GEOLOGIC, ENVIRONMENTAL AND NORMATIVE ISSUES IN THE NATURAL OCCURRENCES OF ASBESTOS (NOA) MANAGEMENT

GAIA M. MILITELLO
Dipartimento di Scienze della Terra, dell'Ambiente e della Vita (DISTAV), Università di Genova, Corso Europa 26, 16132 Genova

Asbestos, a substance included among group I carcinogens, is considered one of the most dangerous types of dust for human health (IARC, 1987; 2012).

This term has a commercial meaning, used to identify six minerals represented by hydrous silicates belonging to the serpentine group (chrysotile) and amphiboles (amosite that is the fibrous variety of grunerite, crocidolite that is the fibrous variety of riebeckite, anthophyllite, tremolite and actinolite), which are easily separable in thin, flexible fibres, resistant to traction and heat and almost chemically inert.

The chemical-physical properties of asbestos have made them, in the past, one of the most important inorganic materials for industrial purposes and technological applications. However, the extraction, use and marketing of these minerals have been prohibited, due to the proven harmful consequences, mainly affecting the respiratory system, that asbestos fibres can cause. Consequently, Asbestos Containing Materials (ACM) have been declared harmful to health and banned in Italy by Law No. 257 of 27/03/1992 and subsequently in the entire European Commission by directive 1999/77/EC as identified as a mutagenic agent, the cause of pleural mesothelioma and other asbestos-related pathologies.

More than 52 countries, including the 28 member States of the European Union, have banned or restricted the use of asbestos in accordance with the campaign carried out by the World Health Organization (WHO) to stop the use of all types of asbestos (Bloise *et al.*, 2020). However, countries such as China, India, Russia, Kazakhstan, Zimbabwe, Brazil, and Canada and Colombia are still producers and consumers of asbestos.

Actually, the mere occurrence of asbestos does not necessarily represent a risk but becomes dangerous if it disperses fibres in the surrounding environment (Gaggero *et al.*, 2017), for example, due to mechanical or thermal stress. However, the asbestos management is ruled by quite obsolete legislation therefore, controversies over the health impact, identification asbestos criteria and regulatory limits are still ongoing.

The aim of this work was to carry out an accurate review of the technical and legislative critical issues useful for updating the methods in force, regarding the analysis for quantification of asbestos. A significant contribution was also made on cytotoxic and genotoxic tests based on the development of more precise and univocal analysis protocols in the classification criteria for asbestos minerals.

QUANTITATIVE ANALYSIS: METHODS COMPARED

The evaluation of the asbestos hazard is based on proper characterization of asbestos concentration trough a counting criteria used to determine the number of regulated fibres (Belardi *et al.*, 2018), establishing requirements for environmental quality and appropriate destination for excavated products. Currently though, official survey techniques prescribed by the normative for assessing the presence of asbestos in bulk samples, by qualitative and quantitative analysis, do not guarantee a unique and reproducible quantification of asbestos content. Notable variables, depending on the material under examination, reside on the context from which the sample is issued, and a vision on evaluating the investigation objective (Cavariani *et al.*, 2010). Therefore, it was established whether asbestos fibres homogeneously distributed in the different fractions ground from asbestos-bearing lithotypes and was calculated the contribution of fibres from each fraction to the overall concentration in the sample. This observation was made by first referring to the Italian Ministerial Decree (M.D. 06/09/1994), which regulates the counting criteria for asbestos fibres potentially occurring in excavated soil and rocks, suggesting that fibre count must be performed on the particle size < 20 mm. The powdered fractions were characterised using a Scanning Electron Microscope coupled with Energy Dispersive Spectroscopy (SEM-EDS). Subsequently another type of observation was made referring to the still in use (in some cases) Italian normative M.D. 10/08/2012, which specifies that analyses must be performed on the < 2 mm fraction and the concentration (mg/kg) correlated with the weight of the whole sample < 20 mm. However, the fibre counts yielded asbestos concentrations 50-60% lower compared with total asbestos analyses according to the new D.P.R. 120/2017. The whole analysis set demonstrated that asbestos concentration results are strongly dependent on the type of rock, the grain size prepared for analysis, and the criteria used to count fibres. Moreover, in the case of occurring asbestos in rocks the use of D.P.R. 120/2017 is considered more suitable for determinations and technical-scientific investigations. In such cases, the definition of a particle as part of the skeleton (2-20 mm fraction) is pointless if it is generated artificially according to the mechanism of rock comminution. Therefore, it is considered more appropriate to express the concentration of the fibres in terms of total content, without referring to the skeleton. This is the most precautionary result because it provides a determination of the total fibres released by a rock. Consequently, there is a need to standardize the worldwide normative regulations to manage asbestos-containing materials by re-evaluation of sample preparation and quantification of asbestos (Militello *et al.*, 2019a).

IN WHAT PERCENTAGES ARE ASBESTIFORM AND NON-ASBESTIFORM MINERALS PRESENT WHEN GROUND?

In addition to the known six minerals classified as asbestos, in nature exist also amphiboles and antigorite and lizardite (polymorphs of serpentine), which have the same composition of asbestos groups but not the same morphology. These minerals are chemically and geometrically (length > 5 μm, width < 3 μm and length: diameter > 3:1) but not morphologically analogous to regulated asbestos and are identified as cleavage fragments (Fig. 1).

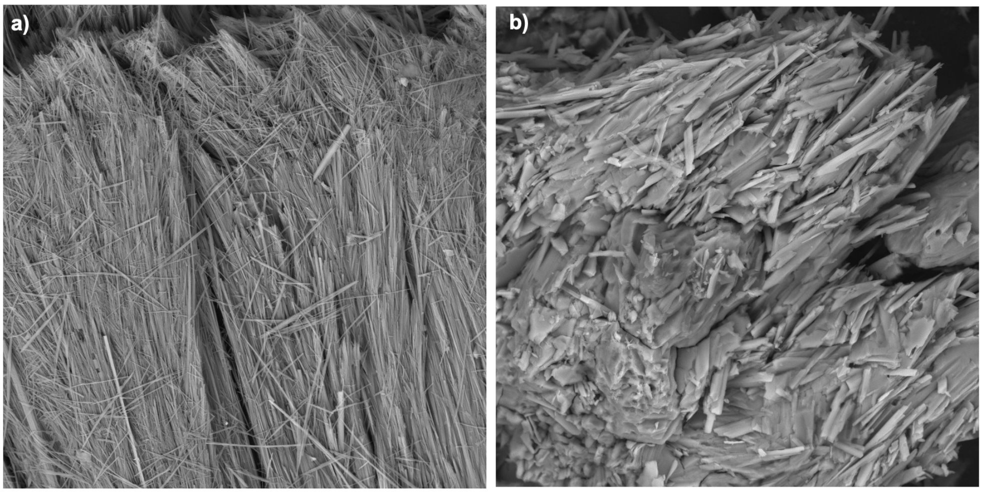

Fig. 1 - SEM images. Representative asbestiform (a) and non-asbestiform (b) amphiboles of the tremolite-actinolite series. Magnifications: 2000X; high vacuum: 20 kV; detector: back-scattered electrons.

In fact, the worldwide normative and scientific gaps also concern the classification criteria of asbestos. If the asbestiform amphiboles have been clearly identified and banned, acicular/prismatic amphiboles are not univocally regulated as asbestos (Militello *et al.*, 2021). Also, among serpentine polymorphs, the fibrous antigorite and lizardite sometimes occur very similar in morphology to the polymorph chrysotile and therefore are not easily distinguishable, especially if associated. Moreover, non-asbestiform particles are a potentially major source of exposure worldwide, so adverse health effects may derive (Hwang *et al.*, 2014). Research and debates are in progress about the composition and morphology of the various types of asbestos and the related fibrous minerals not classified as asbestos, such as erionite, fibrous lizardite and antigorite (Cardile *et al.*, 2007; Ballirano *et al.*, 2018). For instance, antigorite is regulated as asbestos only in the New Caledonia legislations (Petriglieri *et al.*, 2015).

The goal of this part of work was to compare, at different investigation scales, by a multi-instrumental approach (Optical microscope - OM; Scanning Electron Microscope coupled with Energy Dispersive Spectroscopy - SEM-EDS; Transmission Electron Microscope - TEM) the morphological features and the influence of textural constraints in massive samples, which determine the origin of fibrous and asbestiform or fibrous but not asbestiform products, of amphibole and serpentine group minerals. Moreover, a new application of the synchrotron radiation X-ray microtomography, not yet used before for asbestos detection, was adopted (Militello *et al.*, 2019b). This semi-destructive technique was chosen because it can help to better observe the arrangement of the fibres in the three dimensions and facilitate the comparative description of cleavage fragments.

A subsequent part of the work was focused on the SEM-EDS analysis of samples containing tremolite-actinolite amphiboles with asbestiform and non-asbestiform habit, subject to mechanical stress by grinding for three different time intervals, to assess how different time lengths of comminution control geometry and morphology of the particles. The comminution is fundamental for the following quantitative determination of fibres. When mechanical stress is applied to rocks containing pristine prismatic or acicular amphiboles, these mineral phases can break, originating particles with dimensions and geometrical ratios that would label them as asbestos, the recast of concentration takes thus to false positives or false negatives, generating a critical issue.

However, the preparation methods described in the Italian Ministerial Decree (M.D. 06/09/1994) and other ministerial protocols from other countries, lack precise coding, thus not guaranteeing comparable and reproducible intra- and inter-laboratory results. According to Ham *et al*. (2019 and references therein), quantitative analysis and the accuracy of the results also depend on the experience of the analyst. Consequently, quantitative calculations can produce high variance results, inhibiting a risk estimate based on univocal data.

Preliminary results showed that the different behaviour of asbestiform and non-asbestiform amphiboles is confirmed in a distinct distribution length: width ratio of fibres, as effect of the comminution, but a geometric overlap of particle sizes is also evident (Militello *et al*., 2020). This could cause an incorrect classification because it can lead to over- or underestimation of the concentrations.

Overall, these results highlight how comminution is a critical phase in the preparation of the samples. The shape of produced particles could depend on lithology, type of the instrument (in particular, the specific breakage mechanism in a grinding device), the time applied for milling the sample and the relative application of the stress. In order to obtain comparable quantitative results, the present data strongly support the need to select the same equipment in each laboratory and to standardize the method of sample preparation.

More data on the shape and the size of particles are the benchmarks to new perspectives into the study of morphological features and mutagenic mechanisms of a presently misunderstood material.

CYTOTOXICITY AND TRANSFORMATION TESTS

Lastly, bioassays were carried out to better understand the different carcinogenic potential of serpentine and amphibole minerals as a function of the crystalline habit, *i.e.*, the aspect ratio and the morphology of the fibres. The literature review did not reveal strong evidence indicating that cleavage fragments have the same or greater carcinogenic potential than asbestos. Different published data suggest that the asbestos fibres toxic effect increases with length, despite some notable exceptions. However, the extent to which a mineral with asbestiform habit affects its toxicologic or carcinogenic mechanisms relative to that of a cleavage fragment of the same mineral remains a matter of debate.

The same samples previously used were prepared to perform biological assays. The capacity of several asbestiform and non-asbestiform powders to cause both cytotoxic effects and carcinogenic potential in BALB/c 3T3 cells was then monitored and evaluated. The fibres/cell interaction was very well represented by SEM and the images in Fig. 2 are among the most representative.

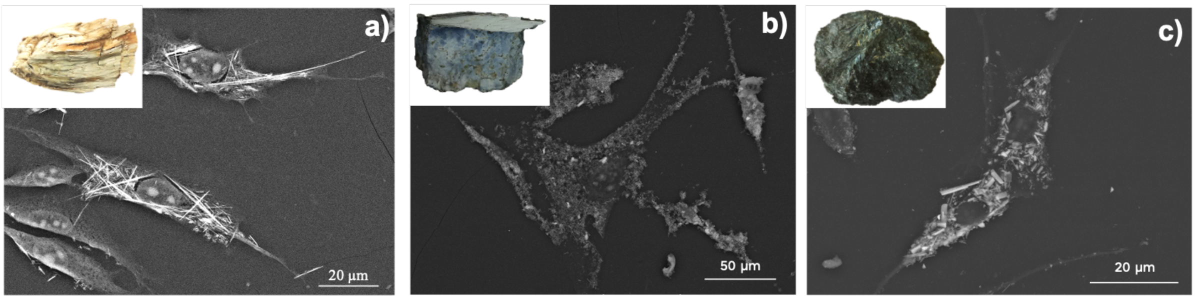

Fig. 2 - Interaction between sample powders of (a) Amphibole vein in serpentinite - F3, (b) Serpentinised peridotite with lizardite vein - A3/S1, (c) Actinolite schist - A5/P3 and the cells. High Vacuum; 20 kV; Detector: Back scattered electrons.

The data presented in this study for *in vitro* experiments highlighted that cleavage fragments of amphiboles and lizardite could induce cytotoxic effects and transformation process as carcinogenic potential. In particular, the toxic effect is shown in the graphs of Fig. 3 in which the concentration of the powders is shown on the abscissa and the vitality values of the cells as a function of the concentration administered on the ordinate. The interpolation of the data obtained for each sample through a regression line allowed us to identify the dose in which no effect is observed (NOEL), mean lethal dose (IC50) and the dose that caused 90% of mortality IC90. The comparison of the results allowed us to identify toxic effects in the sample A3/S1 and A5/P3 like those reported for cells exposed to asbestos F3 at the same concentrations.

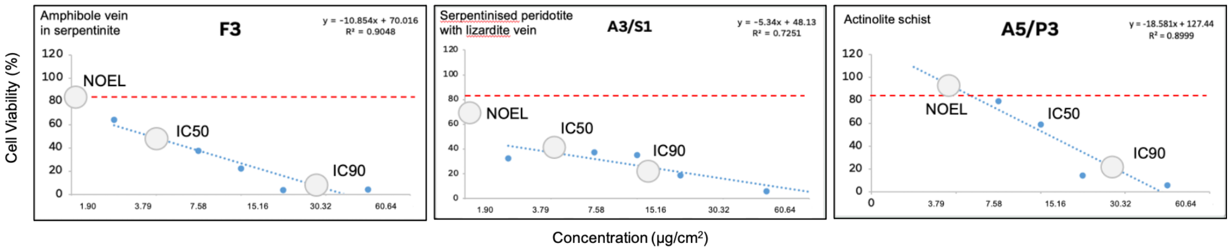

Fig. 3 - Graphs showing the different cell viability of BALB/C3 expressed as % of viability as compared with untreated controls of the corresponding cell lines. BALB/C3 cells were exposed to different concentrations either to asbestos or cleavage fragment.

The demonstration of the cytotoxic and transforming activity of fibres in the 3T3 model could constitute a starting point for further studies that may lead to the extrapolation of the risk related to exposure and categorization of fibres by the relevant regulatory institutions.

It is necessary to debate what aspect ratio is to be considered as lower and upper limits of a range. The risk of developing chronic diseases by inhalation of fibres is linked to different factors as particle morphology, size, physical-chemical properties and bio-persistence correlated to the crystalline habits and can produce relevant consequences to the human respiratory system. More constraints on this issue plays an essential role in establishing health risk because, as part of the analysis for environmental monitoring, acicular fibres could influence the final calculation of the asbestos concentration.

CONCLUSIONS

This work provided advances in the knowledge of cleavage fragments thanks to the multidisciplinary approach of the project, which integrated geological and mineralogical data with bioassays data. The new results achieved have provided useful indications for future research also in other areas than those investigated. A complete petrographic characterization was carried out, through microscopic and spectroscopy techniques, of the phases that may occur with asbestiform and non-asbestiform fibrous habit, *i.e.*, minerals of the serpentine group (antigorite, chrysotile and lizardite) and minerals of the amphibole group (actinolite and tremolite). The observations under OM allowed to investigate the textures and the spatial relations between the mineralogical phases. Under the SEM the aspect ratios and the textural relationships between the various phases were characterised, but the discrimination among the serpentine polymorphs was impossible specially when occurring in the host rock. The TEM analysis permitted the high-resolution discrimination for cleavage fragments and allowed an effective distinction between asbestos minerals. However, the macroscale habit does not always correspond to that observed at the microscale (*i.e.*, not repeated with fractal geometries). For example, when the elongated particles are very thin it may not be possible, even with TEM, to differentiate adequately asbestiform fibres from prismatic crystals or cleavage fragments.

Above all, the characterization of non-asbestiform fibres and the evaluation of their hazard is important because these minerals are more common than asbestos in many geologic environments, and disturbance can result in the release of prismatic or acicular single crystals or cleavage fragments resembling asbestos fibres.

To date, workers may not be exposed to asbestos, but they can be for non-asbestiform amphiboles. Considering only the geometry, both prismatic and asbestiform amphiboles and serpentine may show inhalable elements with width < 3 μm, length > 5 μm and width length ratio > 1:3. Furthermore, although studies confirm that amphiboles are not carcinogenic if not in size greater than 1:20, because they are not easily engulfed by macrophages, more in-depth studies are necessary, especially because carcinogenicity of the cleavage fragments is not completely excluded.

Although asbestos minerals have been banned, their non-asbestiform analogues are not yet univocally regulated as asbestos, despite their geometric ratios fit within the concept of fibres. We demonstrated that, under the same chemical composition, the morphological characterization of the phases could not be disregarded to evaluate carcinogenicity.

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