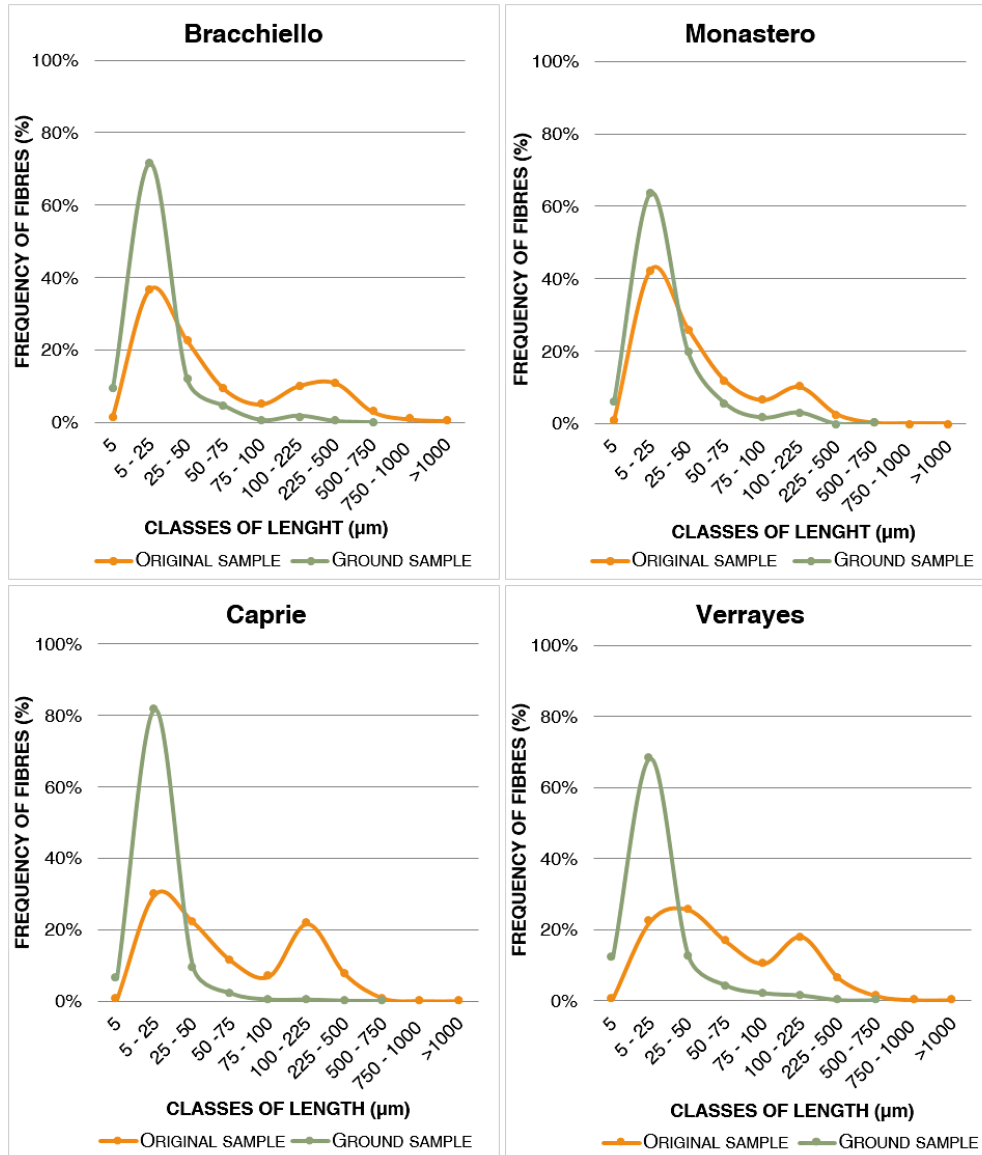
Figure 53: Percentage frequency of particles in each sample, before and after grinding
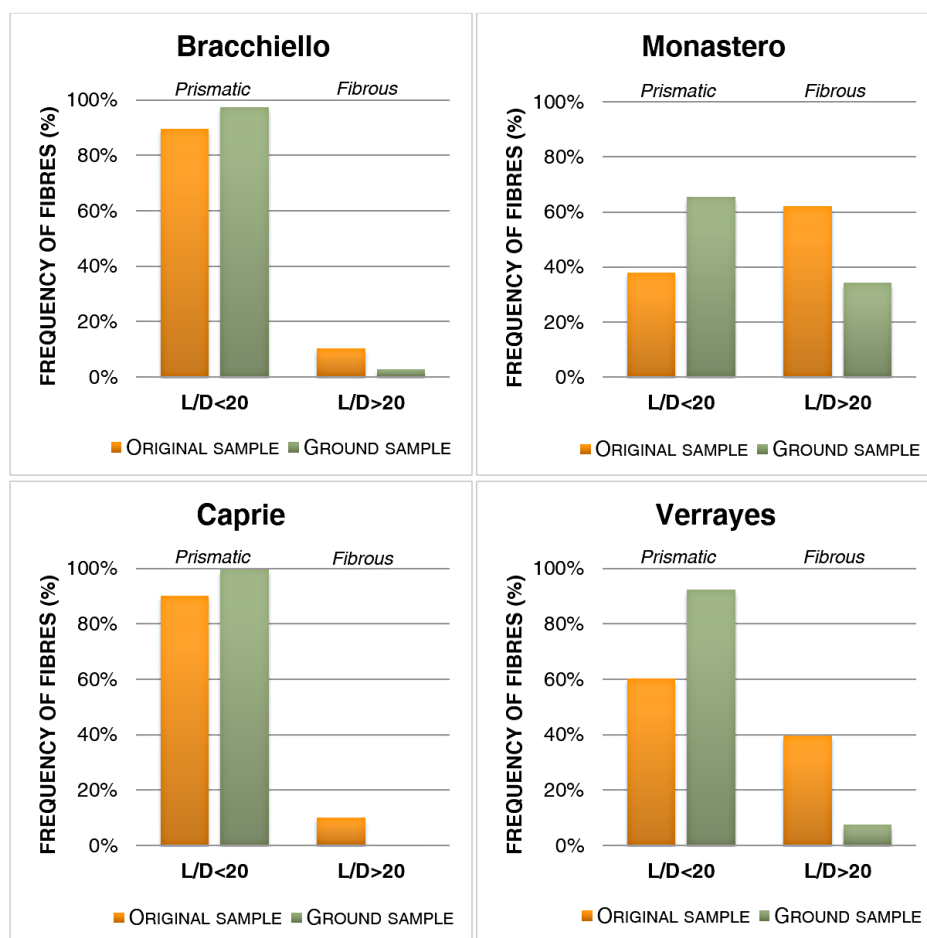
Figure 54: Amounts of fibrous and prismatic component according to HSE [23] before and after grinding

4.4 DISCUSSION

4.4.1 GRANULOMETRIC ANALYSIS

The results from the granulometric analysis, shown in *Figure 53*, demonstrate a similarity in the reduction of component length. The original samples are characterized by a great frequency of particles located in the 5–25 µm and 100–225 µm classes. These two classes delineate two obvious peaks and there is one in the 75–100 µm class with a lower frequency. The grinding action is shown with the green curve. This process highlights an attenuation of the second peak (100–225 µm) and a growth in the first (5–25 µm). The long components have a reduction of their length and there is an increase of short elements inside the 5–25 µm class. In short, particles undergo a perpendicular to the length break during grinding and this action causes a reduction of their length. This case is different from the classic asbestos breaking method, which is defined as a longitudinal split of the fibres.

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The elements with lengths lower than 5 μm were not considered, because they are not covered in the definition of 'respirable' fibre by the World Health Organization. The graphs in *Figure 54* show a high concentration of ground particles around 25 μm. The maximum diameter is 2.5 μm, ten times higher than the limits of PCOM. Therefore, PCOM is a reliable instrument for this kind of analysis. Moreover, the SEM analysis on the ground samples has permitted checking the accuracy of the previous analysis. This is especially valuable to avoid errors related to the particle dimensions.

4.4.2 DIMENSIONAL ANALYSIS BASED ON THE HEALTH AND SAFETY EXECUTIVE DEFINITION

The results of the dimensional analysis, shown in *Figure 54*, illustrate the variation in the fibrous and prismatic components before and after the grinding process. Looking at the original sample, the yellow bars, Bracchiello and Caprie present more accentuated prismatic components in comparison to the Verrayes sample. The Monastero sample is characterized by the presence of a fibrous component. After the grinding process, the green bars, there is an increase of the prismatic elements, which is especially marked for Verrayes and Monastero (an increase of 32% and 27%) and slight for Caprie and Bracchiello (an increase of 9% and 6%).

4.5 CONCLUSIONS

The aim of this study was the evaluation and analysis of tremolite behaviour submitted to anthropic (or natural) mechanical actions, which subsequently contribute to the release of fibres into the environment. It is important to underline that tremolite in nature can be present in two crystalline habits: asbestiform (fibrous) or non-asbestiform (prismatic). So, the objective is pointed towards the evaluation of morphology changes in fibres or prism particles after a grinding process. The dimensional analysis, which is based on a direct measurement of the 'particles' size (length and diameter), has allowed the study of these changes by means of the definition from the Health and Safety Executive. HSE distinguishes the fibrous component from the prismatic using the ratio between length and diameter (L/D higher than 20 defines a particle as fibrous).

The results of this research indicate that the prevalent morphology of an amphibolic mineral, in this case, a tremolite asbestos, can change habit. This is the case for the Monastero sample, which initially appeared fibrous and, after the grinding process, its fibrousness was reduced because the production of prismatic elements took over. The other samples, that initially owned a high content of prismatic particles, are subject to further increases in their prismaticity. The

dimensional analysis allows realistic and reliable results to be obtained for both the sample's composition and of the effects of grinding.

Regarding the carcinogenicity aspects of asbestos, the production of prismatic components in an amphibolic mineral by means of grinding action (digging or earthmoving), can generally be a safer situation for the exposed subject, such as workers and inhabitants. This is an interesting theme focused on the relationship between the crystalline particle breaking methods and their impact on the respiratory system. In fact, many experimental studies carried out by different authors previously cited proved a lower rate of carcinogenicity for a non-asbestiform amphibole than an asbestiform amphibole.

This study can be used for future surveys, in application to another typology of amphibolic minerals, such as actinolite, and comparing an asbestiform mineral with a non-asbestiform one. Furthermore, it would be also interesting and useful to provide an evaluation about the potential effects on human health and, therefore, obtain a correspondence with the other authors in term of carcinogenicity of these non-asbestiform tremolites.

Chapter 5

5. STRATEGIES AND BEST PRACTICE FOR THE SAMPLING AND THE QUANTIFICATION OF ASBESTOS IN DIFFERENT CASES

5.1 INTRODUCTION

This chapter defines the procedures to be adopted for the sampling of excavation materials according to the different excavation techniques and the various lithologies envisaged. It should be noted that the analytical tests carried out on the excavated materials, according to the methods and frequencies defined in this chapter, have the sole purpose of ascertaining the presence of natural asbestos in the massive samples, in order to define the protocols to be adopted for the monitoring of airborne asbestos and the mitigation measures in the management of these materials, in order to prevent the dispersion of asbestos fibers in the air. The aforementioned Asbestos Management Protocol (Protocollo Gestione Amianto, PGA) describes the frequencies and the types of massive samples that are taken depending on the type of excavation and the type of material in which the work is going on.

With regard to excavation methods, the following three cases are envisaged:

- Traditional excavation with a demolition hammer;
- Excavation in traditional with explosive;
- Mechanized excavation with TBM/EPB (Tunnel Boring Machine/Earth Pressure Balance).

Regarding the lithologies, the following three cases are envisaged:

- Excavation in Green Stones or Formation of Molare/Brecce di Costa Cravara;
- Excavation in sedimentary arenaceous-marly lithologies with the presence of asbestos deriving from Piedmontese Tertiaty Basin (BTP);
- Excavation in rocks different from the previous ones.

In particular, in the case of traditional excavation (with demolition hammer and explosive) the sampling of the material will be performed directly from the excavation face, in the case of excavation in mechanized with TBM/EPB cutter the sampling of the material will be performed by the conveyor belt.

The sampling frequencies have been defined according to the lithology being excavated. In particular for the lithologies made up of Green Stones and Molare/Brecce di Costa Cravara Formation, characterized by the presence of asbestos in mineral matrix with high variability both in terms of concentration and in terms of localization, the following sampling frequencies are provided:

- Every three advances, in the case of traditional excavation with a demolition hammer. Each advance has a variable length between 0.80 and 1.40 m;
- Every advance (about 3 m), in the case of traditional excavation with explosives;
- Every working day (of excavation), in the case of excavation in mechanized.

The sedimentary arenaceous-marly lithologies of the BTP showed a constant presence of natural asbestos with concentrations below to the limits provided for the management of the material as a by-product. In the case of excavation in this lithology it is therefore planned to perform the sampling every 50 m both for the traditional excavation and for mechanized excavation. All other lithologies have detected the absence of natural asbestos. In the case of excavation in this lithology, therefore, sampling is required every 100 m approximately both for the traditional excavation and for mechanized excavation. It is reminded that Ministerial Decree 161/2012, concerning the environmental characterization of the material in progress, provides that the sampling has to be carried out every 500 m of progress.

In the following paragraphs it will be examined in depth the frequency and the different types of sampling, providing some useful analytical considerations. It should be emphasized that, in addition to the various samples previously mentioned, all the exploration cores and surface probes made during the design phase and all the sampling carried out in parallel with the environmental control agencies must be added to the list.

5.2 WORK PLANNING: SURFACE INVESTIGATION AND EXPLORATIVE CORE SAMPLES

In the design phase of a tunneling work, the main objective is to have a representative number of samples along the designated route, which will allow the development of a geological map as precise as possible and a correct planning of the works.

Competent geologists perform superficial probes that can provide a description of the lithologies that will be crossed, moreover are carried many cores sample to the depth to which the gallery will pass out involving the whole future excavation face.

Typically if the excavation face will have a height of about 8/10 meters there will be for each point investigated a series of 10 core samples of one meter each (*Figure 56*).

The first analysis must be macroscopic and carried out by a geologist who recognizes the different lithologies and any variations in depth.

Figure 55: Part of a core sample

The next step is the analysis of the asbestos content. The different pieces of the original sample are considered as 10 different samples. Obviously the information obtained from the first samples are very important also for the following samples, because they provide indications on the possible presence of asbestos. The first phase it must be the observation of the unaltered sample and, in case of identification of fibrous material, the identification by PCOM analysis of the type of asbestos. The core sample is generally clean and compact, in this case, after drying, it is recommended to grind the entire sample before the quartering. The

grinding of the entire sample is extremely important to make it as homogeneous and representative as possible.
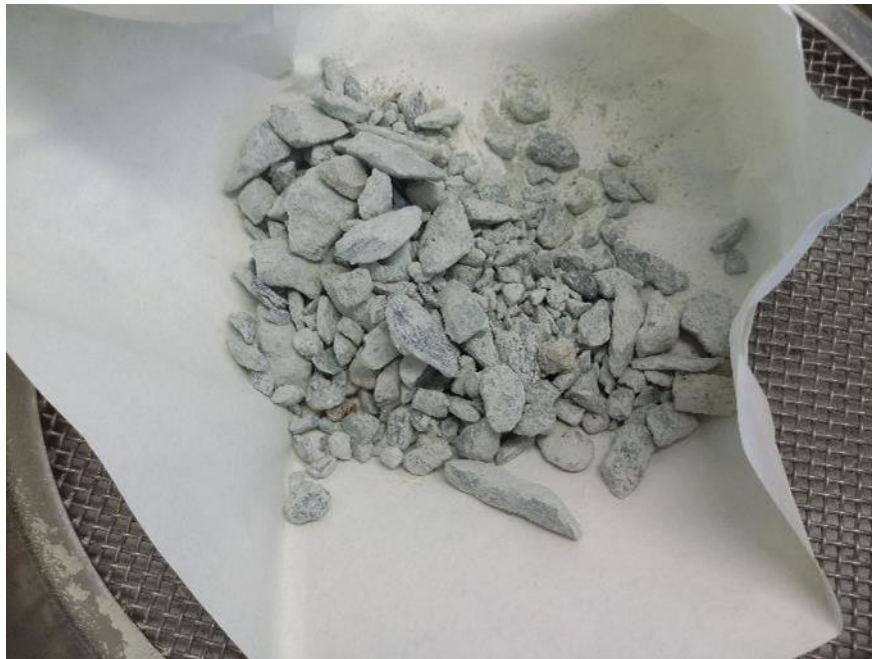
Figure 56: Ground sample

Although hypothesizing a homogeneous distribution of the asbestos in the area surrounding the sample under analysis can then prove to be ineffective, it is certainly useful to homogenize at least the meter of sample taken. In this case we believe that the analysis through the PCOM methodology is more advantageous and does not present particular difficulties.

The soundings and the cores carried out during the planning of the work play a fundamental role. In fact, through the study of these samples it is possible to elaborate a section of the tunnel route's geological map. The knowledge of lithology is important both for the prediction of possible environmental problems related to the presence of asbestos and to be able to choose the most suitable excavation technologies for the different lithologies. As already explained above, the work of the Terzo Valico dei Giovi goes through many different lithologies that can change suddenly, in the following figures (*Figure 58 and Figure 59*) some geological sections of the work in question are shown.
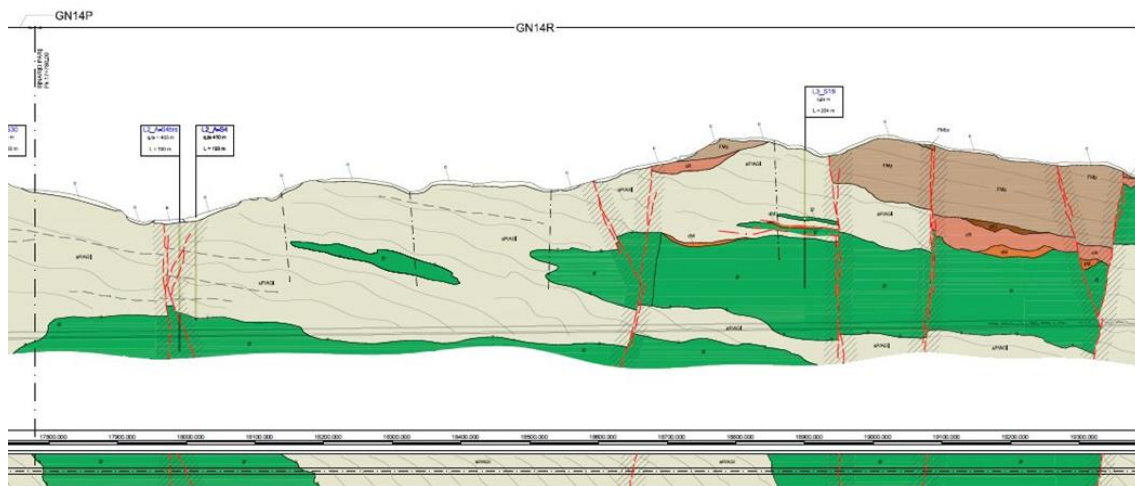
Figure 57: Geological map section with green stones (www.terzovalico.it)
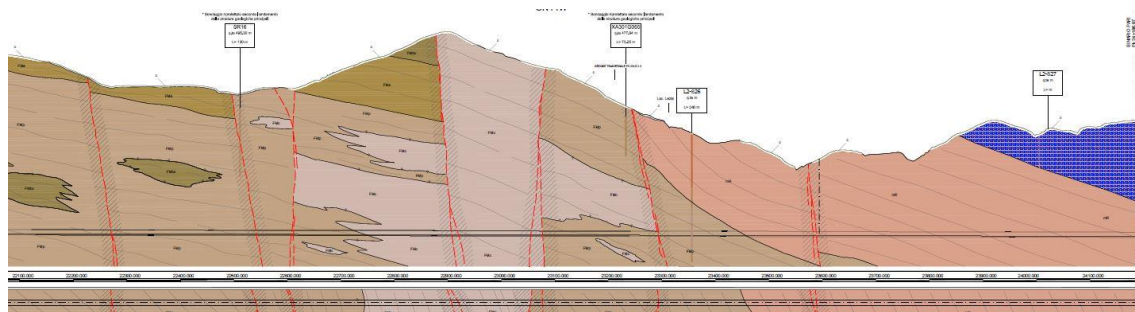
Figure 58: Geological map section (www.terzovalico.it)

5.3 DURING THE OPERA: TRADITIONAL EXCAVATION

The traditional excavation is performed by:

1. Excavation with a demolition hammer;
2. Excavation with explosives.

5.3.1 CONTROL OF THE EXCAVATION FRONT

With the traditional excavation technique (both in the case of excavation with demolition hammer and in the case of excavation with explosives) the excavation face is constantly accessible, so the geologist can visually verify the lithology involved. The excavation face sampling is performed at each "break down

advancement": it is a complete processing cycle, which, in the case of excavation with a demolition hammer, corresponds to a cycle of excavation, material's extraction, positioning of supporting beam and spritz, or, in the case of excavation with explosive, corresponds to a cycle of explosion, material's extraction, positioning of supporting beam and spritz.

For the traditional excavation with demolition hammer, each break down corresponds to a progression between 0.8 and 1.4 m. For the traditional excavation with explosive, each break down corresponds to a progression of about 3 m.

For each single "break down" the geologist at the front verifies and certifies the homogeneity of the outcropping lithotype on the excavation face with respect to the previous characterization, through the drafting of a report. Beyond that, to any significant variation of the lithotype involved in the excavations, the geologist provides a complete characterization of the rock mass.

Therefore, the geologist at the construction site compares the excavation front with the one previously found, and even in the presence of a single geological variation, without lower limits of size of the new outcropping rock, certifies the variation of lithology. This means that the appearance of a portion of green stone, even small, is considered a lithological variation for the application of the Asbestos Management Protocol and is attested in the drafting of the front of the excavation report.

The sampling at the front allows to check the lithology present and to identify the actual danger level, confirming or not the predictive value indicated by the geological model.

At the first discovery of Green Stones at the excavation face (intended as a lithological passage from the absence of green stones to the presence of green stones at the front stop), the excavation front is secured and a sampling is carried out.

 For this purpose, samples of Green Stones are taken. In this case, as for the core samples, the procedure that we consider the most correct is using PCOM with a previous grinding of the entire sample.

5.3.2 SAMPLING METHODS ON THE EXCAVATION FRONT

In the excavation with traditional methods (both in the case of excavation with a demolition hammer and in the case of excavation with explosives), the sampling of the material is performed directly from the excavation face with the hammer used in excavation operations (assuming that also in the case of blasting there is a hammer to perform the operations of removal from the front).

The composite sample (primary sample) is made by taking the material from 8 points (increments) of the excavation face, located according to a reference grid (C. Clerici 2008). In the case of homogeneous excavation, the points are located according to a systematic criterion as shown in the following image (*Figure 60*).
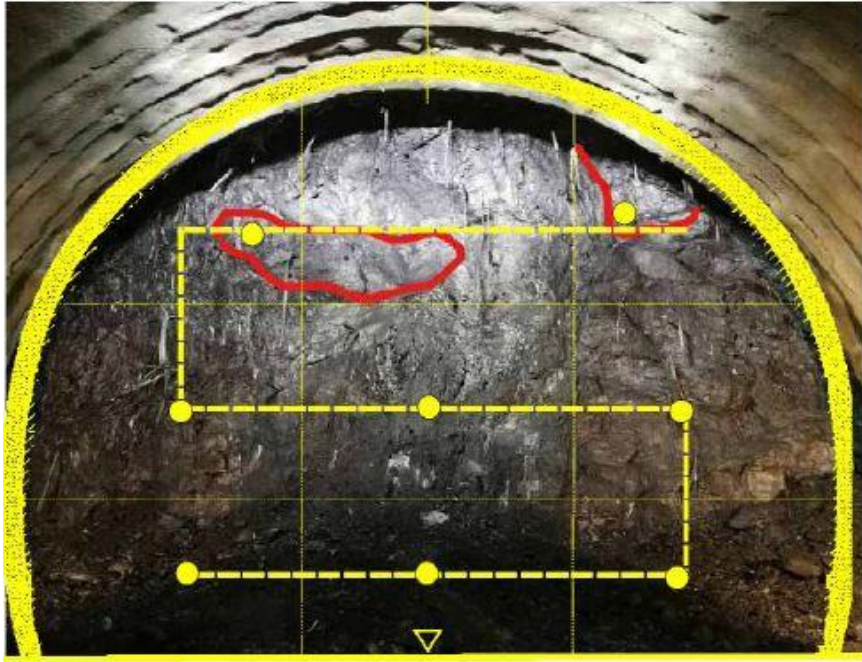
Figure 59: Sampling grid (PGA, 2018)

This method of sampling, however, is indicative in the case that the lithology is uniform for the entire front. In the case in which the geologist detects the presence of green stones or of different lithologies, the sampling points can be moved in order to guarantee the most homogeneous sampling. An example is provided in the *Figure 61*.
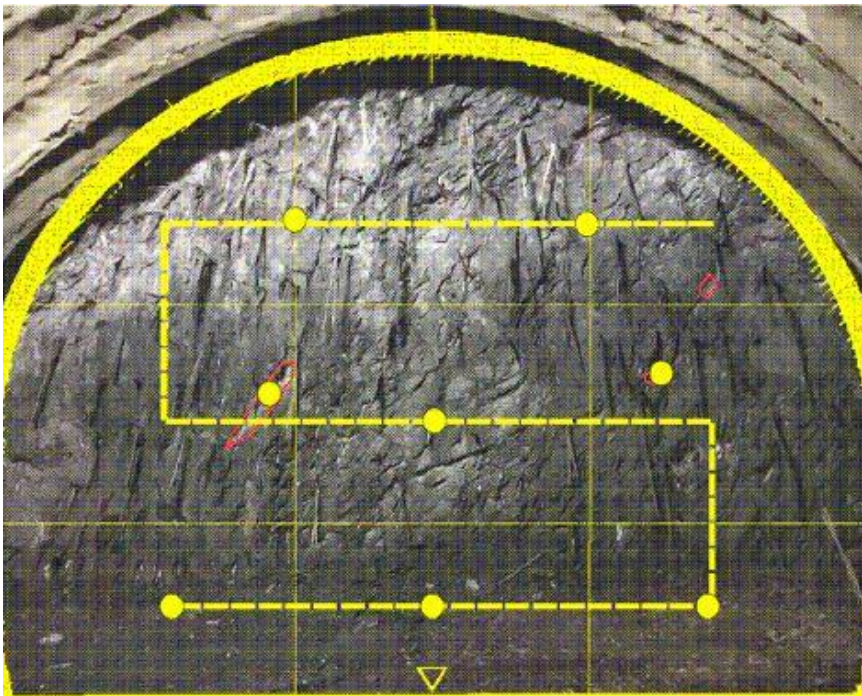
Figure 60: Modified sampling grid (PGA, 2018)

As shown in the *Figure 62*, the material removed with the hammer is collected in a bucket positioned immediately below the tip of the demolition hammer. Subsequently to the realization of the mixed sample an in-field quartering is performed up to a sample that does not exceed the 3 kg.

Figure 61: Front sampling (PGA, 2018)

Although the microscopic analysis requires a much lower quantity, it is useful for analysts to have a substantial quantity of material (*Figure 6*3) to observe it macroscopically and proceed with a further comminution before the next quartering.

Figure 62 : Laboratory sample

5.3.3 SAMPLING ON REMOVED SOIL

In the case of sampling by sorting from the excavated material placed near excavation front or in the site storage area (*Figure 63*) a withdrawal of at least 8 increments, 4 located on the surface and 4 inside the pile, to form a single representative composite sample is carried out.

Figure 63: Storage of excavated material (PGA, 2018)

5.4 DURING THE OPERA: TUNNEL BORING MACHINE (TBM) EXCAVATION

5.4.1 CONTROL OF THE LITOLOGY

The mechanized excavation technique involves the use of the TBM and the excavation front is not accessible, therefore the lithology can not be guarantee by direct observation of the excavation front. In this case the verification of the excavated lithology is performed by observing the debris carried on the conveyor belt on the back of the cutter head. Surely, preliminary investigations play a fundamental role in this assessment.

For this purpose, the geologist collects samples of the extracted material directly from the belt. The sample is repeatedly washed to remove the fine fraction and then subjected to detailed observation in the field by the same geologist.

5.4.2 SAMPLING METHOD

The sampling for asbestos presence analysis, as for the lithology evaluation, can not be carried out on the excavation front.

Sampling will be performed directly on the conveyor belt, before the excavated material reaches the concrete storage tank. A "flow rate sampler with flow deviation" will be used, placed in the hopper at the end of the conveyor belt. The hopper is equipped with a tilting flap designed so as to open inwards and cut off all

the flow of falling debris, causing it to deviate towards the sample collection tank (*Figure 65*).
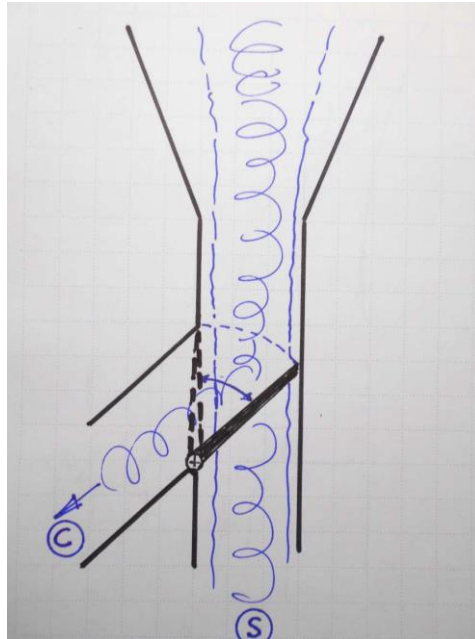
Figure 64: Scheme of sampling with flow deviation from the conveyor belt (PGA, 2018)

The sample will be made up of several increments: for this purpose, an operator delegate for sampling will open the sampler by combining different amounts of material into the sample collection tank or big bag, until the quantity calculated as representative is reached.

The collected sample is composed by wet material with homogeneous granulometry and of a considerably smaller size compared to the front sample. The sample will consist in monochromatic mud (due to the presence of perforation liquids)(*Figure 66*), and, even after the drying process, the macroscopic observation could not provide sufficient information.

Figure 65: Sampling from the collection tank

In this case the best methodology for the analysis is certainly using SEM. The material is homogeneous and after the drying process an aliquot is ground to reach the optimum size. The SEM analysis allows to observe the material without pretreating it, while, for the PCOM analysis, in this case, it would be necessary to wash the sample several times, preserving all the water used in order to collect all the material. The procedure would therefore be much longer and more complex.

Chapter 6

6. FINAL CONSIDERATIONS

Starting from the purpose of this work, which was the investigation of the problem concerning the quantification of the content of Naturally Occurring Asbestos within rock samples deriving from excavation activities, it is necessary to remind that the regulatory framework is extremely inadequate both from the analytical point of view and for the strategies and best practice for the managing of the natural material containing asbestos.

This work starts with the validation of a quantification methodology using the PCOM. The ministerial Decree 6/9/1994 affirms that PCOM is a technique reliable only for a qualitative evaluation of the asbestos presence.

The decree is full of inconsistencies and it is possible to find paragraphs in which the PCOM methodology is criticized and others in which a quantitative use is also assumed. However, no indication is provided regarding the analysis. Our methodology is based on a correct separation of the granulometric classes in order to solve the problem of the depth of field investigated by the PCOM, in fact a homogeneous granulometric distribution decreases the possibility that small fibers are in classes of greater granulometry. The correct identification of asbestos is guaranteed by their unique properties and optical characteristics. Certainly PCOM has a lower resolution than SEM but the portion of sample analyzed is much more representative. In the third chapter the effectiveness of the PCOM technique was evaluated, comparing it to the SEM one for 150 samples with low asbestos content and the results were satisfactory. There is a good correlation between the results obtained with the two techniques both in the case of absence of asbestos and in the low quantity range. The discordant cases are discussed with the description of the most frequent causes of error, such as the presence of out-of-scale objects and the difficult distinction of serpentine polymorphs (antigorite) with the SEM technique. Out of scale objects are a source of error in the SEM methodology due to the limited amount of analyzed material. The presence of a bundle of asbestos, in this case, can lead to values much higher than the actual content of the material itself. The distinction between antigorite and chrysotile with the SEM technique is based only on a morphological evaluation due to the fact that the EDS spectrum of the two minerals is extremely similar. Using PCOM the discrimination is easier and it is based on the optical properties typical of each mineral.

It is possible to affirm that both methods have advantages and disadvantages but surely the success of the analysis passes through the competence of the operator and the knowledge of the various possible sources of error. It must be emphasized that the first step for a correct characterization of a material, especially if it derives from a tunnel excavation, is its macroscopic observation and the knowledge of all the possible preliminary information: geological maps, exploratory scanning and airborne monitoring.

The behavior of tremolite, an amphibole that can occur both in prismatic and fibrous crystalline habit, if subject to grinding, was also studied. The study aims to verify if the grinding process, which is part of the analytical process (and which also simulates a working process during the removal or breaking of asbestos-containing rocks), could modify the initial crystalline habit or otherwise increase the component fibrous of the mineral itself. The analysis showed that the grinding does not lead to an increase in the mineral fibrousness nor to the creation of fibers if the starting mineral was of prismatic habit.

As a final conclusion for this work and from our experience it can be stated that there is no a perfect technique for this type of analysis. Each sample is different and each laboratory has its own experience. However, I believe that for a correct analysis of a rock sample it is always necessary to start from the macroscopic observation, and, if fibrous minerals are observed, try to define the typology by using Phase Contrast Optical Microscopy.

Once this information is obtained, if the sample comes from a probe, from a sampling at the excavation front or from a heap, the PCOM technique is excellent because it guarantees a continuous observation of the sample in the different phases of analytical preparation and greater representativeness.

If, on the other hand, the sample is in the form of mud, loose soil, or belongs to clay/silt/sand formations with a fine and homogeneous particle size, then the SEM technique seems to be more suitable and effective above all for analysis times, taking into account that a particle size division would be useless.

The flowsheet of these processes is reported in

Figure 66

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                        ┌─────────────────┐
                        │ NATURAL SAMPLES │
                        └─────────────────┘
                    ┌────────────┴────────────────┐
          ┌──────────────────┐          ┌──────────────────┐
          │ BEFORE           │          │ DURING           │
          │ TUNNELING        │          │ TUNNELING        │
          │ (PROJECT PHASE)  │          │ (WORK PHASE)     │
          └──────────────────┘          └──────────────────┘
                    │                  ┌─────────┴──────────┐
          ┌──────────────────┐   ┌───────────────┐   ┌───────────────┐
          │ SURFACE          │   │ TRADITIONAL   │   │               │
          │ INVESTIGATION    │   │ EXCAVATION    │   │ TBM EXCAVATION│
          │                  │   │(DRILLING AND  │   │               │
          │ EXPLORATIVE      │   │BLASTING AND   │   │SAMPLES FROM THE│
          │ CORE SAMPLES     │   │ HAMMER        │   │CONVEYOR BELT  │
          │                  │   │ EXCAVATION)   │   │ (MUD)         │
          └──────────────────┘   │               │   │               │
                    │            │EXCAVATION FRONT│   │SAMPLES OF     │
          ┌──────────────────┐   │ SAMPLES       │   │SILT/CLAY/SEND │
          │ MACROSCOPIC      │   │               │   │               │
          │ OBSERVATION      │   │DEPOSIT OF     │   │               │
          │                  │   │MATERIAL SAMPLES│  │               │
          └──────────────────┘   │               │   └───────────────┘
                    │            │EXPLORATION    │           │
          ┌──────────────────┐   │FRONT CORES    │   ┌───────────────┐
          │ PCOM             │   └───────────────┘   │ SEM           │
          │ METHODOLGY IS    │           │           │ METHODOLGY IS │
          │ SUGGESTED        │   ┌───────────────┐   │ SUGGESTED     │
          └──────────────────┘   │ PCOM          │   └───────────────┘
                                 │ METHODOLGY IS │
                                 │ SUGGESTED     │
                                 └───────────────┘
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Figure 66: Suggested methodology flowsheet

Moreover I hope that, starting from the circumstance of the discussion of this thesis, the collaboration between research groups of different Universities could increase hugely. Fortunately in the last years several interesting studies have been carried out and I'm sure that many are moving forward. For our part we have the purpose of develop our methodologies and our knowledge of the topic, dealing with new instruments and techniques (Raman, FTIR, XRD) trying to improve ourselves and to be of help to scientific and civil society.

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