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# Preliminary evaluation on interaction between *Galdieria sulphuraria* and chrysotile

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ABSTRACT

Asbestos, a naturally occurring fibrous silicate, was extensively utilized in construction between 1965 and 1987 due to its fire-retardant, sound-absorbing, electrical insulating, and heat-resistant properties, as well as its mechanical flexibility. By the 1990s, the deleterious health effects of airborne asbestos fibers became evident, ranging from asbestosis to mesothelioma, prompting widespread bans on its mining and use internationally. Despite extensive efforts to identify and secure asbestos-containing materials in buildings, a significant challenge persists: the safe disposal of deteriorating materials, which must adhere to stringent regulatory safety standards.

This paper presents preliminary findings on utilizing *Galdieria sulphuraria*, a multi-extremophilic red microalga, for the phycoremediation of chrysotile asbestos. This specific microorganism was selected for its remarkable ability to maintain intracellular homeostasis under extreme conditions (pH 0–4, temperatures up to 56 °C) and its established feasibility for industrial-scale cultivation, making it particularly suitable for integration into waste management protocols. Multiple experimental iterations consistently demonstrated chrysotile degradation under varying conditions. To quantify magnesium removal from asbestos fibers, scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM-EDS) was employed. Concurrently, inductively coupled plasma optical emission spectrometry (ICP-OES) detected solubilized magnesium in the liquid phase, with concentrations increasing progressively over a two-week period. Results demonstrate significant alterations in chrysotile's optical properties, morphological characteristics, and structural composition, particularly the removal of its outer brucitic layer, verified through phase contrast optical microscopy (PCOM) and X-ray powder diffraction (XRPD). This biological approach offers a promising alternative to conventional physicochemical treatments, operating efficiently at ambient temperatures while minimizing secondary waste generation.

## 1. Introduction

Asbestos is the generic term for a group of naturally occurring fibrous silicates belonging to the serpentine and amphibole mineralogical series (Deer et al., 1992). These minerals are characterized by their high tensile strength, flexibility, resistance to heat and chemical degradation, and electrical insulation properties. Historically, asbestos was widely utilized in various applications, including fiber-cement roofing, vinyl flooring, packing materials, ventilation ducts, chimneys, mastics, and tars (Virta Robert, 2002). Despite widespread bans in developed countries, asbestos continues to be produced and used globally, with approximately 90 % of world production concentrated in Russia, China and Kazakhstan (Thives et al., 2022). The health implications associated with asbestos exposure have become increasingly evident since the 1970s (IARC, 1977; Mossman et al., 1990). Asbestos-induced diseases, including asbestosis, lung cancer, and malignant mesothelioma, are primarily attributed to the inhalation of respirable fibers that can persist in lung tissue for decades (Selikoff et al., 1964; Kamp et al., 1992). The carcinogenic potential of asbestos is multifactorial, involving fiber dimensions, surface reactivity, biopersistence, and crucially, iron content and iron-bodies formation (Pollastri et al., 2015). The presence and speciation of iron, including both oxidation state and coordination environment, are critical factors affecting asbestos toxicity (Walter et al., 2019).

Chrysotile, commonly known as white asbestos, represents over 95 % of commercially developed asbestos deposits globally (Whittaker and Zussman, 1958; Ross et al., 2008). As a fibrous variety of magnesium silicate within the serpentine group, chrysotile possesses a distinctive structure consisting of a tetrahedral silicate sheet $(Si_2O_5)_n^{2n-}$ (T) combined with an octahedral brucite-like layer $[Mg_3O_2(OH)_4]_n^{2n-}$ (Parson

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et al., 1992). The dimensional mismatch between these layers is accommodated through curling, resulting in the formation of cylindrical structures with outer diameters of 22–27 nm and inner diameters of 7–8 nm (Yada, 1971; Ballirano et al., 2017). In this configuration, the silicate layer invariably occupies the inner position, while the brucite layer forms the exterior of the cylinder (Wicks and Whittaker, 1975; Whittaker, 2009).The iron content in chrysotile, while relatively low compared to amphibole asbestos varieties, becomes particularly significant due to the mineral's high dissolution rate, which progressively exposes structural iron at the fiber surface (Pollastri et al., 2015; Wylie, 2017). This available surface iron catalyzes the formation of reactive oxygen species (ROS), particularly hydroxyl radicals, which are implicated in the pathogenesis of asbestos-related diseases (Kamp et al., 1992; Fubini et al., 1999; Gazzano et al., 2005).

Despite its widespread historical use in buildings, vehicles, and industrial applications, contemporary chrysotile management presents significant challenges due to stringent disposal regulations and safety protocols. Consequently, substantial research efforts have been directed toward transforming chrysotile into non-toxic and potentially valuable secondary materials. Current remediation approaches primarily employ thermal, mechanical, or chemical treatments. Thermal methods require temperatures exceeding 1000°C to achieve complete dehydroxylation and recrystallization (Gualtieri, 2000; Leonelli et al., 2005), while mechanical treatments involve intensive grinding to disrupt fiber structure (Iwaszko et al., 2018). Chemical approaches typically utilize strong acids to extract magnesium from the brucitic layer (Rozalen et al., 2013; Sugih et al., 2022). The effects of acid attacks on chrysotile fibers have been studied extensively since 1958, when acids were initially used in mineral processing to remove carbonate or iron ore residues from extracted fibers (Whittaker and Zussman, 1958; Speil and Leineweber, 1969). Once the toxicity of asbestos was understood, further studies tested various acids at room temperature (pH = 1) over reaction periods of up to 30 days, establishing a scale of efficiency for degrading the brucitic layer. Among the tested acids, oxalic acid proved most effective, forming glushinskite precipitate and outperforming sulfuric and nitric acids (Rozalen et al., 2013). Other experiments involved hydrochloric acid at different temperatures (Necasova and Buchta, 2019) or the use of additives to achieve similar results at lower temperatures (Paolini et al., 2019). It has demonstrated that chrysotile asbestos carcinogenicity can be significantly reduced by removing iron with organic acids (Chao et al., 1994; Foresti et al., 2009) and magnesium with oxalic acid (Gold et al., 1997), with these studies showing that iron removal reduces radical release and correspondingly decreases DNA and lipid damage.

While effective, these conventional methods often necessitate substantial energy inputs, generate secondary waste streams, and pose operational hazards. Biological remediation strategies, particularly those utilizing microorganisms, represent a promising alternative approach. Various bacterial and fungal species have demonstrated capacity to interact with and potentially degrade asbestos fibers through mechanisms including acidification, chelation, and enzymatic activity (Mohanty et al., 2018; Bhattacharya et al., 2021). Recently, Borges et al. (2022) investigated the breakdown of chrysotile through bacteria, particularly *Aspergillus niger*, which produces metabolites including gluconic, citric, and oxalic acids. As with mineral acids, magnesium passes into solution, but the biological process is slower (Borges et al., 2022). Mohanty et al. (2018) did not obtain equally significant results with bacterial treatment, likely due to insufficient concentration of organic acids produced by the bacteria and consequently high pH. A similar approach was employed by Gadd (2004) and Martino et al. (2004), in which fungi attach to the surface with mycelium, inhibiting fiber diffusion and releasing metabolites capable of chelating iron or producing acids capable of degrading chrysotile, a phenomenon evaluated by the abundance of magnesium in solution.

Lichens, living in symbiosis with fungi or green algae, produce oxalic acid, which again breaks down chrysotile (Favero-Longo et al., 2013). Some cereal plants have also been studied for their production of phytosiderophores near their roots, which are capable of chelating iron and small quantities of other metals (Christofidou-Solomidou and Willenbring, 2018). This is particularly interesting as iron content varies depending on the type of asbestos—while very low in chrysotile, it is higher in other varieties such as crocidolite (Wylie, 2017).

Among microbial candidates, extremophilic microorganisms offer particular advantages for asbestos remediation due to their resilience in challenging environmental conditions and diverse metabolic capabilities. *Galdieria sulphuraria*, a unicellular red microalga belonging to the Cyanidiophyceae family, exemplifies such extremophilic potential. This organism demonstrates remarkable adaptability, thriving in environments characterized by high temperatures (up to 56°C), acidic conditions (pH 0-4), and elevated concentrations of toxic metals (Gross and Oesterhelt, 1999; Oesterhelt et al., 2007). *G. sulphuraria's* exceptional tolerance is attributed to several adaptive mechanisms, including specialized membrane transporters, metal-binding proteins, and compartmentalization strategies (Curien et al., 2021). Moreover, this microalga exhibits metabolic versatility, capable of photoautotrophic, heterotrophic, and mixotrophic growth, offering significant advantages for large-scale cultivation and industrial application (Iovinella et al., 2023; Kharel et al., 2024).

Recent studies have demonstrated *G. sulphuraria's* capacity to sequester heavy metals from aqueous solutions, suggesting potential applications in environmental remediation. Research on its genetic resources has revealed pathways involved in metal homeostasis and horizontal gene transfer (HGT), enhancing its ability to recover rare earth elements (REEs) like cerium, yttrium, europium, and terbium under acidic conditions (Iovinella et al., 2023). This alga is also capable of metal sequestration through extracellular polymeric substances (EPS) and surface functional groups, evidenced by cadmium and zinc bioremediation studies, where up to 30 % metal removal efficiency was achieved in strongly acidic matrices (Kharel et al., 2024). A compelling advantage of *G. sulphuraria* is its ability to remain metabolically active under a broad temperature range, including moderate ambient conditions around 22–25 °C (Curien et al., 2021). This contrasts with many other acidophilic or thermophilic microorganisms that require higher temperatures (35–60 °C) to maintain robust metabolic rates (Choi et al., 2023). However, its interaction with asbestos minerals, particularly chrysotile, remains unexplored.

The present study aims to address this knowledge gap by investigating *G. sulphuraria's* potential for chrysotile degradation, with specific focus on the removal of the fiber's brucitic layer and consequent alteration of its morphological and toxicological properties.

## 2. Materials and methods

### 2.1. Chrysotile

High-purity raw chrysotile from a Canadian mine (Lake Asbestos) was characterized using phase-contrast optical microscopy (Leica DMLP microscope equipped with a Leica DFC 290 digital camera). Chrysotile fibers exhibit characteristic flexibility and distinctive optical birefringence. Under phase contrast optical microscopy, the fibers exhibit a transition from blue coloration with an orange halo to light blue with a pink halo at 90° intervals of rotation. This diagnostic optical behavior is observed when fibers are mounted in an immersion medium with a refractive index of $n = 1.55$, which provides optimal contrast for visualization of the birefringent properties inherent to the chrysotile crystal structure.

The SEM D8350 FEI QUANTA INSPECT microscope, equipped with an EDS analysis system, was used to characterize the chrysotile fibers. The analysis confirmed the fibers' flexibility and revealed their elemental composition, with magnesium being more abundant than silicon. This compositional analysis is consistent with the theoretical stoichiometry of chrysotile [$Mg_3Si_2O_5(OH)_4$], where the magnesium-to-silicon ratio reflects the mineral's layered silicate structure.

The chemical composition of the samples was determined using an inductively coupled plasma atomic emission spectrometer (ICP-OES Perkin Elmer OPTIMA 2000 DV). Three replicates of the original chrysotile were digested with 2.5 mL of sulfuric acid, 2.5 mL of phosphoric acid, and 1 mL of hydrofluoric acid. The mixture was processed in a microwave oven, and the resulting product was filtered through 0.45 µm filters. Magnesium, silicon, aluminum, and iron content of the samples were measured using the ICP-OES, with calibration based on standard solutions (nebulizer: 0.65 L/min, RF Power: 1400 W, pump: 1.50 mL/min).

X-ray powder diffraction (XRPD) analysis was carried out using a Rigaku (Tokyo, Japan) SmartLab SE diffractometer. The instrument utilized Copper K-alpha radiation (CuKα) at 40 kV and 30 mA, with a scanning range of 5–90° 2θ, a step width of 0.01°, and a scan speed of 2°/min. The system was equipped with a D/teX Ultra 250 (H) detector and operated using the Rigaku SmartLab Studio II software package. Powder samples for analysis were manually prepared under a fume hood from the chrysotile fibers using an agate mortar.

### 2.2. Galdieria sulphuraria strain

The extremophilic red microalga *G. sulphuraria*, strain 074 W, was selected from acidic hot spring environments for its pre-adaptation to high proton concentrations (pH <2) and metal resistance. The nutrient medium was based on a modified version of Allen's (Allen, 1959) medium, prepared without magnesium to encourage algal adhesion to the chrysotile fibers. The visible light provided by cool fluorescent lamps delivered a constant irradiance of approximately 150 µmol $m^{-2}$ $s^{-1}$ to the culture flasks, with illumination limited to daytime hours (18 h of light and 6 h of dark). Fresh biomass samples were diluted to an optical density (OD) at 750 nm ranging from 0.3 and 0.8. These samples were incubated in darkness at 35 °C for 20 min in triplicate. The dark-adapted photosystem II maximum quantum yield of photochemistry (QY, $F_v/F_m$) was measured at 455 nm with an AquaPen-C AP-C 100 instrument (Photon Systems Instruments, Czech Republic) and used to assess at the end of the experiment the vitality of the strain.

### 2.3. Experiment setup

A total of six Erlenmeyer flasks (200 mL capacity) were used, allowing direct comparison among:

- Algae-Only Control: 3.5 g/L wet matter of *G. sulphuraria*, no chrysotile;
- Chrysotile-Only Control: medium without algae, 250 mg chrysotile;
- Test Group: 3.5 g/L wet matter of *G. sulphuraria* and 250 mg chrysotile.

To facilitate interaction between the algae and chrysotile, mechanical agitation was avoided. Instead, gentle manual movement was applied daily to prevent excessive sedimentation and to minimize shear-induced fragmentation.

This study bridges extremophile biotechnology with chrysotile fiber degradation at near-ambient temperature (22°C) and strongly acidic pH (~2).

### 2.4. Experimental monitoring

Algal viability was confirmed weekly via fluorescence microscopy (Zeiss Axio Scope.A1). To monitor the experiment over time, one growth medium sample and one fiber sample were collected weekly. The medium was extracted from each flask using a syringe, filtered through a 0.2 µm filter, and analyzed for magnesium content using inductively coupled plasma optical emission spectroscopy (ICP-OES) measurements.

Fibers were collected using a serological pipette, filtered using a vacuum pump, and washed with distilled water. They were analyzed with PCOM, SEM-EDS, and X-ray powder diffraction. Powder samples for analysis were manually prepared from the chrysotile fibers by milling them in an agate mortar. The fibers remaining after treatment with *G. sulphuraria* were analyzed after microwave digestion with ICP-OES.

This strategic integration addresses three major objectives:

- Ecological relevance: operating at temperatures feasible in outdoor or unheated remediation contexts;
- Analytical rigor: employing complementary techniques (SEM-EDS, XRPD, PCOM, ICP-OES) to track fiber transformation;
- Comparing a purely acid-mediated dissolution control against a *G, sulphuraria*–driven process, highlighting the alga's specific contribution to magnesium mobilization and fiber degradation.

## 3. Results and discussion

The SEM photo (Fig. 1) shows how algae tend to adhere to the chrysotile fibers and cover most of the surface, while mosses and lichens are found to grow spontaneously on rocks containing asbestos, but only in certain areas and under certain conditions (Favero-Longo et al., 2013; Berry et al., 2024). The fibers recovered over the weeks, even when washed using a vacuum pump, always have some algae attached.

Photographs of the pH 2 chrysotile-containing growth medium and those of the chrysotile-containing *G. sulphuraria* and growth medium observed as the weeks progress (one week for each row of photographs) document a progressive birefringence loss. The optical properties of chrysotile changed from the typical blue color with orange halo to a yellow color tending toward white, and it is possible to note the presence of increasingly smaller fragments. Different kinetics can be observed between the chrysotile in the culture medium and that in contact with the algae; in fact, after the first week, the photograph of the culture medium still demonstrates blue fibers with a pink halo. It can be assessed that the fibers in contact with the algae decrease in size more rapidly (Fig. 2).

The fibers filtered from the growth medium and those from the algae-containing medium were also observed under electron microscopy, and again a decrease in fiber length was observed (Fig. 3A). Fragility was also assessed by placing the fibers in an agate mortar and crushing them with a few blows, and it was observed that in both cases the fibers fragmented into small prisms, although larger grains were observed in the case of the fibers immersed in the growth medium (Fig. 3B).

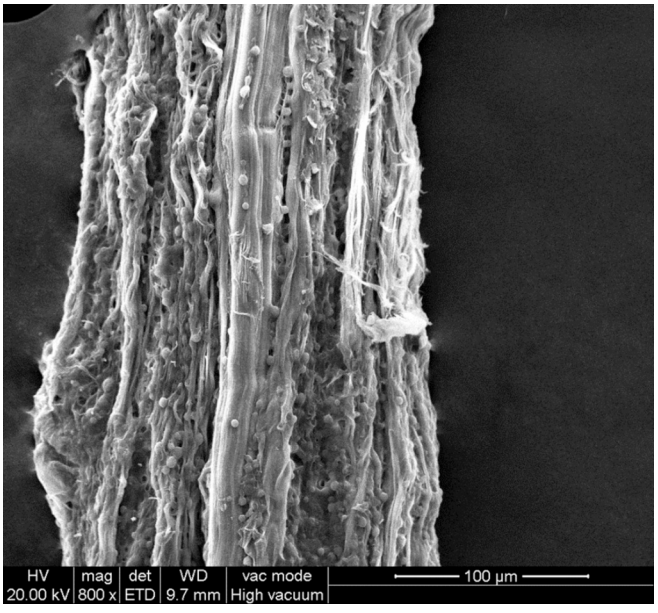


**Fig. 1.** Interaction between chrysotile fibers and algae.

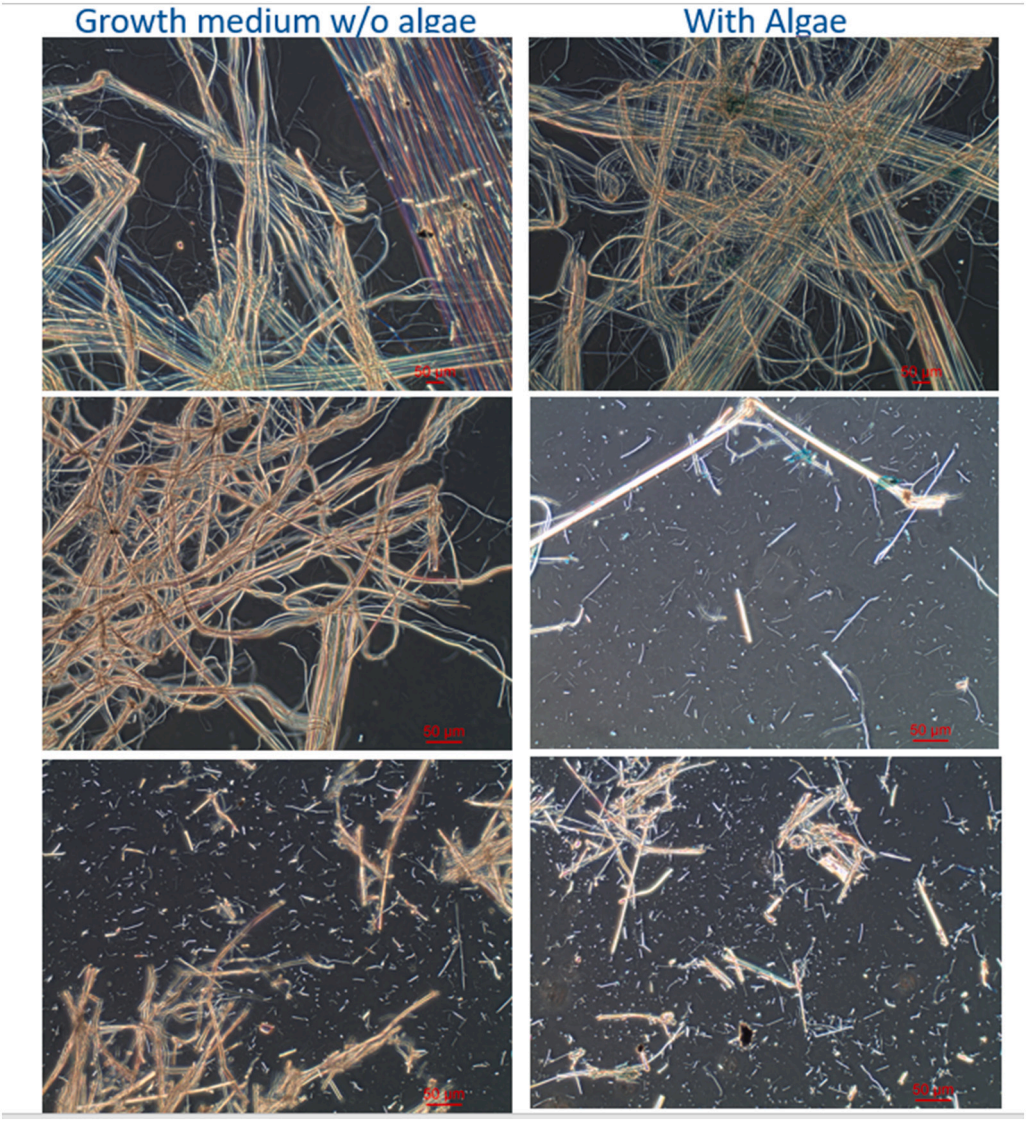


**Fig. 2.** Weekly progression of chrysotile degradation at pH 2. Left: growth medium alone; right: *Galdieria sulphuraria* culture. Top to bottom arrangement shows weekly evolution from blue-orange birefringent fibers to yellow-white fragments, with accelerated kinetics observed in the presence of algae. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

According to the World Health Organization (WHO), respirable asbestos fibers are defined by specific dimensional criteria that determine their potential for pulmonary penetration and health impact. These criteria include: length $\geq$ 5 µm, diameter $<$ 3 µm, and aspect ratio (length/width) $>$ 3:1. The fibrogenicity and carcinogenicity of asbestos fibers depend on several fiber parameters, including dimensional characteristics. Fibers meeting the WHO definition are considered to have the greatest potential for penetrating the respiratory system and causing health effects, including pulmonary fibrosis, lung cancer, and mesothelioma. These dimensional specifications are internationally recognized and utilized for occupational and environmental asbestos exposure assessment, providing a standardized framework for risk evaluation and regulatory compliance. Short asbestos fibers ($<$ 5 µm in length) are subject to macrophage-mediated clearance from the human body and exhibit reduced toxicity compared to longer fibers, as reported in most literature (Moreau et al., 2014). SEM revealed biofilm-mediated transverse fracturing, reducing fiber lengths to $<$5 µm below WHO's respirability, mirroring fracture modes typically associated with high-temperature ($>$80°C) acid leaching (Suquet, 1989).

The EDS spectrum recorded keeping the number of Si counts fixed for all spectra demonstrates a steady decrease in the Mg peak, related both to time and to the difference between the fibers filtered from the growth medium and those from the algal culture. In Fig. 4, the black spectrum is obtained from the fibers extracted from the growth medium after one week, while the blue spectrum is that obtained from the fibers taken from the algal culture after one week; a considerable variation can be seen between the height of magnesium peaks. The same trend is observed for the following weeks, and from the fourth week for fibers in algae solution and fifth week for both solutions, the height of the magnesium peak is close to zero, indicating that the octahedral brucite layer is breaking down (Essih et al., 2024).

A primary consideration is whether the magnesium released from the chrysotile fibers dissolved into the solution. To answer this, a few milliliters of solution were taken from each flask every week and analyzed using ICP-OES.

The analysis reveals distinct patterns of magnesium concentration over the seven-week experimental period. In the algal solution without chrysotile (control), magnesium concentrations remained consistently low throughout the experiment, showing no significant variation from the baseline levels of approximately 30 mg/L. In contrast, solutions containing chrysotile exhibited a progressive increase in magnesium concentration over time. The growth medium with chrysotile showed a

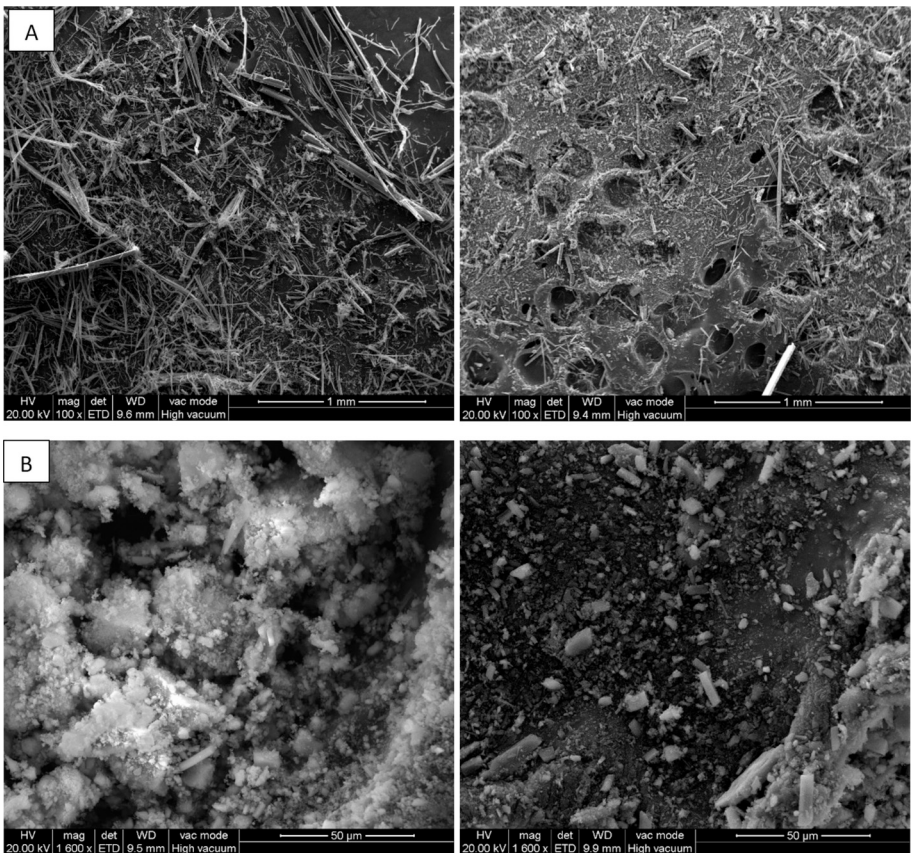


**Fig. 3.** Observation of fibers after three weeks of testing A and five weeks of testing B. On the left Fibers tested without the presence of algae, on the right fibers tested with the presence of *Galdieria sulphraria*. Raw indicated with A are gained directly form the flask, raw indicated by letter B represents fibers crushed with mortar.

steady accumulation of magnesium, reaching concentrations of approximately 370 mg/L by the seventh week. The algal solution containing chrysotile demonstrated a similar trend but with higher magnesium levels, reaching peak concentrations around 350 mg/L, representing approximately 10 % higher abundance compared to the growth medium alone.

Notably, during the final two weeks of the experiment, the rate of magnesium increase in the algal solution was lower than that observed in the growth medium. This phenomenon may be attributed to two possible mechanisms: first, the magnesium could be utilized by algae for photosynthetic processes; alternatively, and more likely, the amorphous silica formed during the destruction of the brucitic layer tends to adsorb magnesium ions from solution (McCutcheon et al., 2015). This latter explanation is consistent with the known affinity of silica surfaces for divalent cations and suggests that the dissolution products of chrysotile may influence the bioavailability of released magnesium.

At the conclusion of the experiment, the viability of the algae was observed using fluorescence microscopy, and assessed using QY value recorded around 0.33 in line with good health of the culture.

In XRPD spectra, the peaks marked with the green dot are typical of the chrysotile spectrum, those marked with the red diamond are typical of silicon oxide spectrum, and the black pentagon is probably due to the presence of a mineral based on iron and silicon. Therefore, it is possible to observe that the original chrysotile changes from a crystalline spectrum to silicon oxide spectrum in the growth medium, meaning that it loses the brucitic layer, and from original chrysotile to an amorphous spectrum in the presence of the algae (Fig. 5). Previous studies generally needed 70–80 °C to achieve such near-complete amorphization (Essih et al., 2024).

To further validate the obtained results, the fibers remaining after the six-week experiment (probably the thickest) were filtered, digested in a microwave oven, and analyzed using ICP-OES.

The compositional analysis of chrysotile samples revealed significant changes in mineral structure over a seven-week experimental period, with notable differences observed between samples maintained in medium alone versus those exposed to algae.

Silicon (Si): Showed moderate transformation with substantial release from the chrysotile structure into the medium. Algae presence accelerated silicon mobilization compared to the control.

Magnesium (Mg): Exhibited the most dramatic transformation with nearly complete release from the original mineral structure. The majority of magnesium was found in the medium phase after seven weeks, with algae further enhancing this mobilization.

Aluminum (Al): Demonstrated moderate transformation with approximately equal distribution between the transformed chrysotile and medium phases. Algae slightly enhanced aluminum release.

Iron (Fe): Showed intermediate transformation behavior with significant release into the medium phase. Algae presence promoted iron mobilization, though iron was retained more strongly than magnesium.

The results indicate preferential elemental release following the sequence: Mg > Fe > Al > Si, reflecting different bonding environments within the chrysotile lattice. Algae-containing samples showed enhanced transformation, suggesting biological processes accelerate chrysotile weathering.

Without algae, 13 % of magnesium remains; with algae, only 4 % of magnesium remains. These results are in agreement with those of McCutcheon et al. (2015), who obtained 84 % Mg removal for a moderate concentration of acid, the same amount obtained with the culture medium without algae (McCutcheon et al., 2015).

The preferential removal of magnesium from chrysotile fibers by *G. sulphuraria* likely involves both passive and active processes. The acidic extracellular environment generated by the microalga (pH

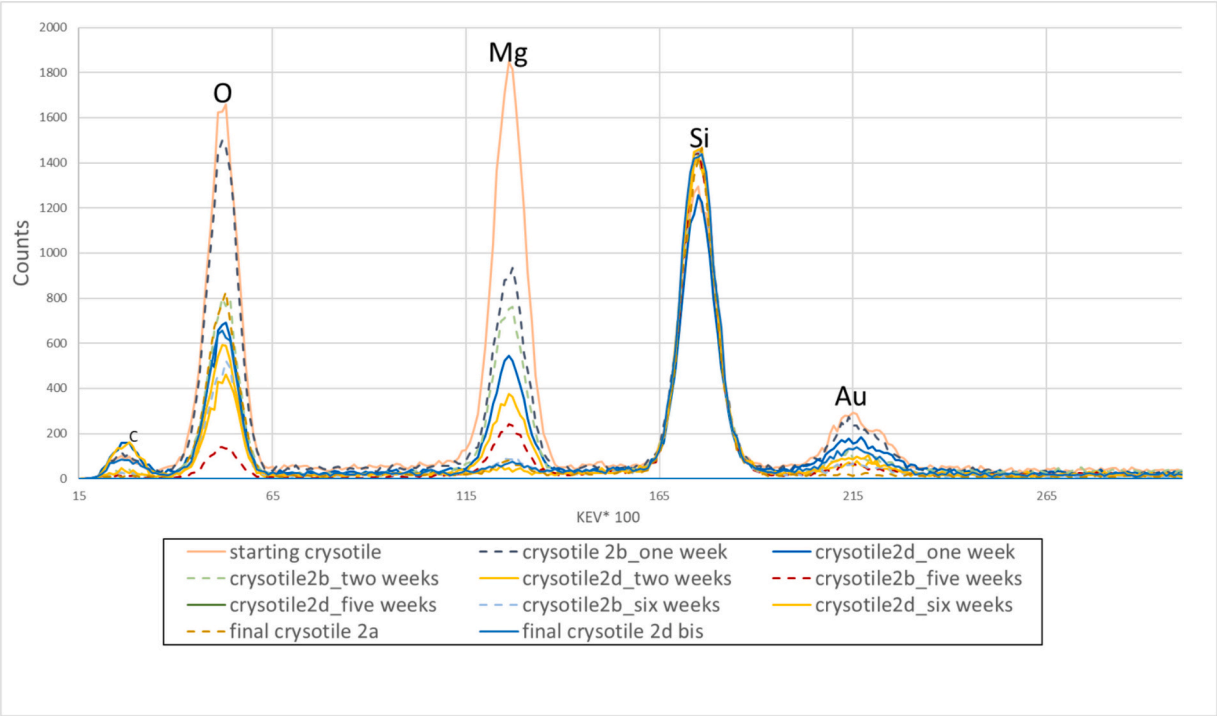


**Fig. 4.** EDS spectra of chrysotile fibers at different alteration stages over time**.** Spectra show the elemental composition evolution of chrysotile fibers extracted from growth medium (dashed lines) and algal culture (solid lines) over six weeks. Main peaks correspond to carbon (C), oxygen (O), magnesium (Mg), silicon (Si), and gold (Au, from SEM-EDS coating). The progressive decrease in the Mg peak indicates degradation of the octahedral brucite layer, with more pronounced reduction in fibers exposed to algal culture compared to growth medium alone. From week 4 (algal culture) and week 5 (growth medium), the Mg peak is nearly absent, indicating complete alteration of chrysotile crystal structure. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

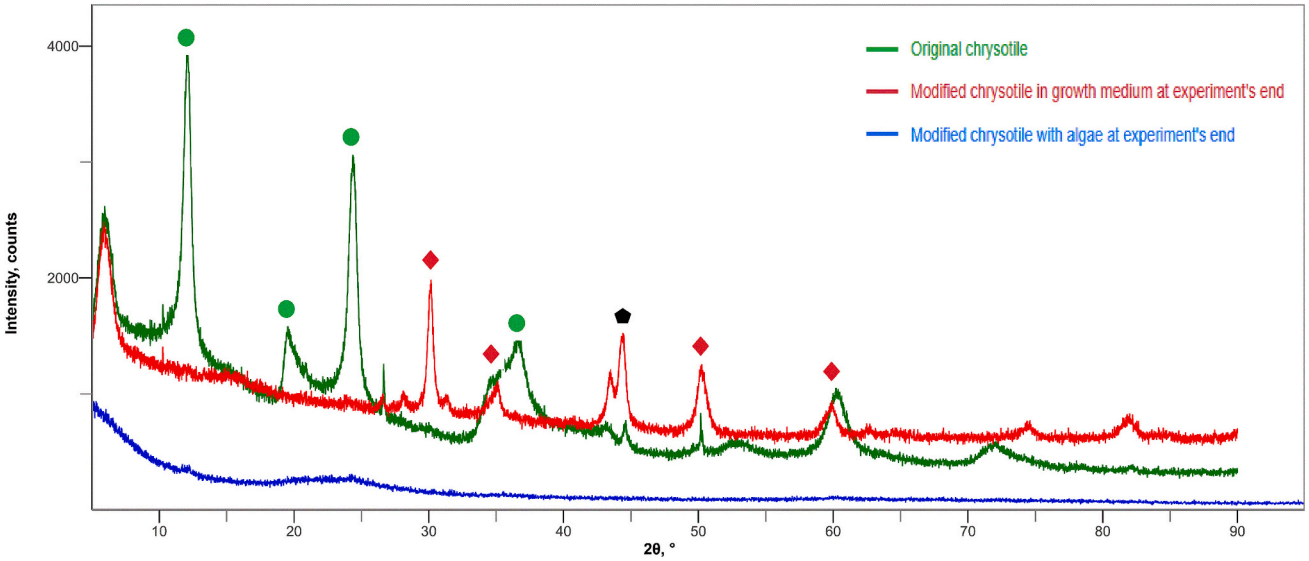


**Fig. 5.** XRPD spectra of original chrysotile, of modified chrysotile with growth medium w/o algae at experiment's end and of chrysotile with algae at experiment's end.

approximately 2) undoubtedly contributes to the dissolution of the brucitic layer, consistent with previously documented acid-mediated degradation mechanisms (Rozalen et al., 2013; McCutcheon et al., 2015). However, the enhanced magnesium removal observed in the algal treatment compared to the acidic medium control (96 % ± 0.1 versus 87 % ± 1.8) indicates additional biological factors at play.

Extracellular polymeric substances (EPS) produced by *G. sulphuraria* likely play a significant role in this enhanced degradation. Microalgal EPS are complex polymers comprising polysaccharides, proteins, nucleic acids, and lipids, with demonstrated capacity for metal binding through various functional groups including carboxyl, hydroxyl, sulfate, and phosphate moieties (Naveed et al., 2019). The synthesis and composition of these biopolymers are dynamically regulated in response to environmental stressors, potentially including exposure to mineral fibers (Danouche et al., 2021). The observed adhesion of *G. sulphuraria* cells to chrysotile fibers, visualized through SEM imaging (Fig. 3),

suggests direct contact-mediated interactions that may facilitate localized degradation.

The pronounced reduction in iron content (approximately 92 % removal in the algal treatment) represents a particularly significant finding with implications for toxicity reduction. Iron associated with asbestos fibers, particularly at the fiber surface, has been implicated in catalyzing the formation of reactive oxygen species (ROS) through Fenton-like reactions, contributing substantially to asbestos-induced cellular damage (Kamp et al., 1992; Fubini et al., 1999). Previous studies have demonstrated that selective removal of iron from chrysotile fibers can significantly reduce their oxidative potential and cytotoxicity (Mohanty et al., 2018). The mechanism of iron removal by *G. sulphuraria* remains to be elucidated but may involve direct assimilation (as iron is an essential micronutrient for photosynthetic organisms), chelation by organic acids or specialized metabolites, or sequestration by EPS components.

The structural transformation of chrysotile from crystalline to amorphous form at moderate temperature (22 °C), as confirmed by XRPD analysis, represents a substantial advantage over conventional thermal treatments typically requiring temperatures exceeding 600 °C (Zaremba et al., 2010). Experiments conducted with chrysotile in an acidic environment at room temperature do not have the same efficiency as Galdieria algae, except for oxalic acid (Rozalen et al., 2013). This energy-efficient transformation has significant implications for both the environmental footprint and economic viability of asbestos remediation processes. Furthermore, the altered morphology of treated fibers—characterized by increased brittleness and tendency to fracture transversely rather than longitudinally—suggests reduced potential for generating respirable fibers, a critical consideration for risk mitigation during handling and processing.

The integration of G. sulphuraria-based remediation with other biological systems offers intriguing possibilities for enhanced performance. For instance, co-cultivation with heterotrophic microorganisms capable of producing organic acids or siderophores might synergistically accelerate fiber degradation. Previous studies have demonstrated effective asbestos biotransformation by various fungi (e.g., Penicillium species) and bacteria (e.g., Pseudomonas species) operating through distinct mechanisms (Martino et al., 2004; Choi et al., 2023). The exceptional acid tolerance of *G. sulphuraria* makes it uniquely suited for such consortia approaches. Field-scale implementation might employ sequential or parallel biological treatment regimes targeting different aspects of fiber degradation and detoxification.

## 4. Conclusions

This study demonstrates the efficacy of *G. sulphuraria* in the degradation and detoxification of chrysotile asbestos through a predominantly biological mechanism.

Phycotreatment with *G. sulphuraria* induces significant alterations in chrysotile's morphological and optical properties, transforming its fibrous structure to a more prismatic form with increased transverse fragility, thereby potentially reducing airborne hazard potential.

The process achieves near-complete amorphization of chrysotile at ambient temperature (22 °C), representing a substantial energy advantage over conventional thermal treatments that typically require 600 °C for comparable results.

Quantitative analysis confirms exceptionally high magnesium removal efficiency (96 %), surpassing the performance of acidic medium alone (87 %) and establishing a new benchmark for biological asbestos remediation.

Significant reduction in fiber-associated iron (92 % removal) suggests potential mitigation of oxidative stress mechanisms implicated in asbestos toxicity, addressing the primary carcinogenic pathway identified in recent research (Chao et al., 1994; Pollastri et al., 2015).

The microalga remains viable and photosynthetically active throughout the remediation process, demonstrating resilience to potential toxic effects of asbestos components and enabling sustained treatment performance.

*G. sulphuraria* offers several strategic advantages over conventional biological remediation approaches, including superior acid tolerance eliminating the need for pH adjustment, metabolic versatility allowing operation in diverse cultivation modes, established large-scale cultivation protocols, and potential for biomass valorization.

The low-temperature (22 °C) operation enables potential co-cultivation with other acid-tolerant microorganisms adapted to moderate temperatures, such as certain Penicillium species that also contribute to chrysotile degradation through organic acid secretion (Borges et al., 2022). This synergistic approach aligns with the World Health Organization's call for cost-effective asbestos mitigation strategies in developing regions (WHO, 2014).

Future research directions should include optimization of process parameters for scaled implementation, investigation of remediation mechanisms through transcriptomic and metabolomic approaches, comprehensive toxicological assessment of treated materials, and exploration of potential valorization pathways for remediation byproducts within a circular economy framework. The exceptional efficiency demonstrated by *G. sulphuraria* in chrysotile degradation warrants consideration of this organism for broader applications in mineral fiber remediation, potentially extending to other hazardous silicate materials.

Several limitations of the current study warrant acknowledgment and suggest directions for future research. First, the laboratory-scale experiments conducted here provide proof-of-concept but require scaling validation for practical implementation. Process parameters including biomass-to-fiber ratio, cultivation duration, and system configuration require optimization for maximal efficiency and cost-effectiveness. Second, while chrysotile represents the most abundant commercial asbestos variant, investigation of *G. sulphuraria's* interactions with amphibole asbestos minerals (e.g., crocidolite, amosite) would provide a more comprehensive assessment of remediation potential. Third, formal toxicological evaluation of treated fibers through standardized in vitro and in vivo models would provide critical validation of hazard reduction.

The potential for valorization of remediation byproducts merits consideration within a circular economy framework. The magnesium-enriched culture medium resulting from chrysotile degradation could potentially serve as a nutrient source for subsequent algal cultivation or agricultural applications. Similarly, the silica-rich residual material might find applications in construction materials, ceramics, or as a soil amendment, consistent with emerging approaches to asbestos waste repurpose (Borges et al., 2022). The algal biomass itself, cultivated in a controlled remediation system, could potentially be harvested for various applications including biofuels, pigments, or biofertilizers, further enhancing the economic sustainability of the remediation process.

## CRediT authorship contribution statement

**Giovanna Zanetti:** Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Davis Alpe:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Paola Marini:** Writing – review & editing, Validation, Resources, Project administration, Funding acquisition. **Mariachiara Zanetti:** Writing – review & editing, Validation, Resources, Methodology, Funding acquisition. **Vincenzo A. Riggio:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Conceptualization.

## Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a

potential conflict of interest.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biteb.2025.102388.

## Data availability

Data will be made available on request.

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