Potentially toxic elements (PTEs) associated with asbestos chrysotile, tremolite and actinolite in the Calabria region (Italy)

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Chemical Geology
https://doi.org/10.1016/j.chemgeo.2020.119896

Potentially toxic elements (PTEs) associated with asbestos chrysotile, tremolite and actinolite in the Calabria region (Italy)

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Abstract

Potentially toxic elements (PTEs) host in asbestos elongate mineral particles is one of the factors which have been invoked to explain their toxicity/pathogenicity effects.

This study quantifies and compares these elements in terms of major, minor and trace element concentrations (Si, Mg, Ca, Al, Fe, Mn, Cr, Co, Ni, Cu, Zn, Be, V, As, Rb, Sb, Ba, Pb, Sr) in various types of asbestos using micro X-ray fluorescence (μ-XRF) and inductively coupled plasma mass spectrometry (ICP-MS), in order to understand how they contribute to asbestos-related diseases. Chrysotile, tremolite asbestos and actinolite asbestos extracted from the Gimigliano-Mount Reventino Unit (Calabria Region, Southern Italy) were used for this study.

Among the investigated minerals high concentrations of Cr (171 ppm) and Be (2.9 ppm) were found in tremolite asbestos and chrysotile respectively. When calculating the pseudo-total concentrations of trace elements in the samples, the largest amounts were detected in tremolite asbestos, followed by actinolite asbestos and chrysotile. However, since other metals such as Mn and Fe (minor elements) are known to induce toxicity, and considering their input to the overall balance, actinolite contained the largest amount of PTEs and in this case chrysotile proved to be more toxic than

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tremolite asbestos. Furthermore, the potential leaching of PTEs, released by chrysotile, tremolite and actinolite asbestos-containing rocks, into the soil and water supply is also discussed. Since asbestos elongate mineral particles can be widespread in the environment (i.e. rocks, soil, water), it is essential to quantify the toxic elements present in asbestos elongate mineral particles in order to prevent asbestos-related diseases. The knowledge obtained from this study will provide us with a better understanding of asbestos-related lung cancer.

Keywords asbestos, toxic elements, elongate mineral particles, trace elements

1. Introduction

Asbestos has been classified as a carcinogenic substance (Group 1) by the International Agency for Research on Cancer (IARC 2012). Five types of asbestos belong to the amphibole group (actinolite asbestos, amosite, anthophyllite asbestos, crocidolite and tremolite asbestos) while chrysotile belongs to the serpentine mineral group (IARC, 2012). The industrial exploitation of asbestos for the fabrication of Asbestos Containing Materials (ACMs) began between 1860 and 1875; however, starting from the late seventies, asbestos demand declined due to the health risks associated with asbestos exposure (Alleman and Mossman, 1997). It is important to note that due to the widespread use of ACMs (e.g., hospitals, schools, gyms, cinemas, industrial plants) during the peak period of asbestos consumption around 1977 (Park et al., 2012), the handling of asbestos-containing materials is still a matter of global concern (Paglietti et al., 2012; Spasiano and Pirozzi 2017; Cannata et al., 2018).

Inhalation of contaminated air is the most common route of asbestos exposure (WHO, 2006). Once the asbestos elongate mineral particles are entered in the body, lungs or stomach (Di Ciaula, 2017), they can cause various chronic diseases that mainly affect the respiratory system. For this reason, asbestos has been banned in more than 50 countries (International Ban Asbestos Secretariat 2016; Spasiano and Pirozzi 2017).

However, since many studies have revealed that amphibole asbestos elongate mineral particles are more dangerous than chrysotile (WHO, 2015), many countries including Russia, China, Brazil (Frank and Joshi, 2014; U.S. Geological Survey, 2016) deem chrysotile to be "safer to use" for industrial purposes. Instead, countries like Canada and Colombia have recently changed their regulations and have introduced a ban on the production, use and sale of asbestos (CEPA, 1999; International Ban Asbestos Secretariat, 2019).

Another aspect to consider is that asbestos may also occur in the environment as natural components of rocks and soils (natural occurrence of asbestos; Gunter, 2018) that are often excavated for numerous civil engineering projects such as housing settlements, railway lines, motorways, etc. These anthropic activities may cause the release of dust particles containing asbestos elongate mineral particles, which pose a significant risk to human health; indeed, it has been widely demonstrated that the onset of diseases such as mesothelioma, lung cancer and asbestosis is linked to asbestos exposure (IARC, 2012; Harper, 2008; Baumann et al., 2015; Bloise et. al., 2017a). Moreover, ACMs disturbance caused by natural risks (e.g. earthquakes, hurricanes) and anthropic disasters (Perkins et al., 2007; Kashimura et al., 2015) may contribute to the release of elongate mineral particles (NIOSH, 2011; Gunter, 2018; Oberdörster and Graham, 2018).

The mechanisms associated with asbestos elongate mineral particles toxicity depend on various factors such as fibre dimensions, biopersistence, surface reactivity, chemical composition and the ability to generate Reactive Oxygen Species (ROS) (Roggli, 1989; Weiss, 1999; Mossman et al., 2011; Huang et al., 2011; Pugnaloni et al., 2013; Baumann et al., 2013; Liu et al., 2013; Turci et al., 2017; Jablonski, 2017). In addition to parameters such as morphometry, biodurability and surface activity, some authors (i.e. Schoonen et al., 2006), consider elongate mineral particles as complex crystal-chemical reservoirs that may release PTEs (e.g. Fe, Ni, Co, Cr) into the intracellular or extracellular environment during dissolution.

Indeed, there is epidemiologic and experimental evidence that PTEs such as some heavy metals are carcinogens and pose a significant threat to human health (e.g., Nemery, 1990; Censi et al., 2006;

Censi et al., 2011a, b). Moreover, some researchers theorize that asbestos elongate mineral particles may play only a passive role in producing diseases as carriers of trace elements (Dixon et al., 1970; Upreti et al., 1984; Nemery, 1990; Bowes and Farrow, 1997; Wei et al., 2014; Bloise et al., 2016a). There has recently been much debate on the role of cancer-causing agents, but it was only in the early 70s that some studies invoked the PTEs as an indicator of asbestos pathogenicity (Cralley et al., 1968; Dixon et al., 1970; Bloise et al., 2016a). It has been proved that high concentrations of trace elements in asbestos are capable of inducing lung cancer (Schreier et al, 1987; Wei et al., 2014). These studies are in agreement with the study conducted by Gazzano et al. (2005) who proposed using synthetic stoichiometric chrysotile elongate mineral particles (free of any toxic elements) as a negative control for toxicological studies since synthetic chrysotile elongate mineral particles do not have any *in-vitro* toxic effects.

In a pioneering study, Cralley et al. (1968) reported data showing the possible role of toxic elements in inducing asbestos-related cancer in bovines and textile workers. Gross et al. (1969) showed that toxic elements induced lung cancers in rats after they were exposed to asbestos dust containing large amounts of Ni, Cr and Co. Medical researchers focused on the physical properties of asbestos as a cause of lung cancer, but plant and animal ecologists have long claimed that trace metals associated with asbestos are the major causes of oxidative stress-induced cell death. For example, a high toxic metal concentration (i.e., Mn) was found in fish exposed to asbestos rich sediments (Schreier et al., 1987), while Pascal and Tessier (2004) showed that Mn is cytotoxic to human normal bronchial epithelial cells in vitro, and Hasegawa et al. (2008) demonstrated that high concentrations of Mn and Cu may increase one’s risk of developing lung cancer. Among the asbestos trace elements known to be hazardous to human health, Ni is considered the most hazardous because it damages DNA (IARC, 1984; Caicedo et al., 2007). Several studies have been published on the capability of Ni to generate ROS and on its toxicity at intracellular sites (Nackerdien et al., 1991; Salnikow et al., 2000; Kawanishi et al., 2001; Chen et al., 2003; Horie et al., 2009). More specifically, Ni can cause a variety of adverse health effects, such as lung fibrosis and cancer of the respiratory tract (Oller et al., 1997;

Seilkop et al., 2003; Leyssens et al., 2017). The adverse health effects of Cr^{3+} found in chrysotile are mainly due to the production of ROS (Scharf et al., 2014). Cr^{3+} can induce tissue damage, necrosis, and inflammation in patients with Cr associated adverse local tissue reactions. However, Cr^{3+} is not deemed to be as dangerous as Cr^{6+}, which is very harmful to humans due its high mobility and bioavailability. As for Co, although no previous studies have linked Co exposure to lung cancer, it is well-documented in literature that if Co is absorbed into the muscle tissues or the bloodstream, it can cause several neurological, cardiovascular and endocrine deficits (Leyssens et al., 2017). Zn is considered to be an essential element for the human body if people are exposed to small amounts. However, as demonstrated by Vanoeteren et al. (1986), excessive concentrations of Zn can seriously affect human health. The normal (Vanoeteren et al., 1986) Zn values reported for humans lie between 1 and 30 ppm.

Pb is a cumulative toxicant that can also have serious consequences for human health; in fact, if absorbed by the body it affects multiple body systems including the respiratory and digestive systems (Boskabady, 2018). Several researchers claim that Be stimulates the formation of ROS, similarly to the redox reactions observed for Fe and Ni, thus increasing asbestos carcinogenesis and lung cancer mortality rates (e.g. Dixon et al., 1970; Mancuso, 1970; Nackerdien et al., 1991; Salnikow et al., 2000; Kawanishi et al., 2001; Chen et al., 2003; Verma et al., 2003).

Asbestos minerals can host a large number of PTEs (i.e., Fe, Cr, Ni, Zn, Mn, Co and Rare Earth Elements) (Scambelluri et al., 1997; Tiepolo et al., 2007; Vils et al., 2008). Previous data reported in the literature concerning ophiolite-derived soils (e.g., Bloise et al., 2016b; Punturo et al., 2018) revealed the presence of a significant amount of PTEs such as Mn, Cr, Co, Ni. It is important to note that the leaching of asbestos due to soil deposits in streams and the weathering of asbestos-bearing rocks, reduces the concentrations of PTEs in the elongate mineral particles and increases them in the soil (Holmes et al., 1971; Kumar and Maiti, 2015). In this regard, it has been demonstrated that some acids alter the mineral surface of asbestos and remove considerable amounts of PTEs (Lavkulich et al., 2014). Therefore, resembling the original contents, soils developed from ophiolitic rocks are characterized by high concentrations of PTEs, in particular Mn, Cr, Ni and Co (Lyon et al., 1968; 1970; Atzori et al., 1999; Oze et al., 2004; Smith et al., 2007; Rajapaksha et al., 2012; Kelepertzis et al., 2013; Vithanage et al., 2014; Baumeister et al., 2015; Bloise et al., 2016b). Mistikawy et al. (2020), hypothesized that ultramafic rocks are rich in PTEs that can become bioavailable or easily mobilized. It is well known that in many countries, such as Italy, Argentina, India and Canada, asbestos large scale mining operations have caused toxic heavy metal pollution in soils, water, and the atmosphere (Cavallo et al., 2013; Kumar and Maiti, 2015). These heavy metals generally exceed, by up to one order of magnitude, the maximum concentration limits imposed by environmental agencies and governments (Caillaud et al., 2009; Tashakor et al., 2014). For example, it has been documented that in the GMRU, the concentration of some PTEs such as Cr, Ni, Co, V in serpentinite-derived soils, exceed the regulatory thresholds for public, private and green residential use (e.g., Punturo et al., 2018). These toxic elements can be mobilized, discharged into various terrestrial environments and absorbed into the body, thus representing a significant threat to public health. Also Cr contamination is a significant environmental challenge in areas where ophiolitic outcrops are abundant.

Moreover, it is important to note that water interacting with ophiolite rocks is generally characterized by high concentrations of PTEs derived through the dissolution of primary solid phases (e.g., serpentines and amphiboles). Apollaro et al. (2011) claim that ophiolites are known sources of dissolved Cr as well as of Ni, Cu, Zn and Pb in the waters coming from the ophiolitic outcrops of the GMRU. Ophiolitic rocks contain large amounts of Cr^{3+} in their minerals (e.g., chrysotile, tremolite-actinolite asbestos, spinels) which is oxidized to Cr^{6+} due to the weathering process and become highly mobile. Therefore, the characterization of asbestos minerals represents the first step for assessing contaminated soil and groundwater in ophiolitic outcrops.

In this scenario, the aim of this study was to carry out a systematic and comparative analysis of PTEs in terms of major, minor (Si, Mg, Ca, Fe, Al and Mn, as oxide) and trace elements (Cr, Co, Ni, Cu, Zn, Be, V, As, Rb, Sb, Ba, Pb, Sr) concentrations in three types of asbestos (i.e., chrysotile, tremolite and actinolite) using micro X-Ray Fluorescence (μ-XRF) and Inductively Coupled Plasma mass spectrometry (ICP-MS), in order to understand how they contribute to numerous health problems. Chrysotile, tremolite asbestos and actinolite asbestos were selected for this study and the samples were collected from Gimigliano-Mount Reventino Unit (GMRU) (Calabria Region, Italy) (Zakrzewska et al., 2008; Punturo et al., 2015). In this area several deposits of serpentinite and metabasite rock containing chrysotile, tremolite asbestos and actinolite asbestos have been mapped and reported (Bloise et al., 2012; 2014; 2016b; Bloise and Miriello, 2018; Bloise et al., 2020). According to the Italian National Mesothelioma Register, 70 mesothelioma deaths, caused by occupational and environmental exposure to asbestos minerals, were recorded in the Calabria region between 1993 and 2015 (INAIL, 2015), thus confirming the presence of asbestos in the environment. For example, Campopiano et al. (2017) identified tremolite asbestos in the pulmonary tissue of goats, sheep and two boars living near disused quarries in the Calabria Region. Recently, Colombino et al. (2019) described a case of malignant peritoneal mesothelioma caused by asbestos exposure (chrysotile and tremolite/actinolite) in a wild boar living in the same area, thus demonstrating a relationship between the neoplasia and exposure to natural occurrence of asbestos outcrops in the Mount Reventino (Calabria Region). Since there is strong evidence of a relationship between lung cancer and asbestos and co-occurring PTEs (Dixon et al., 1970; Nemery, 1990; Wei et al., 2014), the comparative evaluation of the amount of PTEs contained in the samples enabled us to determine which elements play a role in asbestos toxicity. Moreover, until now no studies have evaluated PTEs concentration associated with actinolite asbestos, in this regard, this paper attempts to fill this gap in academic literature.

2. Materials and methods

The Calabria region (southern Italy) is characterized by wide occurrence of ophiolitic rocks which are used as building and ornamental stones; "Verde Calabria" is the commercial name for this local stone (Bloise et al., 2014; Punturo et al., 2015; Bloise et al., 2016b; Punturo et al., 2018). The exploitation sites of these rocks, some of which are still active, are located in the Gimigliano-Mount Reventino Unit (GMRU) (Punturo et al., 2018). In this study, asbestos minerals coming from ophiolitic outcrops belonging to the GMRU have been investigated; specifically:

a) Chrysotile, from a road cut close to San Mango D'Aquino town. This specimen, also contains minor impurities of antigorite (Punturo et al., 2015); b) tremolite asbestos from an abandoned metabasite quarry located in the area of Mount Reventino (Bloise and Miriello, 2018); c) actinolite asbestos from an abandoned quarry close to the towns of Conflenti. This specimen contains very minor impurities of talc and cordierite (Bloise, 2019). The complete and exhaustive mineralogical and physical characterization of the asbestos considered in this study, can be found in: (Punturo et al., 2015; Bloise and Miriello, 2018; Bloise, 2019).

Elongate mineral particles were manually selected and disaggregated with the aim of choosing those without any evident chemical alteration or impurities by using optical microscope Zeiss Axioskop 40 under reflected light conditions.

Major and minor elements (Si, Mg, Ca, Fe, Al, Mn) as oxide were quantified by means of micro X-ray fluorescence (μ-XRF) analysis. The equipment used is a Bruker M4 Tornado spectrometer, equipped with two X-ray tubes (Rh and W) and two SDD detectors, active area of 60mm^2. The Rh tube has a polycapillar optic to concentrate the radiation in a spot <20μm (Mo-Kα). The data for each point has been acquired in vacuum conditions (2 mbar) using Rh radiation with the generator operating at 50 kV and 150 μA, using two detectors to increase the intensity of the received signal. The acquisition time for each measurement was 60 s. In this study, sixty spot analyses were performed on each sample. This technique allows to study micrometric compositional variations. To improve the elements detection, each point was measured twice, once without a primary filter for the quantification of major elements and another time with a primary filter composed of three superimposed layers of Al (100 microns), Ti (50 microns) and Cu (25 microns). This produces a significant reduction of the background up to 15 keV, improving sensitivity to minor and trace elements, especially those ones whose spectral lines are between 4 and 14 keV. The acquired spectra were processed with the software ESPRIT M4 v. 1.5.2.65 to obtain a semi-quantitative analysis expressed as wt.% of major elements (oxides) and ppm for minor elements.

Cr, Co, Ni, Cu, Zn, Be, V, As, Rb, Sb, Ba, Pb and Sr were determined by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Fe and Mn analyzes were also obtained through ICP-MS as well as µ-XRF. Analyses were conducted by means of ICP-MS AGILENT 7800. For this analysis, 0.1 g of sample powder is digested with HNO_3 + HF in high pressure (90 bar) vessels in Milestone Microwave. The accuracy (i.e., the relative difference from reference values) was generally better than 8%. To calibrate the equipment, the analytical service has used certified standard dilutions (Panreac) of the different elements (1000 mg/l each) grouped in a multi-elemental patron. Data collection was possible by using the common experimental procedure used in the Mass Spectroscopy Laboratory of the University of Calabria (Italy) (e.g., Bloise et al., 2016a). Cr, Co, Ni, Cu, Zn, Be, V, As, Rb, Sb, Ba, Pb and Sr were determined by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS), due to the very low detection limit of this equipment (Table 2) compared to µ-XRF.

3. Results

3.1. Major and minor elements

Major and minor elements (Si, Mg, Ca, Fe, Al and Mn) as oxide were measured in chrysotile, tremolite and actinolite asbestos (Fig.s 1, 2 and 3) contained in rocks that belong to the Gimigliano - Mount Reventino Unit (Calabria Region, Italy) by means µ-XRF. As regards the major elements in chrysotile, SiO_2 and MgO contents were 46.41 wt.% and 48.78 wt.% respectively, with minor amounts of CaO (1.37 wt.%) and FeO (3.22 wt.%) (Fig. 4a, Table 1). These values are in agreement with those detected in chrysotile and reported in literature (Morgan and Craley, 1973; Bloise et al., 2016a).

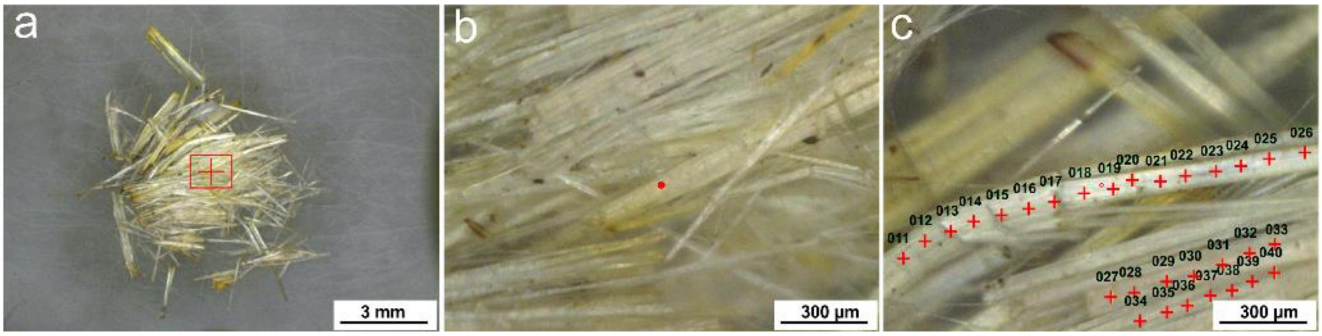

Fig.1. Micro-X-ray fluorescence (μ-XRF) images of chrysotile from GMRU. a) Straw-yellow chrysotile elongate mineral particles, from San Mango D'Aquino (Calabria Region, Italy). b) chrysotile bundles, c) chrysotile elongate mineral particles with some analysis points, note (top left corner) the curvature that suggests the flexibility of the elongate mineral particle.

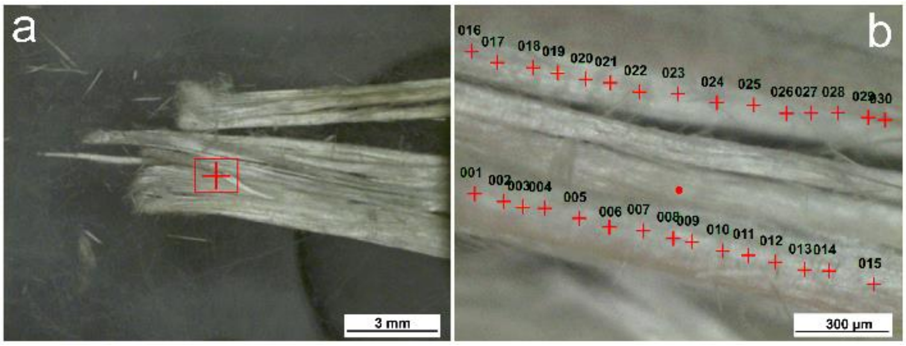

Fig. 2. Micro-X-ray fluorescence (μ-XRF) images on the tremolite asbestos from GMRU. a) white tremolite asbestos, from an abandoned quarry in Mt. Reventino (Calabria Region, Italy), b) tremolite asbestos with some analysis points.

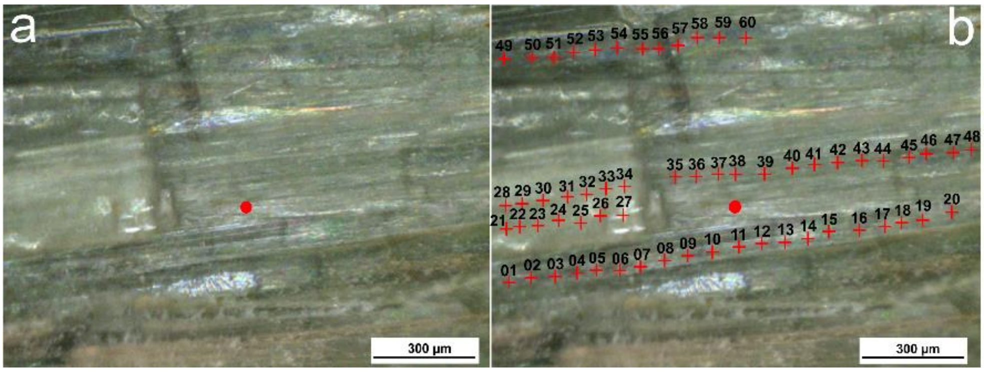

Fig. 3. Micro-X-ray fluorescence (μ-XRF) images of actinolite asbestos from GMRU. a) green actinolite asbestos, from an abandoned quarry close to the town of Conflenti (Calabria Region, Italy), b) actinolite asbestos with some analysis points.

(wt.%)	Ctl	Tr	Act
MgO	48.78 (0.72)	27.12 (3.90)	22.96 (0.56)
SiO_2	46.41 (0.82)	55.79 (5.81)	54.06 (0.70)
CaO	1.37 (0.96)	14.99 (1.86)	11.29 (0.64)
FeO	3.22 (0.82)	1.80 (0.20)	9.07 (0.76)
Al_2O_3	0.02 (0.01)	0.34 (0.16)	0.38 (0.12)
MnO	0.17 (0.05)	0.20 (0.05)	0.75 (0.11)

Table 1. Average values of major and minor element concentrations (wt.%) in the investigated asbestos elongate mineral particles from GMRU obtained by µ-XRF. Ctl = chrysotile, Tr = tremolite asbestos, Act = actinolite asbestos. Standard deviations in brackets.

For the amphiboles, the tremolite asbestos samples show SiO_2 and MgO average values of 55.8 wt.% and 27.12 wt.%, while in actinolite asbestos samples the content is 54.06 wt.% and 22.96 wt.% (Fig. 4b, Table 1) respectively. CaO was more abundant in tremolite asbestos, with values of 15.0 wt.%, compared to actinolite asbestos whose concentration was 11.3 wt.%. As expected, actinolite asbestos showed higher FeO contents, reaching 9.07 wt.% (Fig. 4c; Table 1) versus 1.80 wt.% of tremolite asbestos and 3.22 wt.% of chrysotile (Fig. 5). As far as, the minor elements are concerned, the data revealed a Mn content >1000 ppm in all of the samples as well as Al in tremolite asbestos and actinolite asbestos (Fig. 5, Table 1). In particular, the data showed a Mn content of 0.17 wt.% in chrysotile, 0.20 wt.% in tremolite asbestos and 0.75 wt.% in actinolite asbestos. Al, was present in small amounts in chrysotile (0.02 wt.%) and was more abundant in tremolite asbestos (0.34 wt.%) and actinolite asbestos (0.38 wt.%) (Fig. 5).

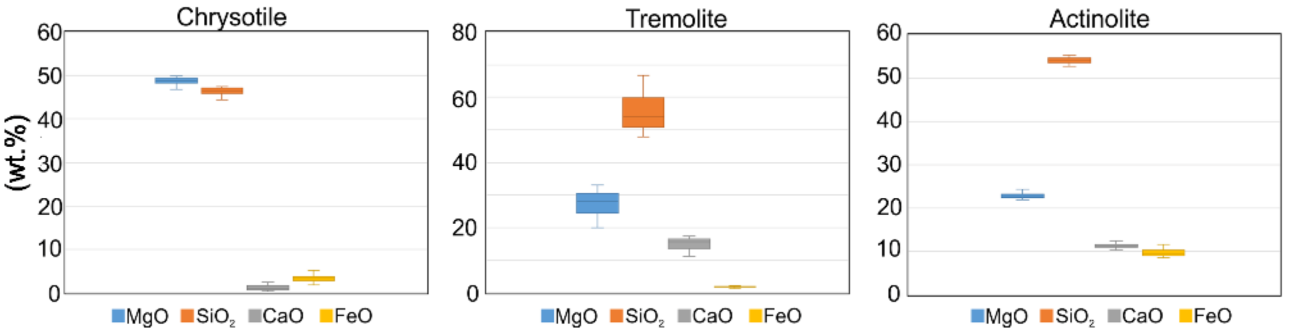

Fig. 4. Box plots showing statistical parameters for contents (wt.%) of MgO, SiO_2, CaO, FeO, in chrysotile, tremolite and

actinolite asbestos from GMRU. Statistical parameters are based on 60 analyses. Vertical lines show the range in contents, the horizontal line inside the box represents the median value.

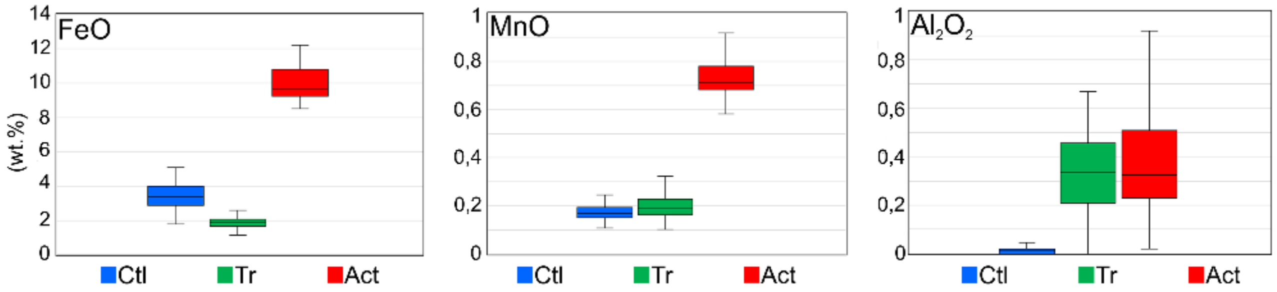

Fig. 5. Box plots showing statistical parameters for contents (wt.%) of FeO, MnO and Al_2O_3, in chrysotile, tremolite and actinolite asbestos from GMRU. Statistical parameters are based on 60 analyses. Vertical lines show the range in contents, the horizontal line inside the box represents the median value.

3.2. Trace elements

Table 2 shows the concentrations of the trace elements (Cr, Co, Ni, Cu, Zn, Be, V, As, Rb, Sb, Ba, Pb, Sr) in each sample, obtained using ICP-MS. The results show that the trace metal concentrations found in chrysotile, tremolite and actinolite asbestos differ (Fig. 6). Fe and Mn whose concentrations were also acquired by means ICP-MS were in line with the data acquired by means µ-XRF.

(ppm)	**Concentration range in human lungs***	**Ctl**	**Tr**	**Act**	**D.L.**
Fe**	40 - 500	25000	14000	75100	0.01
Mn**	0.01 - 3	1186.8	678.2	3874.2	0.01
Cr	0.002 - 0.50	5.50	170.91	14.86	0.20
Co	0.002 - 0.1	1.89	22.64	4.69	0.05
Ni	0.01 - 1.00	4.32	308.63	14.50	0.09
Cu	1 - 5.00	10.52	24.53	19.39	0.06
Zn	1 - 30.00	65.47	28.42	46.56	1.90
Pb	0.02 - 0.50	2.40	4.44	23.29	0.04
V	0.0005 - 0.50	6.88	7.07	11.06	0.10
Be	0.0001 - 0.03	2.90	0.30	1.10	0.01
As	0.001 - 0.10	7.00	1.20	1.50	0.40
Rb	0.5 - 10.00	0.70	0.40	0.70	0.30
Sb	0.002 - 0.10	0.20	0.20	0.60	0.01

Ba	>1.10	11.40	14.8	14.1	0.07
Sr	0.01 - 1.00	10.70	200.0	47.0	0.03

Table 2. Trace element concentrations (ppm) in the investigated asbestos elongate mineral particles from GMRU obtained by ICP-MS. Ctl = chrysotile; Tr = tremolite asbestos; Act = actinolite asbestos. *Indicative baseline data for some trace elements in normal human lung tissues (Vanoeteren et al., 1986). D.L. = detection limit; **Minor element.

Special attention has been paid to the content of some metals (i.e. Cr, Co, Ni, Cu, Zn) whose summation Σ(Cr, Co, Ni, Cu, Zn) was the highest in tremolite asbestos, reaching a value of 555 ppm, followed by actinolite asbestos (100 ppm) and chrysotile (88 ppm). In particular, Cr concentration was 5.5 ppm in chrysotile, 171 ppm in tremolite asbestos and 15 ppm in actinolite asbestos, while Co showed lower values of 1.89 ppm in chrysotile, 22.64 ppm in tremolite asbestos and 4.69 ppm in actinolite asbestos. The highest concentrations of Ni and Cu were found in tremolite asbestos, followed by actinolite asbestos and chrysotile. Ni content was 4.32 ppm in chrysotile, 308.63 in tremolite asbestos and 14.50 in actinolite asbestos, while Cu was present at a concentration of 10.52 ppm in chrysotile, at 24.53 ppm in tremolite asbestos and at approximately 19.39 ppm in actinolite asbestos. Zn were detected in chrysotile reaching values of 65.47 ppm, followed by actinolite asbestos (46.56 ppm) and tremolite asbestos (28.42 ppm).

Figure 7 shows the concentration (ppm) patterns of trace elements Be, V, As, Rb, Sb, Ba, Pb, and Sr found in chrysotile, tremolite and actinolite asbestos. It is important to note that the concentrations of the trace elements V, Rb and Ba were quite similar in all three samples or showed slight differences (e.g. Be, Sb). However, As, Pb and Sr data highlight more heterogeneous values. In fact, the highest concentration of As was detected in chrysotile with a value of 7.0 ppm, while similar concentrations were found in tremolite and actinolite asbestos: 1.20 ppm and 1.50 ppm respectively. As regards the Pb and V values, the data revealed that the highest concentrations were detected in actinolite asbestos followed by tremolite asbestos and chrysotile. Indeed, Pb content was approximately 2.40 ppm in chrysotile and reached 4.44 and 23.29 ppm in tremolite and actinolite asbestos respectively. V, was present at concentrations of approximately 6.88 in chrysotile, 7.07 ppm in tremolite asbestos and

11.06 ppm in actinolite asbestos. The Sr content, was higher in tremolite asbestos in which reached 200.0 ppm compared to actinolite asbestos (47.0 ppm) and chrysotile (10.7 ppm). For Be content, it was 2.90 ppm in chrysotile which was higher than the Be concentrations found in tremolite asbestos (0.30 ppm) and actinolite asbestos (1.10 ppm). As a resume, the obtained data highlight that chrysotile sample contained the highest concentrations of As (7.0 ppm) and Be (2.9 ppm), tremolite asbestos had the highest amount of Sr (200 ppm) and Ba (14.8 ppm) while actinolite asbestos is characterized by the highest values of Pb (23.29 ppm) and V (11.06 ppm) (Fig.7).

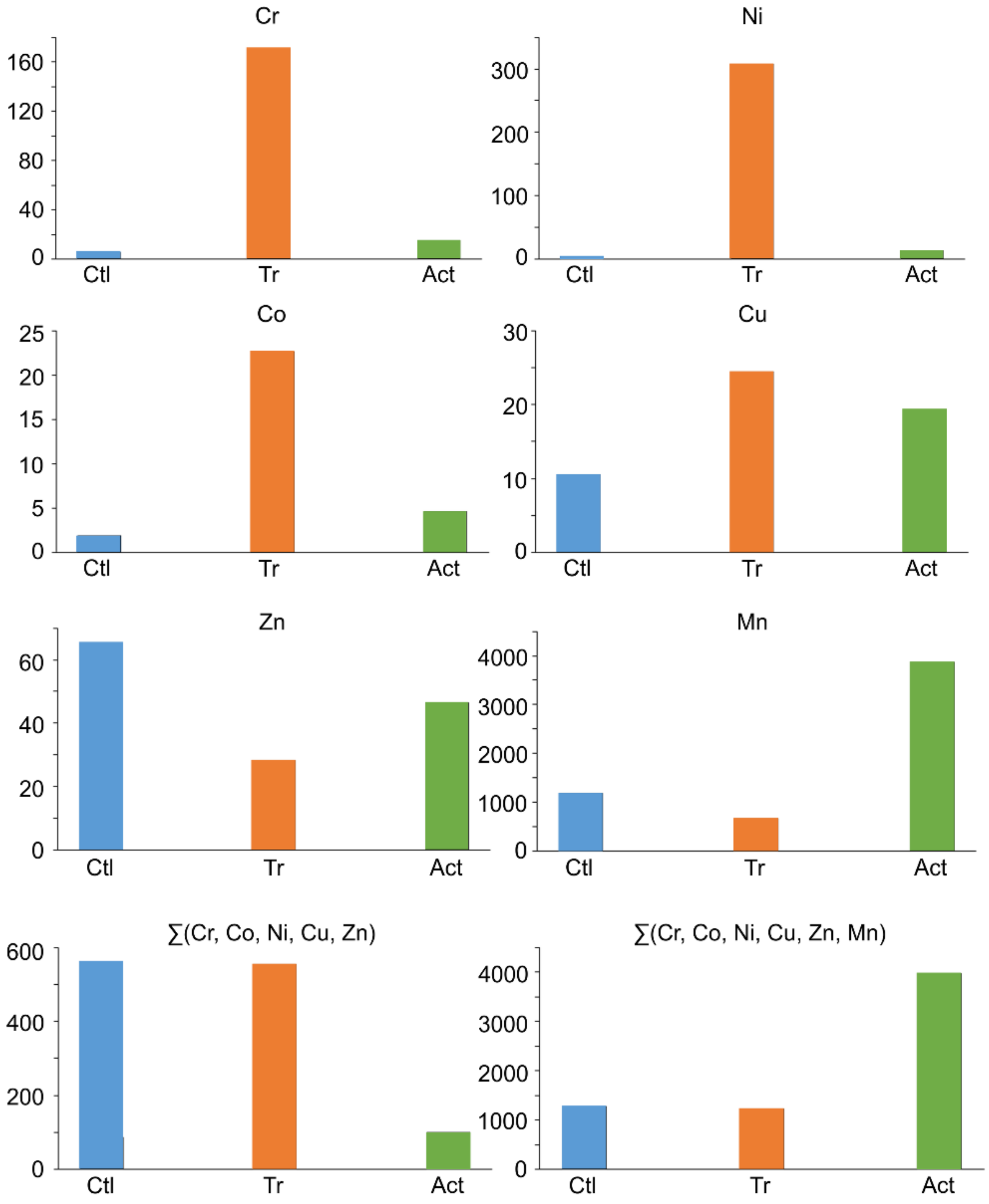

Fig. 6. Concentration of heavy metals (ppm) in the investigated asbestos elongate mineral particles from GMRU. Ctl = chrysotile; Tr = tremolite asbestos; Act = actinolite asbestos. Each element has an error, measured as relative standard deviation (RSD%), of around 1%.

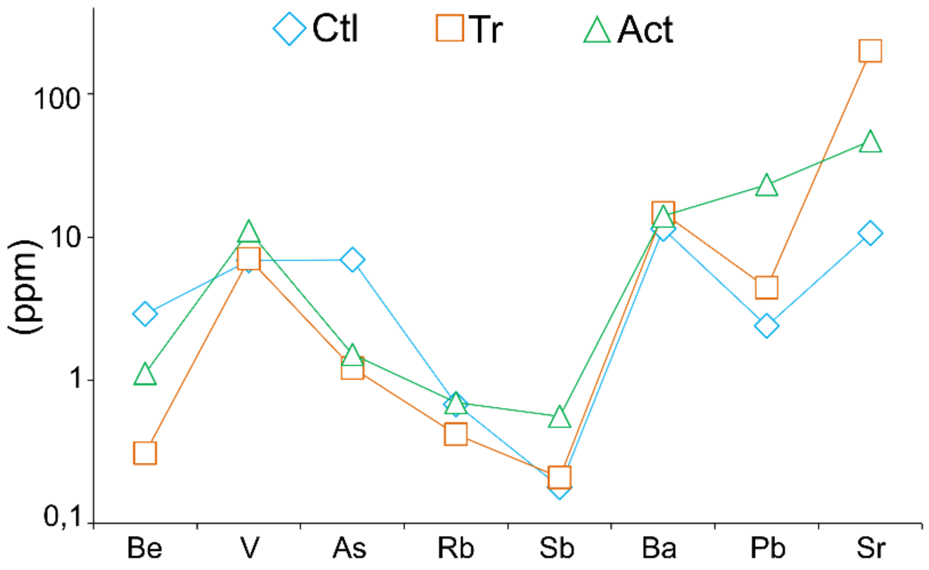

Fig. 7. Trace elements concentration (ppm) patterns in the investigated asbestos elongate mineral particles from GMRU. Ctl = chrysotile; Tr = tremolite asbestos; Act = actinolite asbestos. Y-axis is in logarithmic scale. Each element has an error, measured as relative standard deviation (RSD%), of around 1%.

4. Discussion

4.1. Potentially toxic elements for the human body

Due to the scientific evidence demonstrating the relationship between lung cancer mortality and cumulative exposure to PTEs, the asbestos toxicity model proposed by Gualtieri (2018) states that it is essential to quantify the toxic elements present in the asbestos. In this study, lower concentrations of the trace metals Cr, Co and Ni were found in the chrysotile samples than those found in the tremolite and actinolite samples. The role of Mn in the balance content of toxic metals in the studied samples is crucial. Indeed, as illustrated in Fig. 6, considering the contribution of Mn (∑ Cr, Co, Ni, Cu, Zn, Mn), the sample containing the largest amount of trace metals was actinolite (3974 ppm) followed by chrysotile (1274 ppm) and tremolite (1233 ppm). However, without taking the contribution of Mn into consideration, the highest concentrations of trace metals were found in tremolite at a total value of 553 ppm, followed by actinolite (100 ppm) and chrysotile (88 ppm). Therefore, the Mn content influences the most hazardous fibre definition related to the content of PTEs. The other trace elements like Be, V, As, Rb, Sb, Ba, Pb and Sr are present in different amounts in the samples. Considering the total amount of these elements (Table 3) in the samples, tremolite showed the highest value (228 ppm) followed by actinolite (99 ppm) and tremolite (42 ppm). The high concentration of Be in chrysotile (3 ppm) may have an effect on the interaction of the elongate mineral particles with the biological system. In addition to Be, the highest concentrations of As which is considered a cytotoxic element, were found in chrysotile (Fig. 7). Indeed, numerous studies have examined the relationship between arsenic exposure and intake and increased lung cancer mortality (Nackerdien et al., 1991; Salnikow et al., 2000; Kawanishi et al., 2001; Chen et al., 2003; IARC, 2012). There is still much debate on the genotoxic properties of vanadium, previous studies have found associations between airborne vanadium particles in the air and lung cancer (Leonard and Gerber, 1994; Rodríguez-Mercado et al., 2011). Another aspect highlighted by Gualtieri et al. (2019) is that the contents of V released from asbestos fibres are very low and should not represent a major health concern. The differences in PTEs (Be, V, As, Pb, Sb, Ba, Pb and Sr) concentration patterns of chrysotile, tremolite asbestos and actinolite asbestos were mainly attributed to the geochemical variable involved in their genesis (e.g., pressure, temperature, availability of elements) in different places. Nevertheless, Be, V, As, Pb, Sb, and Ba showed similar concentration patterns for both tremolite asbestos and actinolite asbestos, except for Pb and Sr. As regards chrysotile, in addition to the geochemical variability involved in its genesis, the different concentration from amphiboles is also due to crystallographic differences among them (Ballirano et al., 2017). Indeed, compared to amphibole, chrysotile showed lower concentration pattern of Sb, Ba, Pb and Sr (Fig. 7), due to the fact that elements with large ionic radii (> 1Å, e.g., Sb, Ba, Pb and Sr) cannot replace Si or Mg in tetrahedral or octahedral sites, since elements with large ionic radius (> 1Å) in chrysotile were hosted to the hollow core present within its fibrils, which act as trapping locations for elements with large ionic radii (Ballirano et al., 2017). Differently, elements such as Be, V and As (ionic radius < 1Å) can be hosted into the crystallographic sites of both amphiboles and chrysotile, and therefore variation of these elements is linked to their geochemical availability rather than to crystallographic reasons.

(ppm)	Σ(Cr, Co, Ni, Cu, Zn)	Σ(Cr, Co, Ni, Cu, Zn, Mn)	Σ(Cr, Co, Ni, Cu, Zn, Mn, Fe)	Σ(Be, V, As, Rb, Sb, Ba, Pb, Sr)	Σ(Cr, Co, Ni, Cu, Zn, Mn, Fe, Be, V, As, Rb, Sb, Ba, Pb, Sr)
Ctl	88	1275	26275	42	26317
Tr	555	1233	15233	228	15461
Act	100	3974	79074	99	79173

Table 3. Sum (Σ) elements concentration (ppm) of Ctl = chrysotile; Tr = tremolite asbestos; Act = actinolite asbestos, from GMRU.

4.2. The role of iron

In addition to the metals mentioned above, several researchers have suggested that, even in trace amounts, iron plays an important role in asbestos-induced cytotoxicity (Shukla et al., 2003).

There are experimental evidence which demonstrate that iron at the surface of elongate mineral particles (Pollastri et al., 2015; Andreozzi et al., 2017) as well as iron content and its structural coordination are essential factors which prompt the formation of toxic hydroxyl radicals with cyto- and genotoxic effects. (Bonneau et al., 1986; Fantauzzi et al., 2010; Pacella et al., 2020). In fact, all natural asbestos elongate mineral particles contain iron either as a substitution for Mg (e.g. chrysotile, tremolite) ions or as a constituent of the mineral structure (crocidolite, amosite; Turci et al., 2017). It has also been demonstrated, that surface ferrous ions constitute the catalytic sites where ROS and free radicals are produced (Pezerat et al., 1989; Fubini and Otero Aréan 1999; Favero-Longo et al., 2005; Gazzano et al., 2005). It is important to note that free radical generation may be induced by reactions in the presence of H_2O_2 (Fenton reaction) and in the absence of H_2O_2 (Haber-Weiss Cycle). After chrysotile has been inhaled, it dissolves and releases Fe as well as other metals (i.e. Ni, Cr, Mn; Turci et al., 2010), inducing toxic effects such as ROS production, oxidative stress, DNA damage, lipid peroxidation and protein modification (Jomova and Valko, 2011). When amphibole asbestos is inhaled, it suffers a limited surface dissolution creating a persistent physical response and biochemical action (in terms of surface activity of iron prompting ROS production; Bernstein et al., 2013; Turci et al., 2017). Consequently, it is possible to deduce that the fast dissolution rate of chrysotile should prompt increased release of available surface-active Fe in the first stage of dissolution despite the small differences in the Fe concentrations in chrysotile (ideally 0-4 wt%), tremolite asbestos (ideally 0-4 wt%) and actinolite asbestos (ideally 7-10%; Gualtieri, 2018; Gualtieri et al., 2018). Our results showed that the highest concentration of Fe was in the actinolite asbestos (9.07 wt%), followed by chrysotile (3.20 wt%) and tremolite asbestos (1.80 wt%) (Table 1). As shown in Fig. 8 and Table 3, considering the contribution of Fe (∑ Cr, Co, Ni, Cu, Zn, Mn, Fe), the sample containing the largest amount of heavy metals proved to be actinolite asbestos (79074 ppm) followed by chrysotile (26275 ppm) and tremolite asbestos (15233 ppm).

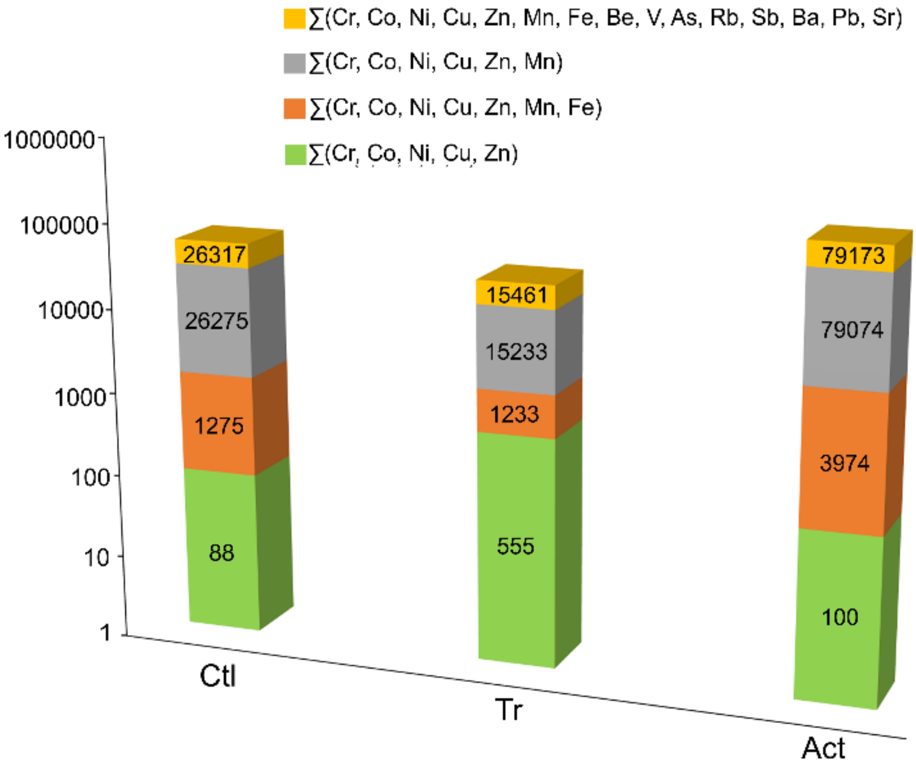

Fig. 8. Sum of PTEs concentrations (ppm) in the investigated asbestos elongate mineral particles samples Ctl = chrysotile; Tr = tremolite asbestos; Act = actinolite asbestos from GMRU. Y-axis is in logarithmic scale.

4.3. Bio-durability

An important factor that influences the degree of fibre toxicity is asbestos bio-durability. In fact, the elevated levels of PTEs in the human body caused by inhaling elongate mineral particles is strictly dependent on various factors, including the solubility of elongate mineral particles. The solubility is defined as the maximum amount of the fibre that dissolves in body fluids (Turci et al., 2017). It determines the bio-durability, that is the residence time of the elongate mineral particles in the organism, and therefore it is probable that adverse interactions occur between the mineral fibre and its biological surroundings (Turci et al., 2017). Asbestos changes in the chemical properties below pH 8.0 (i.e., Bernstein et al., 2013), and PTEs associated with asbestos elongate mineral particles can be released into the lungs. As demonstrated by many authors (Hesterberg et al., 1998; Bernstein et al., 2013; Rozalen et al., 2014; Gualtieri et al., 2018; Gualtieri, 2018), the solubility of chrysotile is higher than that for amphibole asbestos. For example, in a recent study Gualtieri et al. (2018) carried out an *in-vitro* comparative study of chrysotile asbestos (UICC, standard Chrysotile "B", Canadian NB #4173-111-1) and amphibole asbestos (i.e., crocidolite and amosite) at pH 4, in order to determine their dissolution rates. Chrysotile undergoes fast dissolution (few months) and releases its PTEs cargo into the lung environment in a short period of time, while amphibole slowly releases its toxic cargo over a long time span (tens of years). Therefore, in our case, it is highly probable that PTEs contained in chrysotile elongate mineral particles are rapidly released into the extracellular medium (Bloise et al., 2016a). In this scenario, under the hypothesis that the other parameters are equal (e.g., fibre dimensions, biopersistence, surface reactivity), our samples with various concentrations of PTEs in their structure, should show different *in-vitro* toxicity levels. Observing the concentrations of ∑ Cr, Co, Ni, Cu, Zn, Mn, Fe, Be, V, As, Rb, Sb, Ba, Pb and Sr which were considered representative of the family of all of the toxic elements present in these natural elongate mineral particles, actinolite asbestos (79173 ppm) proved to be the sample with the highest concentration of PTEs, followed by chrysotile (26317 ppm) and tremolite asbestos (15461 ppm) (Fig. 8; Table 3). Moreover, it should also be considered that chrysotile showed higher values of Be and As compared to other samples, and it is less bio-durable than amphibole asbestos. These potentially toxic elements present in asbestos elongate mineral particles could play an important role in the pathogenesis of human lung cancer (Dixon et al., 1970; Nemery, 1990; Wei et al., 2014). If these trace elements accumulate in sufficient amounts in the lungs, via fibre dissolution, they may cause lung cancer (e.g., mesothelioma and bronchogenic carcinoma) (Dixon et al., 1970; Nemery, 1990; Wei et al., 2014), as it will alter the baseline levels of these elements in normal human lung tissue, which has not been affected by disease (Table 2; Vanoeteren et al., 1986).

4.4. Toxic elements in the environment

A considerable number of PTEs could be partly contained in the structure of amphibole and chrysotile asbestos as isomorphic substitutions in certain crystallographic sites (Morgan and Cralley, 1973; Bloise et al., 2009a, 2010; Ballirano et al., 2017) and partly as components of magnetite and chromite contamination in asbestos (Kumar and Maiti, 2015; Bloise et al., 2020a).

The Calabria region is characterized by numerous areas of ophiolite outcrops (Punturo et al., 2004; Cirrincione et al., 2015; Ricchiuti et al., 2020) which are potential sources of asbestos elongate mineral particles and PTEs (e.g. Mn, Cr, Co and Ni) and are released into the environment due to naturally occurring processes (drainage, leaching) and also due to anthropic activities (mining, excavation, landscape modification), resulting in the contamination of soil, water and air (e.g., Bloise et al., 2016b; Cannata et al., 2018; Gwenzi, 2019; Ricchiuti et al., 2020).

The relatively high concentrations of PTEs found in the studied asbestos samples (Fig. 8; Table 3) makes them potentially available for subsequent accumulations in water and soil that can adversely affect human health. These toxic elements could be leaching off ophiolitic host rocks and their respective sediments causing a significant increase in PTEs concentrations in soils and groundwaters. A strong correlation has been found between high levels of PTEs in the air, water and soil and human disease (e.g., Gwenzi, 2019) as they can trigger deadly pathologies.

4.5. Cross-country comparison of asbestos samples

Even if different analytical techniques were used, we compared our data to those obtained by other authors for the same mineral species, in order to gain a better understanding of how toxic elements may contribute to the overall toxicity of the elongate mineral particles. Iron data of actinolite asbestos from GMRU are in line with those ones previously reported in the literature (Table 4; Pollastri et al., 2017; Bloise, 2019). As expected, among the investigated minerals actinolite asbestos is the mineral with the highest iron content (Fig. 9).

The FeO found in the chrysotile samples from GMRU is slightly higher than the amounts found in chrysotile from Canada, Balangero (Italy) and Val Malenco (Italy), (Table 4) studied by Pollastri et al. (2016). As regards tremolite asbestos, the FeO content in the sample analysed for this work was 1.8 wt.% (Fig. 9) which is lower than the values obtained for the tremolite asbestos from Castelluccio (Italy), Maryland (USA), Mount Rufeno (Italy), San Mango (Italy) and Ala Stura (Italy) studied by Pacella et al. (2010) and in line with the data of tremolite asbestos from Decollatura (Italy) (Table 4). Finally, similar amounts of FeO were found in our studied actinolite asbestos (9.07 wt.%), compared to the actinolite asbestos samples studied by Pollastri et al. (2017) and collected from the Aurina Valley which showed a value of 7.77 wt.% (Fig. 9; Table 4).

As regard, Cr, Co, Ni, Cu, Zn and Mn, different results were obtained when we compared the values of the analysed samples, with those reported in literature (Morgan and Cralley 1973; Bloise et al. 2016a), depending on the outcrop where the chrysotile specimens were collected (Table 5). In fact, as shown in Fig. 10a, chrysotile from GMRU is characterized by ∑ Cr, Co, Ni, Cu, Zn, Mn at 1274 ppm, which is lower than UICC chrysotile from Canada (1750 ppm), Balangero (2574 ppm) and Val Malenco (2725 ppm) studied by Bloise et al. 2016a by means ICP-MS (Table 5)

As regards tremolite asbestos, the sample analysed showed the highest PTEs. By comparing our data set with the data obtained for tremolite asbestos from Val D’Ala studied by Bloise et al. (2016a), tremolite asbestos from GMRU contained a total amount of toxic metals (Cr, Co, Ni, Cu, Zn) equal to 555 ppm while in tremolite asbestos from Val D’Ala it reached 685 ppm.

Based on the results of the present study and those reported in literature (Table 5), actinolite from GMRU proved to be the asbestos sample with the highest concentration of PTEs while the similar values observed for the chrysotile samples collected from the GMRU and Canada outcrops (Bloise et al., 2016a) and tremolite asbestos from GMRU and Ala Stura outcrops demonstrated that they could be least toxic samples as they contain the lowest concentrations of PTEs. Nevertheless, it is worth remembering that, due to their different structural matrix, it has been highlighted that chrysotile is not as much biodurable as amphiboles (i.e. tremolite and actinolite asbestos) in simulated lung fluids (Bernstein et al., 2013, Gualtieri et al., 2018; see Bio-durability section). The fast dissolution rate of chrysotile prompts the full release of available PTEs in the host organism in a few months. However, thanks to this comparison, it was observed that Pb, V, As, Sb, Be were not always present in large amounts in our samples (Fig. 10b). As a matter of fact, chrysotile from GMRU revealed a total amount of Pb, V, As, Sb and Be of 19.40 ppm which is higher than those reported for chrysotile collected from Val Malenco (5.70 ppm) and lower than those reported for chrysotile from Balangero (56.10 ppm) and chrysotile UICC from Canada (20.40 ppm). As regards the tremolite asbestos from GMRU and Val D'Ala, the samples contained similar concentrations equal to 13.20 ppm and 13.80 ppm respectively.

These differences in PTEs concentrations may be due to the common chemical variability exhibited by amphibole elongate mineral particles (e.g., Hawthorne and Oberti 2007; Andreozzi et al., 2009) and to the various geochemical/petrological processes involved in the formation of chrysotile and amphiboles (Bloise et al., 2020). It is important to note that small variations in the amounts of PTEs may also be due to the different degrees of accuracy of the instruments used in the various laboratories.

Sample	**FeO**	**Detected by**	**References**
Chrysotile GMRU(Calabria, Italy)	3.22 (0.82)	µ-XRF	This work
Chrysotile Balangero (Piedmont, Italy)	2.9 (5)	EMPA	Pollastri et al., 2016
Chrysotile UICC (Quebec, Canada)	1.6 (3)	EMPA	Pollastri et al., 2016
Chrysotile Val Malenco (Lombardy, Italy)	1.4 (1)	EMPA	Pollastri et al., 2016
Tremolite asbestos Castelluccio (Basilicata, Italy)	2.06 (16)	EMPA	Pacella et al., 2010
Tremolite asbestos Maryland (Maryland, USA)	4.50 (77)	EMPA	Pacella et al., 2010
Tremolite asbestos Mount Rufeno (Latium, Italy)	2.23 (8)	EMPA	Pacella et al., 2010
Tremolite asbestos San Mango (Calabria, Italy)	2.97 (9)	EMPA	Pacella et al., 2010
Tremolite asbestos Ala Stura (Piedmont, Italy)	2.42(25)	EMPA	Pacella et al., 2010
Tremolite asbestos Decollatura (Calabria, Italy)	1.10 (n.d.)	EDS/SEM	Apollaro et al., 2018
Tremolite asbestos GMRU(Calabria, Italy)	1.80 (0.20)	µ-XRF	This work
Actinolite asbestos Aurina Valley (Trentino, Italy)	7.77 (5)	MS	Pollastri et al., 2017
Actinolite asbestos GMRU (Calabria, Italy)	9.07 (0.76)	µ-XRF	This work

Table 4. FeO (wt.%) in the investigated asbestos samples from GMRU (Gimigliano Mount Reventino Unit). Reference data of chrysotile from Balangero, from Canada UICC, from Val Malenco, tremolite asbestos from Castelluccio, from Maryland, from San Mango, from Ala Stura, from Decollatura, actinolite asbestos from Aurina Valley, are shown for

comparison; (estimated standard deviations in brackets). n.d. = not detected; µ-XRF = micro X-ray fluorescence; EMPA = electron microprobe analysis; MS = Mössbauer spectroscopy.

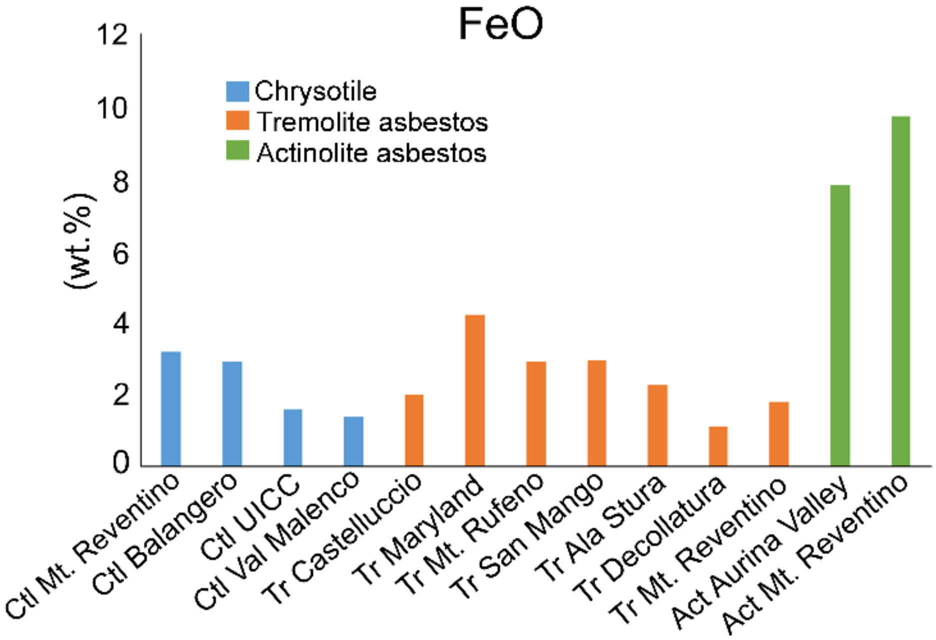

Fig. 9. FeO (wt.%) in the investigated asbestos samples from GMRU (Mt. Reventino). Ctl = chrysotile; Tr = tremolite asbestos; Act = actinolite asbestos. Reference data of chrysotile from Balangero, from Canada UICC, from Val Malenco, tremolite asbestos from Castelluccio, from Maryland, from San Mango, from Ala Stura, from Decollatura, actinolite asbestos from Aurina Valley, are shown for comparison.

(ppm)	**Cr**	**Co**	**Ni**	**Cu**	**Zn**	**Mn**	**Σ(Cr, Co, Ni, Cu, Zn)**	**Σ(Cr, Co, Ni, Cu, Zn, Mn)**
Chrysotile GMRU (Calabria, Italy)	5.50	1.89	4.32	10.52	65.47	1186.80	88	1274
Chrysotile Balangero (Piedmont, Italy)	1078.20	40.40	445.80	14.50	33.10	962.00	1612	2574
Chrysotile UICC (Quebec, Canada)	360.50	45.30	866.10	1.70	17.40	459.00	1291	1750
Chrysotile Val Malenco (Lombardy, Italy)	153.60	52.40	1576.20	29.70	22.70	890.00	1835	2725
Tremolite asbestos GMRU (Calabria, Italy)	170.91	22.64	308.63	24.53	28.42	678.20	555	1233
Tremolite asbestos Ala Stura (Piedmont, Italy)	165.00	26.90	473.00	3.20	17.20	879.90	685	1565
Actinolite asbestos GMRU	14.86	4.69	14.50	19.39	46.56	3874.20	100	3974

(Calabria, Italy)

Table 5. Trace element concentrations (ppm) in the investigated asbestos sample from GMRU obtained by ICP-MS. Reference data of chrysotile from Balangero, from Canada UICC, from Val Malenco, tremolite asbestos from Ala Stura are shown for comparison, as this sample was previously studied by Bloise et al., 2016a, all obtained by ICP-MS.

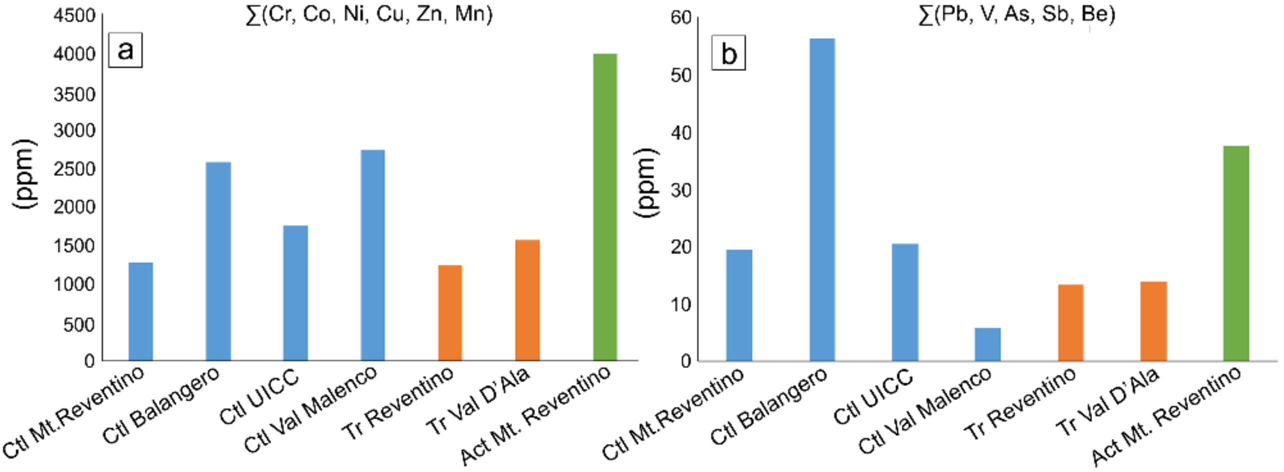

Fig. 10. a) Sum of PTEs (Cr, Co, Ni, Cu, Zn, Mn) concentrations (ppm) in the investigated asbestos samples from GMRU (Mt. Reventino), Ctl = chrysotile; Tr = tremolite asbestos; Act = actinolite asbestos. b) Sum of PTEs (Pb, V, As, Sb, Be) concentrations (ppm) in the investigated asbestos samples from GMRU. Reference data of a chrysotile from Balangero, from Canada UICC (standard Chrysotile “B”, NB #4173-111-1), from Val Malenco, asbestos tremolite from Ala di Stura are shown for comparison, as this sample was previously studied by Bloise et al., 2016a.

6. Conclusions

The purpose of this study was to determine the presence and quantity of PTEs that, as showed by the literature might have a role in asbestos toxicity. To this aim, the chemical data obtained by analysing three natural asbestos samples (chrysotile, tremolite asbestos, actinolite asbestos) from Gimigliano-Mount Reventino Unit (Calabria Region, southern Italy) were examined. The results show that, taking into account the contribution of heavy metals (i.e. Cr, Co, Ni, Cu, Zn) and their toxicity levels, it is possible to speculate that tremolite asbestos is the most toxic form of asbestos among the investigated minerals, followed by actinolite asbestos and chrysotile. The same trend is observed with other trace elements such as Be, V, As, Rb, Sb, Ba, Pb and Sr. However, considering the contribution of other metals such as Mn and Fe to the overall balance (i.e. Mn, Fe, Cr, Co, Ni, Cu, Zn, Be, V, As, Rb, Sb, Ba, Pb, Sr), actinolite asbestos proved to be the most toxic elongate mineral particles among the investigated minerals, while in this case chrysotile should be more toxic than tremolite asbestos. Moreover, it should also be considered that chrysotile showed higher values than Be and As and it is less biodurable than amphibole asbestos and therefore releases its PTEs cargo into the lung environment in a short period of time.

In view of the mesothelioma deaths caused by asbestos exposure in the Calabria region, this study supports the hypothesis that PTEs hosted in asbestos elongate mineral particles could be among factors which contribute to explain their toxicity/pathogenicity effects.

The data obtained for this paper may prove useful for determining the toxicity/pathogenicity potential of asbestos and for interpreting the results of *in-vitro* testing. As regards environmental pollution, it is essential to quantify the PTEs present in asbestos minerals in order to limit exposure and minimize the public health risks for people living in these geological contexts. In humans, exposure to asbestos minerals and toxic elements can cause lung cancer. In conclusion, the concomitant presence of asbestos minerals and PTEs in the environment poses a serious health threat to local populations. Due to the abundance of ophiolite outcrops worldwide, the results obtained from this study will be of global interest.

Acknowledgments

Financial support. The work has received analytical funding from the research group CHARROCK, at University of Salamanca (scientific responsible D. Pereira). The work has received financial support from the FFABR Fund (by the Italian MIUR) (scientific responsible A. Bloise). Part of Research was financed by University of Catania Piano triennale della ricerca (2017-2020) (scientific responsible R. Punturo).

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