Identification and Preliminary Toxicological Assessment of a Non-regulated Mineral Fiber: Fibrous Antigorite from New Caledonia

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Abstract: The rising awareness about the risk due to asbestos environmental exposure has led to a new interest in the investigation of non-regulated mineral fibers. Evidences of chronic diseases have been described in individuals exposed to naturally occurring asbestiform (NOA) minerals in Turkey (erionite), Italy (fluoro-edenite), and USA (winchite/ rictherite). In New Caledonia, an increased incidence of asbestos-related diseases was correlated with the natural occurrence of fibrous serpentines, chrysotile and fibro-lamellar antigorite, in outcrops, roadways and soils. A minor amount of tremolite asbestos was also observed, increasing the health hazard. By adopting a precautionary principle, New Caledonia legislation classified antigorite as regulated asbestos, even if a limited toxicity assessment is available. Caledonian antigorite exhibits a wide range of natural shapes, morphologies and degrees of alteration, as a result of pedogenic alteration induced by sub-tropical conditions. As the alteration increases, lamellar antigorite gradually cleaves into fibrous-like particles assuming a fibro-lamellar habit. An increase in the emission of inhalable (potentially asbestiform) fibers in air was observed. To understand this mechanism, a multidisciplinary mineralogical and geochemical investigation was carried out. Additionally, several *in vitro* tests have been performed on three antigorite samples, subjected to different levels of alteration, to collect preliminary information on antigorite toxicity. Alteration modifies the surface reactivity of antigorite. The circulation of fluids induces a mechanical stress and an elemental exchange at mineral/water interface, promoting the loss of cohesion of the mineral structure and affecting the surface chemistry and toxicity of fibrous (asbestiform) antigorite.

Keywords: fibrous antigorite; NOA; weathering; toxicity; New Caledonia.

1. Introduction

Inhalation is the primary route of exposure of mineral fibers that becomes a cause of concern in the case of exposure from natural deposits of asbestos (IARC, 2012). Asbestos fibers may be released from asbestos-bearing deposits and, without appropriate dust management, may pose a potential health hazard when rocks are crushed or exposed to natural weathering and erosion, or to human activities. Natural contexts are therefore unconfined sites of study with a great intrinsic diversity, not only related to the activities that can cause the suspension of mineral fibers, but also to environmental sources. Lee et al. (2008) emphasize how difficult it is to reliably correlate the presence of mineral outcrops belonging to the carcinogenic mineral fibers (Group I - Carcinogenic to humans, IARC, 2012) and the impact on health. This depends upon the different physico-chemical properties, the amount of the fibers emitted by each source and the local environmental conditions (IARC, 2012; Turci et al., 2016; Erskine and Bailey, 2018). In the past few decades, epidemiological, *in vitro* and *in vivo* studies have linked chronic diseases to the presence of non-asbestos fibrous minerals. A high-profile case is the example of mesothelioma epidemic in Cappadocia (Turkey), where the impact on the health of exposed people was observed before the fibrous minerals responsible for the epidemic could be determined, finally discovered to be fibrous erionite (Carbone et al., 2011), a zeolite more carcinogenic than the six regulated asbestos minerals. This led to a new interest in the scientific community to investigate potentially hazardous non-asbestos fibrous minerals (*e.g.*, balangeroite; Gazzano et al., 2005; Turci et al., 2005). The lack of a comprehensive scientific knowledge on the toxicology of non-regulated fibrous minerals makes it difficult to assess the potential risk due to environmental exposure.

The New Caledonia provides a good example to assess the toxicity of fibrous antigorite, considering the impact of pedogenesis on the formation and release of these fibers into the environment.

2. Asbestos health hazards in New Caledonia

Located in the southwest Pacific Ocean, in a complex set of marginal basins and continental or volcanic ridges along the Circum-Pacific Belt, the island of New Caledonia is one of the world's largest producers of Ni-ores formed by the alteration of ultramafic rocks. The New Caledonia ophiolite complex is one of the largest (300 km long, 50 km wide and 2 km thick) and best-exposed continuous peridotite complexes in the world, covering more than a third of the land area (Ulrich et al., 2010).

The investigation of NOA in New Caledonia started after the diagnosis of asbestos related pathologies in human populations non-occupationally exposed to asbestos (Luce et al., 2004). An excess of malignant mesothelioma, observed in the 1980s in the northern Kanak communities was associated to the use of "Pö", a tremolite-containing whitewash (Goldeberg et al., 1991). Other cases of mesothelioma and pleural cancer were noted through the year 2008 affecting people associated with mining sites and municipalities. Recently, Baumann et al. (2011) linked these cases of lung malignancies with the presence of serpentinite outcrops, rich in chrysotile and fibrous antigorite. Caledonian populations, living and/or working in proximity to natural outcrops, are therefore subjected to a double environmental and domestic exposure. In this scenario, mining companies need to implement the NOA-risk management in order to protect workers, sites and residents.

In the assessment of risk of exposure, an extensive geological survey of the different (fibrous) varieties of amphibole and serpentine present in the outcrops was performed (Lahondère, 2012, and therein). The natural occurrences of asbestos and related fibrous minerals were overlay onto a detailed geological map (Figure 1; DIMENC-SGNC, 2010). As a result, most outcrops of Ni-laterite deposits are found to contain serpentine and amphibole, not infrequently as fibrous (asbestiform) varieties. While tremolite-amphibole is mainly present in central and northern New Caledonia terranes, serpentine chrysotile and fibro-lamellar antigorite occur in peridotites (Lahondère, 2012). The large distribution of fibrous antigorite over a large part of the island make its environmental exposure a potential public health issue for New Caledonia (Laporte-Magoni et al., 2018).

To deal with this occupational and environmental issue, the Government of New Caledonia legislated and promulgated its first regulation on asbestos materials (*Déliberation N°82 du 25 Aout 2010*). In contrast to European and worldwide asbestos regulations, the New Caledonia decree classifies serpentine antigorite as asbestos, on a precautionary basis. It is worth noting that the regulation makes no distinction between antigorite and fibrous (asbestiform) antigorite. Moreover, this legislative text does not specify an analytical method for the identification and quantification of fibers emitted, relying in this respect to French regulation (NF X43 269). It should be noted that no standard samples exist for measurement of airborne antigorite fiber concentration, which has led to some difficulties in asbestos risk prevention and management. Finally, the New Caledonian decree, similarly to the vast majority of asbestos regulations currently enforced, does not provide a guideline about the management of the NOA-risk.

3. NOA occurrences in lateritic units

In Caledonian ultrabasic units the serpentinized peridotite assemblages exhibit the widespread presence of serpentine minerals combined with minor amounts of tremolite-actinolite amphibole. Owing to its ability to better withstand the oxidation processes, fibrous serpentine is commonly found in the saprolitic zones currently mined for nickel (Trotet, 2012).

Serpentine occurs along tectonic structural discontinuities as fractures, faults and shear zones, probably due to different thermodynamic conditions according to the geodynamic context. When exposed to natural weathering, NOA-bearing rocks are subjected to a humid tropical to sub-tropical climate, influenced by trade winds, and an alternating hot-dry and rainy-cool season. Under these climate conditions, natural deposits of asbestos are subject to a secondary process of alteration. As a result, mineral fibers occur with different morphologies, likely connected to different degrees of alteration. In this context, the term *alteration* refers to a physico-mechanical modification in the shape and cohesion of rock fabric. With an increase in the degree of alteration, massive assemblages gradually cleave into lamina or needle-like acicular crystals. This progressive loss of cohesion leads to the disappearance of the original structure, and conversely increases the appearance of individual asbestos-like fibers. Minerals which have been subjected to alteration may vary from prismatic-platy to asbestiform, through acicular-lamellar.

A complete mineralogical-petrological (optical microscopy, SEM, TEM, micro-Raman) and geochemical (major and trace elements) approach was applied to the structural, chemical and morphological characterization of fibro-lamellar antigorite (Petriglieri et al., 2019). Thirty-five rock fragments collected at mining sites (outcrops, quarries, tracks, pits) of different ultrabasic units were analyzed (Figure 1).

4. Fibro-lamellar antigorite

Serpentinized peridotites show a large network of fault planes and veins containing lamellar crystals of antigorite, centimeters to decimeters in length, cross-cut by more or less continuous veinlets of chrysotile. In the less altered areas, generally at the base of the saprolite horizon, antigorite blades show a compacted, moderately hardened appearance, dominated by a pale-green to green color. Platy shaped lamellae are welded and parallel to each other (Figure 2A). Moving up in the regolith profile, antigorite occurs in the form of stacks of laminas exhibiting a progressively more friable aspect. Blades appear fragmented and are associated with fibers. These fibers may originate by the extreme cleavage (fraying) of the same lath-shaped crystals (Figure 2 B,C). Antigorite assumes a fibro-lamellar habit in highly altered horizons. Due to strong mechanical separation and cleavage, antigorite has a completely transformed morphological appearance and is associated with a porous low-density material. The friable nature of these specimens is evident (Figure 2 D). Therefore, antigorite, non-fibrous when fresh, gradually cleaves with pedogenic alteration presenting fibrous-like particles, which are not strictly asbestos fibers according to legal and commercial definitions, but their fibrous-asbestiform nature may have a potential impact on human health.

In the evaluation of morphological and textural features, optical and SEM images of Caledonian antigorite show an intimate intergrowth of lamellar and fibro-lamellar shapes (Figure 3). Several key distinctions relating to the mineralogy, texture and alteration state of antigorite were obtained through the examination of petrographic thin sections. According to Wicks and Whittaker (1977), antigorite is typically recognized for its non-pseudomorphic *interpenetrating* or *interlocking* texture (Figure 3 B). Actually, Caledonian samples consist of randomly orientated aggregates of fibro-lamellae and show a wider variety of shapes and intergrowths. They appear as star and fan formed aggregates (Figure 3 A), lath-shaped lamella (Figure 3 C), and fibrous-lamellar blades (Figure 3 D). Even the same sample can display the co-existence of several different textures. Although the two-dimensional nature of petrographic thin sections makes it difficult to distinguish the crystal habit (*e.g.*, fibrous, acicular and lamellar), polarized light microscopy observations allow one to evaluate the intergrowth of different, fibrous or non-fibrous, phases in their textural context. Samples that appear massive, lamellar and unaltered in hand sample commonly display their fibrous shape at the optical microscopy scale. Increasing the magnification, SEM images display the huge morphological variability exhibited by Caledonian antigorite, which has the form of fibro-lamellar crystals, characterized by the co-existence of both lamellar and fibro-lamellar shapes (Figure 4). Bundles of parallel elongated lath-shaped crystals exhibit the typical habit of phyllosilicate minerals, characterized by the overlapping of platy sheets. However, aggregates of randomly oriented non-elongated blades may also occur. Most particles maintain their lamellar habit displaying a gradually bent, slinky to curvilinear, ending.

5. Impact of pedogenic alteration on fiber release

Physical and mechanical stress appears to be one of the main reasons for the various degree of alteration displayed by the mineral fibers of New Caledonia. As the alteration increases, a gradual increase in distance between closely overlapped fibers and/or fibro-lamellae occurs, resulting in a greater macro-porosity. This is probably related to the circulation of surface water which percolates down and penetrates rocks, permeating cracks, fractures and shear zones. It is proposed that the penetration of fluids within fibrils is thus favored and causes a chemical elemental exchange at the mineral/water interface, creating a severe mechanical stress which results in the complete loss of cohesion of the original structure.

To evaluate the role of chemical element exchange as to its capacity to break apart and disperse antigorite fibers, a preliminary geochemical investigation has been conducted. In this context, the main chemical reactions involved at the crystal/water interface are dissolution, redox reactions, hydration, decarbonation, and the most common, hydrolysis. Thus, the most soluble elements may be leached by water (*e.g.*, Mg), leading to the dissociation of fibrous minerals and consequently favoring the emission of fibers. It should be remembered that the variation of element solubility is strictly related to the type of element and silicate mineral involved in the mineral/water reactions. The study of major and trace element concentrations represents a first tracer of the impact of weathering on altered rocks. Analysis of major and trace elements were conducted using optical and mass spectrometry (ICP-OES and ICP-MS). Chemical signatures of (fibrous) antigorite reveal a systematically lower MgO and higher FeO_{tot} content, compared to what is typically reported in the scientific literature (from 35 to 45, and 2 to 5 wt.% respectively; Deschamps et al., 2013; Cannaò et al., 2016). Additionally, a higher concentration in Cr, Mn, Co, V, Sc, Cu and Ni, was observed. An advanced stage of alteration is observed for all antigorite specimens, also for samples macroscopically observed to be unaltered. These results are consistent with the laterisation process involved in Ni-ore deposit formation (Butt and Cluzel, 2013).

6. Potential toxicity of fibrous antigorite

To date, only preliminary data on the potential toxicity of fibrous antigorite are available (ANSES, 2014, and therein). To better assess its pathogenicity, a set of *in vitro* cell-free and cellular tests were performed. To this purpose, three antigorite samples presenting different levels of cohesion (from low- to highly altered) and containing about 50% fibrous particles were compared with chrysotile (UICC Chrysotile A, Rhodesian) in terms of physico-chemical properties known to modulate asbestos toxicity and cellular responses.

Asbestos toxicity is based on fibrous habit, surface reactivity and high biopersistence, which altogether yield persistent inflammation and DNA damage. For this reason, i) size and morphology, ii) surface reactivity towards free radical release and iron bioavailability, and iii) dissolution in simulated body fluids were investigated. Data acquired were also compared to those obtained from a lamellar antigorite from the western Alps, Italy (Groppo and Compagnoni, 2007).

Size and morphology, including aspect ratio, of the four antigorite samples were carried out by an automated image analysis system (FPIA 3000, Malvern). Morphometrical analysis was performed to discriminate between respirable (L/D >3, D <3 μm), non-respirable fibers (L/D >3, D>3 μm) and non-fibrous particles (L/D <3), according to regulated critical dimensions (IARC, 2012). Caledonian samples are all in the form of fibro-lamellar crystals. After a gentle mechanical stress they fracture easily, releasing elongated fibrous particles, most of which have the dimensional characteristics of respirable fibers (L/D >3, D <3 μm). Antigorite samples were ground in a ball mixer mill (Retsch MM200) for 2-5 min (27 Hz) to obtain a similar size distribution. Agate jars were used to avoid metal contamination. After grinding procedure, particles appear fractured, mainly in the form of acicular or isometric crystals. Caledonian antigorite contains about 40-55% of respirable fibers, compared to 12-15% of the Italian sample. The lamellar Italian antigorite is made up of mostly prismatic fragments. In all Caledonian samples the amount of respirable fibers is not correlated to the alteration status.

Surface reactivity was evaluated by measuring the ability of antigorite to catalyze generation of hydroxyl and carbon-centered radicals in cell-free tests and release iron into solution (bioavailable iron). Mid-to-highly altered antigorite showed the same reactivity in hydroxyl radical release as UICC chrysotile but, opposite to chrysotile, it did not catalyze carbon-centered radical generation and contained smaller amounts of bioavailable iron.

Dissolution was investigated in Gamble's solution, which mimics interstitial fluid within the deep lung, and phagolysosomial simulant fluid. All samples dissolved slower than chrysotile.

Finally, cellular effects were investigated in human epithelial cells (A549) and in murine macrophages (MH-S). Figure 5 shows the release of LDH (lactate dehydrogenase), an intracellular cytosolic enzyme that is released in the culture medium when cell membranes are damaged (cytotoxicity). Highly-altered antigorite showed a similar, dose-dependent cytotoxic effect. On the other hand, less-altered lamellar antigorite, as well as the non-fibrous Italian sample, were not toxic, even at the highest doses. The increasing higher activity of LDH is associated with a higher degree of alteration. Moreover, at high dose (4 times higher than chrysotile), highly-altered samples induced oxidative stress and production of Nitric Oxide, a cytotoxic and pro-inflammatory intracellular messenger. They also damaged DNA in alveolar cells. The unaltered antigorite showed very weak surface reactivity and did not trigger any cellular effect.

7. Conclusions

The comprehensive approach involved in the study of Caledonian fibro-lamellar antigorite delivered three main results:

- Caledonian antigorite exhibits a fibro-lamellar habit, resulting in a greater variability in texture and morphology than unaltered antigorite.
- Pedogenic alteration affects the surface reactions and increases the genesis and release of fibers. The penetration of fluids within fibrils, associated with a chemical elemental exchange at mineral/water interface, causes a progressively internal mechanical stress, ultimately, there is a complete loss of cohesion of the original structure.
- The different reactivity of three antigorite samples in cell-free and cellular tests suggests a role of pedogenic alteration, which modifies surface chemistry, in the potential pathogenicity of fibrous antigorite. Cell-free and cellular tests revealed a lower reactivity of antigorite samples compared to chrysotile. This reactivity is fully absent in the low-altered specimen, suggesting a lower hazard associated with fibrous antigorite. The slow dissolution in simulated

bodily fluids, however, indicates that antigorite biopersistence could be higher than chrysotile. Further research is needed to confirm the lower toxicity of antigorite with respect to chrysotile.

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Figure captions

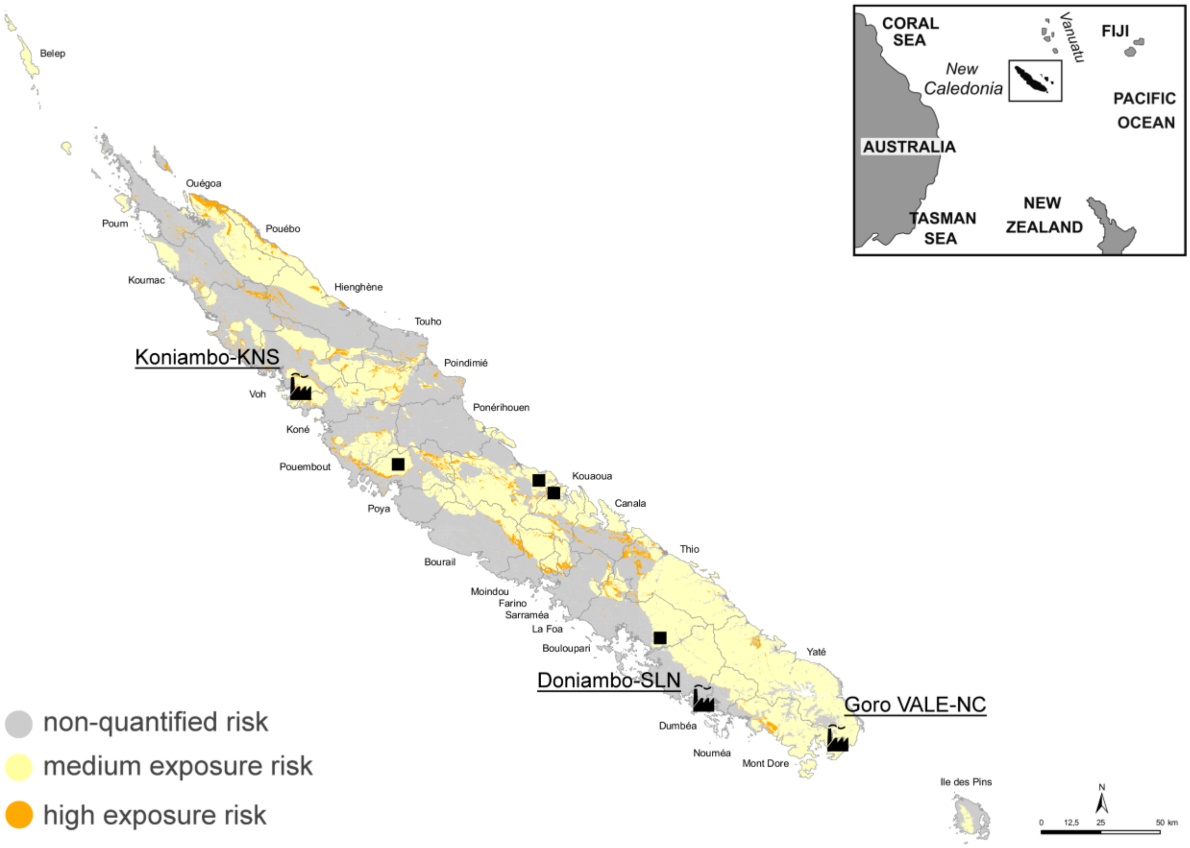
Figure 1. Geological sketch map of natural occurrences of fibrous-asbestiform minerals in New Caledonia. The three major sites of nickel-mining activity are magnified (modified after DIMENC-SGNC BRGM, 2010). Sampling sites are indicated with square.

Figure 2. Macroscopic features of hand-scale antigorite samples. An evident lack of coherence and a very altered appearance characterizes the New Caledonia rock-fragments.

Figure 3. Textures of fibrous antigorite observed by polarized light microscopy (cross-polarizing images). A) star and fan formed aggregates; B) interpenetrating texture; C) lath-shaped lamella; D) fibrous-lamellar blade.

Figure 4. SEM images of different morphologies of antigorite. Most particles maintain their lamellar habit displaying a gradual bent, slinky to curvilinear, ending.

Figure 5. Cytotoxicity LDH released by alveolar macrophages after a 24 h incubation with increasing doses of antigorite samples (Italian non-fibrous Atg and Caledonian low-to-highly altered), 30 µg/ml of chrysotile (Ctl) or 120 µg/ml of synthetic vitreous fibers (MMVF-CTRL).



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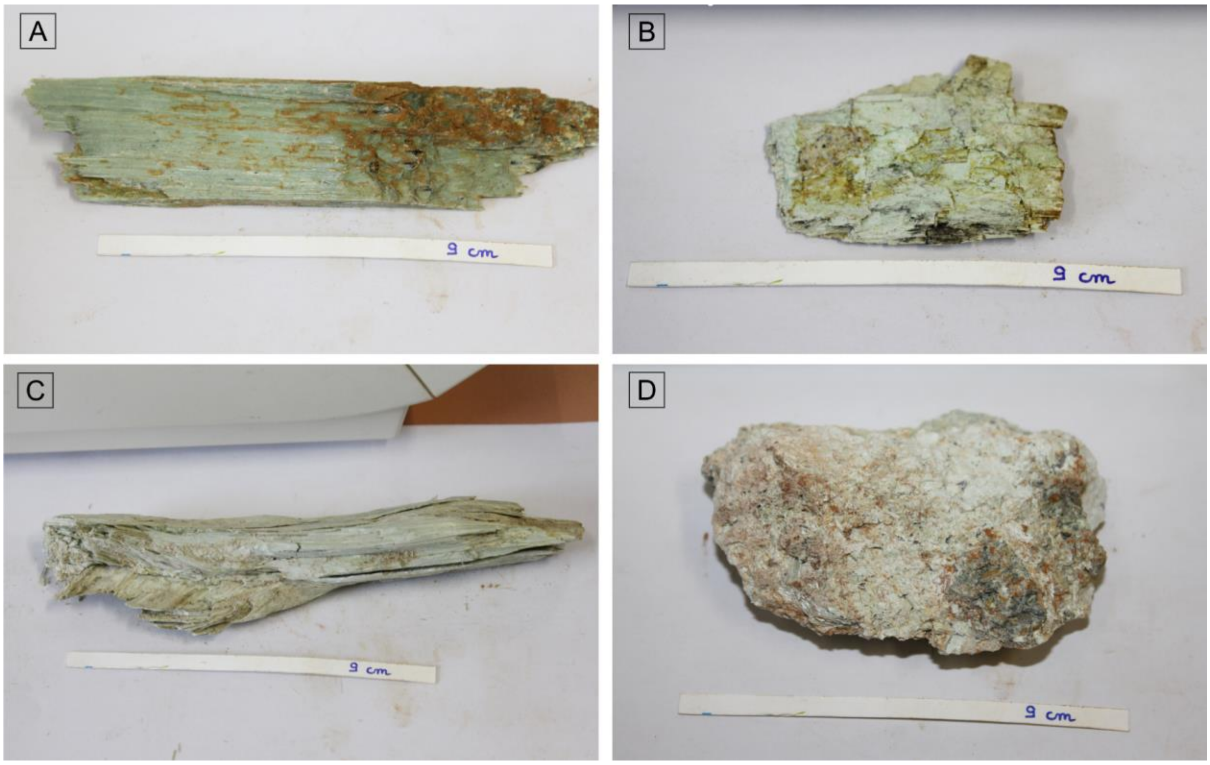

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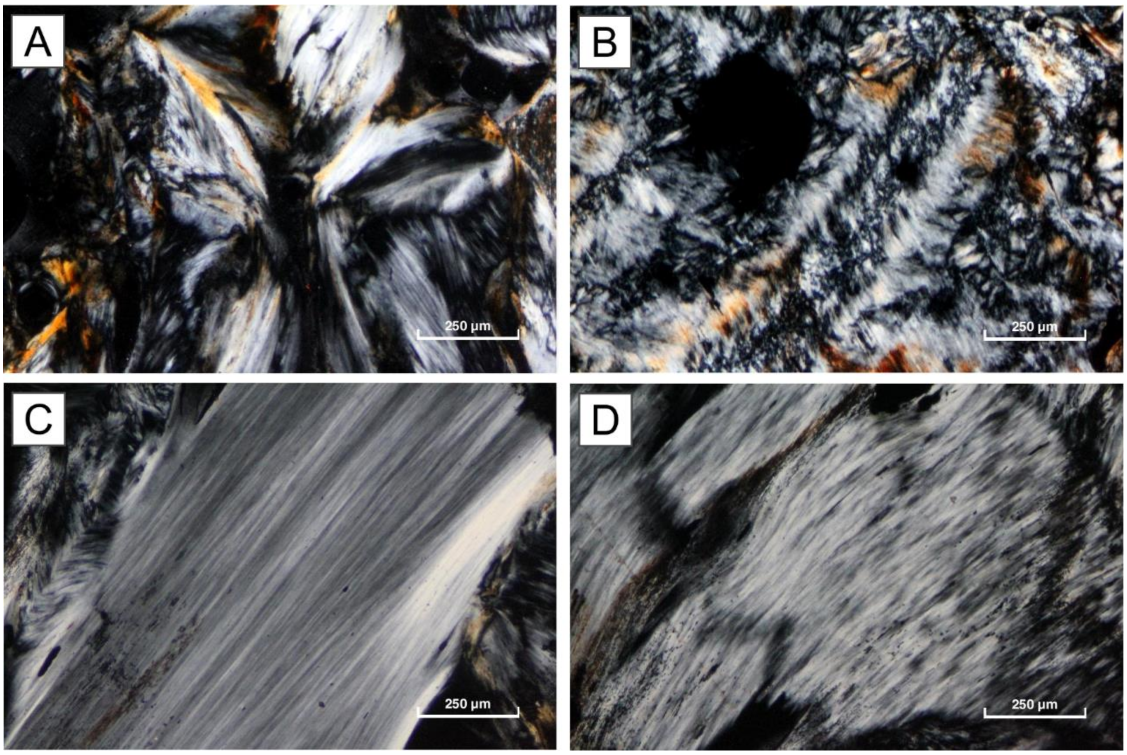

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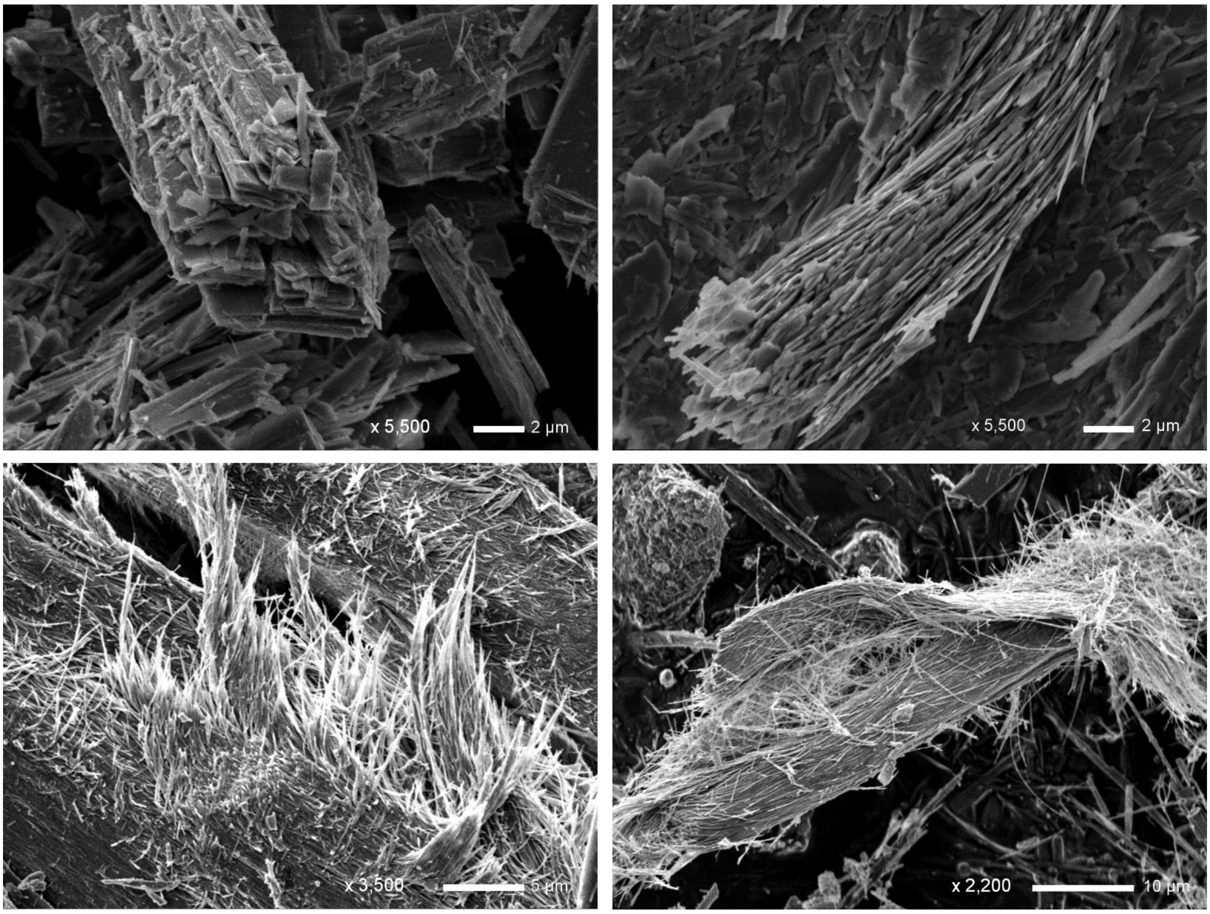

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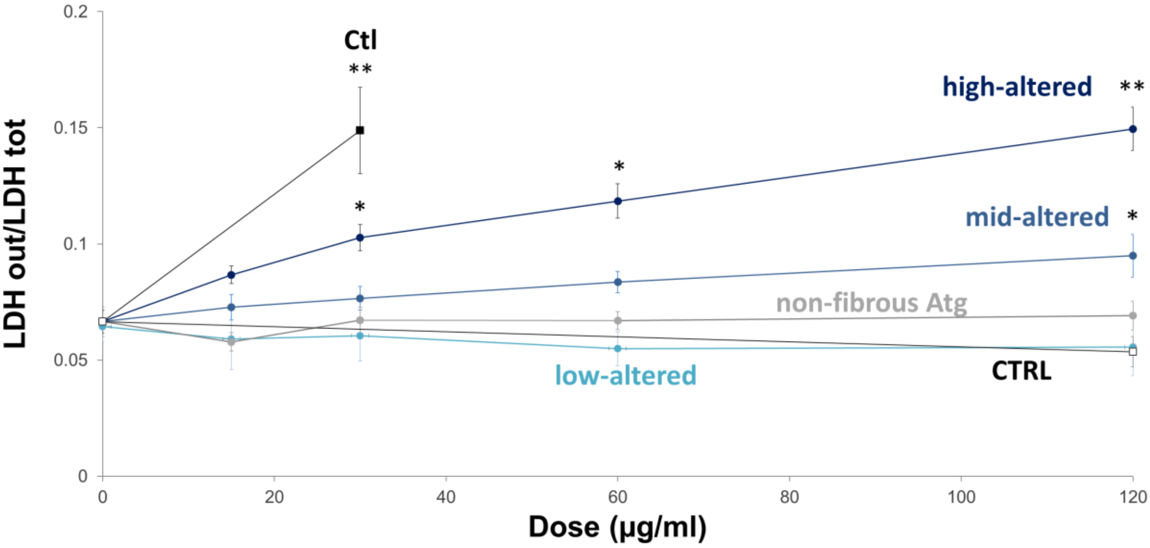

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