# Lichen deterioration of asbestos and asbestiform minerals of serpentinite rocks in Western Alps

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# Lichen deterioration of asbestos and asbestiform minerals of serpentinite rocks in Western Alps

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**Abstract**

Naturally Occurring Asbestos (NOA) on serpentinite outcrops are exposed to abiotic and biotic environmental forces which may affect their toxicity-relevant properties. Lichen colonization and deterioration were examined on monomineralic and polymineralic veins, containing asbestos (chrysotile, tremolite) and/or asbestiform minerals (antigorite, balangeroite, carlosturanite, diopside). The hyphal penetration of *Candelariella vitellina* within the different veins ranged from few hundreds of microns to several millimetres. The various NOA mineral species contacted by lichen hyphae were differently modified in their chemical composition. NOA were incubated in the laboratory with oxalic acid to mimic the dissolution process driven by lichen metabolites bearing acidic and chelating functions, typically pulvinic acid in the case of *C. vitellina*. The incubated fibres were chemically modified to the same extent of those colonized by *C. vitellina* in the field and were used to examine the effects of lichen deterioration on the toxicity-relevant release of Reactive Oxygen Species (ROS). Incongruent dissolution patterns observed in the cases of chrysotile-, chrysotile + balangeroite- and tremolite bearing veins were associated to a significant (even if not complete) inactivation of the surface. Conversely, the veins bearing minerals subjected to congruent dissolution (antigorite, diopside) maintained their reactivity. Lichen colonization may thus be associated to a bioattenuation process on some kinds of fibrous veins, but such effect cannot be generalized to all colonized NOA.

## 1. Introduction

The six commercially relevant asbestos minerals have been strictly regulated (USA, China) or banned (27 EU countries and 39 more) under worldwide workplace health and safety and public health regulations (International Ban Asbestos Secretariat, 2011). Nevertheless, some concern is growing about the widespread presence of Naturally Occurring Asbestos (NOA; US-EPA, 2011) – asbestos minerals found in place in their natural state, not commercially mined or used – which can be released into the air by natural processes (e.g., rock and soil weathering) or human activities (e.g., agriculture, construction), thus becoming a threat to human health (Culley et al., 2010). Shared scientific and procedural basis for assessing NOA occurrence in a given area and evaluating the associated risk has been invoked in order to develop effective public policies and minimize fibre hazard (Lee et al., 2008).

The natural occurrence of asbestos minerals is mostly associated to serpentinite rocks, where the fibres occur within different generations of metamorphic veins (O'Hanley, 1996; Virta, 2005). The largest deposits of asbestos were punctually mined in the serpentinite belt of several ophiolitic complexes around the world (eastern Canada, Russian Urals, California, north-eastern Greece, Cyprus and northwest Italy) (Ross and Nolan, 2003). Beside these wide, but rare commercial deposits, small occurrences of fibre-bearing surfaces are extremely common throughout all the serpentinite outcrops and may act as sources of fibre dispersion in natural environments (Lee et al., 2008).

The invoked characterization of the potential risk associated to NOA in the case of the widespread, but small sources of NOA, should take into account the following points:

(a)

the six regulated asbestos minerals (namely, chrysotile, tremolite, actinolite, anthophyllite, riebeckite-crocidolite and grunerite-amosite) only partially account for the whole set of fibrous minerals comprised in NOA. In fact many studies have reported on asbestiform minerals not commercially exploited, but sharing a fibrous habit with asbestos, whose pathogenicity is mostly unknown (Baris et al., 1987; Comba et al., 2003; McDonald et al., 2004; Turci et al., 2009; Pugnaloni et al., 2010);

(b)

NOA are more than likely to appear in polymineralic veins, including other fibrous minerals (Groppo and Compagnoni, 2007a);

(c)

opposite to airborne fibres generated by human activities, naturally released fibres from serpentinite outcrops have been often affected by abiotic and biotic environmental forces for a long time, which may have modified their toxicity-related physico-chemical features (Favero-Longo et al., 2005a, 2009a; Daghino et al., 2008).

Serpentinites of the Italian Western Alps contain two asbestos minerals: (a) chrysotile, the serpentine asbestos most widely mined worldwide, always showing fibrous habit, and (b) tremolite, an amphibole which only occasionally occurs with fibrous habit (Groppo and Compagnoni, 2007a). Widespread asbestiform minerals are: (c) antigorite, having the same composition of chrysotile, but different crystalline structure, which in most cases displays a lamellar habit, but occasionally a pseudo-fibrous, columnar habit (Groppo and Compagnoni, 2007b); (d) carlosturanite, a serpentine-type phase (Compagnoni et al., 1985), mostly displaying a fibrous habit; (e) balangeroite, a Fe-rich mineral of the gageite group, mostly found with fibrous habit in association with chrysotile (Compagnoni et al., 1983); (f) diopside, a pyroxene which usually displays prismatic habit, but also occurs as a fibre in chrysotile + carlosturanite bearing veins (Ferraris, 1995; Groppo and Compagnoni, 2007a).

The toxicity of asbestos and asbestiform minerals is currently related to several physico-chemical features acting together such as fibrous habit, high biopersistence and redox-reactive iron ions at the surface, generating Reactive Oxygen Species (ROS) (Kane, 1996; Fubini and Fenoglio, 2007; Ballirano et al., 2008).

In mountain areas, physical environmental forces including temperature changes, freezing and thawing, and washing away by rain, were shown in laboratory experiments to induce a partial dissolution process of chrysotile, tremolite and balangeroite and, consequently, to affect their surface reactivity (Favero-Longo et al., 2009a). On the other hand, serpentinite rocks of mountain areas, including western Alps, are widely colonized by lichen-forming fungi (Favero-Longo et al., 2004), well known agents of physico-chemical deterioration on natural and artificial mineral substrata (Warscheid and Braams, 2000; St. Clair and Seaward, 2004; Gadd et al., 2012). Lichens physically support disaggregation processes through their expanding (wetting) and contracting (drying) thalli adhered to the rock surfaces and the penetration of their hyphae along mineral planes of weakness and intergranular voids (Adamo and Violante, 2000; de los Rìos and Ascaso, 2005). The release of molecules with acidic and chelating functions, leaching and/or complexing metal ions, mostly accounts for lichen-driven mineral dissolution and/or neoformation (Adamo and Violante, 2000). Primary metabolites, particularly the well-known chelator oxalic acid, and/or a wide range of slightly soluble secondary metabolites can be involved in these biogeochemical processes depending on species (Gadd, 1999; Chen et al., 2000).

Lichen communities were described on the asbestos-rich serpentinites of the chrysotile mine of Balangero (Torino, Italy) where the fibre rough texture improves bioreceptivity by increasing water retention and, possibly, propagule deposition (Favero-Longo et al., 2006). Chrysotile fibres contacted by the hyphal penetration component (*sensu* Favero-Longo et al., 2005b) of several lichen species, including *Candelariella vitellina* (L.) Müll. Arg., *Xanthoparmelia pulla* (Ach.) O. Blanco, A. Crespo, Elix, D. Hawksw. & Lumbsch,*Xanthoparmelia tinctina* (Maheu & A. Gillet) Hale, *Lecanora rupicola* (L.) Zahlbr. and*Acarospora cervina* A. Massal., were affected in their chemical composition ( Favero-Longo et al., 2009a, b). The octahedral Mg-rich sheets, which compose the serpentine structure in association with tetrahedral silicate sheets, were selectively leached. The finding of oxalates at the interface between the mineral substrate and most of the investigated lichen species suggested oxalic acid as the most common, although not exclusive, leaching agent ( Favero-Longo et al., 2007, 2009a, b). The removal of poorly coordinated iron ions, replacing some magnesium in chrysotile and related to its surface reactivity, upon the incubation with lichen chelants (including oxalic acid and lichen secondary metabolites, as pulvinic and norstictic acid), was shown in laboratory following a biomimetic approach ( Turci et al., 2007). The induced chemical modification was associated to a partial decrease in the surface reactivity of the fibres, suggesting a bioattenuation role for lichens on asbestos-bearing rocks. The work also indicated that the specific chemical feature of each chrysotile source and, in general, of each fibrous mineral should be independently considered with regard to inactivation.

This paper aims to compare the lichen-driven modification of chemical composition and surface reactivity of chrysotile, occurring in monomineralic or polymineralic veins (chrysotile, chrysotile-balangeroite, chrysotile-diopside-carlosturanite), with that of other asbestos (tremolite) and asbestiform minerals (antigorite).

Four types of fibrous veins uncolonized or penetrated by lichen hyphae in the field were examined by means of light polarizing microscopy and scanning electron microscopy (SEM); elemental compositions of the fibres were obtained with energy dispersive X-ray spectroscopy (EDS). Observations and analyses were focused on veins covered by thalli of *C. vitellina*, a lichen species extremely common on serpentinites ( Favero-Longo et al., 2004), which was ubiquitous on all the four selected vein types.

The asbestos and asbestiform fibres below the thalli, occurring in low amount and difficultly separable from other organic and inorganic materials, were not suitable to carry out the tests on the toxicity relevant surface reactivity. Fibres which were similarly modified in their chemical composition to those found below *C. vitellina* in the field were thus produced in the laboratory by incubating fresh fibres with oxalic acid. Although oxalic acid may not be secreted by *C. vitellina* ( Favero-Longo et al., 2007, 2009b, this work), it is commercially available and displays a high solubility (14.3 g/100 ml at 25 °C) which makes it mostly suitable to generally mimic in vitro, in reasonable times, the deterioration driven by lichen metabolites with acidic and chelating functions ( Turci et al., 2007).

The chemical modification of fibres was experimentally characterized in terms of: i) ion release in the supernatant by atomic emission spectroscopy (ICP-AES) and ii) mode of mineral dissolution (congruent *vs.* incongruent) by the SEM-EDS analysis of the cation to silicon ratio in solid residuals. The potency to release ROS in the presence of hydrogen peroxide (Fenton-like activity, Fubini et al., 1995) was finally evaluated for unaltered and leached fibres using the spin trapping technique coupled with electron paramagnetic resonance spectroscopy (EPR).

## 2. Material and methods

## 2.1. Analysis of lichen deterioration of fibrous minerals in the field

The physical interaction and the chemical deterioration effect of lichens on asbestos and asbestiform minerals were investigated on four types of fibrous veins (A-D) exposed at the surface of serpentinite rocks in Western Alps (Table 1):

**Table 1.**

Asbestos and asbestiform minerals of serpentinite rocks in Western Alps: ideal and calculated formulas of the investigated samples.

**Table 1**
Asbestos and asbestiform minerals of serpentinite rocks in Western Alps: ideal and calculated formulas of the investigated samples.

| Minerals | | Abbr. | Ideal formula | Sample (Vein-min.) | Calculated formula |
|---|---|---|---|---|---|
| Serpentines | Chrysotile | Ctl | $Mg_3Si_2O_5(OH)_4$ | A-Ctl | $(Mg_{2.92}Fe_{0.08})[Si_{2.00}O_5](OH)_4$ |
| | | | | B-Ctl | $(Mg_{2.84}Fe_{0.10}Ni_{0.03})[Si_{2.01}O_5](OH)_4$ |
| | | | | E-Ctl | $(Mg_{2.92}Fe_{0.08})[Si_{2.00}O_5](OH)_4$ |
| | | | | F-Ctl | $(Mg_{2.65}Fe_{0.23}Al_{0.13})[Si_{1.96}O_5](OH)_4$ |
| | Antigorite | Atg | $Mg_3Si_2O_5(OH)_4$ | C-Atg | $(Mg_{2.68}Fe_{0.08}Al_{0.06})[Si_{1.95}Al_{0.05}O_5](OH)_4$ |
| | Carlosturanite | Cst | $(Mg, Fe Mn)_{21}(Ti, Si)_{12}O_{28}(OH)_{34}$ | B-Cst | $Mg_{19.74}Fe_{0.66}Mn_{0.34}Ti_{0.97}Si_{11.16}O_{28}(OH)_{34}$ |
| Others | Tremolite | Trm | $Ca_2Mg_5Si_8O_{22}(OH,F)_2$ | D-Trm | $Ca_{2.00}(Mg_{4.84}\ Fe_{0.16})[Si_8O_{22}](OH)_2$ |
| | Diopside | Dio | $Ca(Mg,Fe)Si_2O_6$ | B-Dio | $Ca_{0.95}(Mg_{1.11}Fe_{0.03})Si_{1.95}O_6$ |
| | Balangeroite | Blg | $(Mg,Fe,Mn)_{42}O_6(Si_4O_{12})_4(OH)_{40}$ | F-Blg | $(Mg_{29.32}Fe_{10.77}Mn_{1.32}Al_{0.05})Si_{16.26}O_{54}(OH)_{40}$ |

Vein A, consisting of chrysotile (A-Ctl), from natural, non-mined serpentinite outcrops in the abandoned Balangero mine (Lanzo Valley).

Vein B, consisting of chrysotile (B-Ctl), carlosturanite (B-Cst) and fibrous diopside (B-Dio), from the serpentinite outcrops of Ciampanesio (Varaita Valley, Monviso ophiolitic massif). On the basis of the complete dissolution of the vein sample, the relative amount of chrysotile, carlosturanite and diopside was 47:48:5 (see Supplementary Material 1, also including X-ray diffraction analysis).

Vein C, consisting of fibrous antigorite (C-Atg), from the serpentinites of Mompantero (Susa Valley). The serpentine fibres were recognized as antigorite on the basis of Raman analyses (Groppo et al., 2006; see Supplementary Material 2).

Vein D, consisting of fibrous tremolite (D-Trm), from the serpentinite lenses embedded within calcschists of Jovencaux (Susa Valley).

Serpentinite samples crossed by the veins were taken both from within the outcrops, where the fibres were not exposed to weathering and biodeterioration forces, and from rock surfaces colonized by *C. vitellina*. Petrographic cross sections for each vein type were prepared according to Piervittori et al. (1991), examined under transmitted light, using a polarizing microscope Olympus BX4, and, after coating with carbon, by scanning electron microscopy (SEM) in both the secondary electron and back-scattered electron mode, using a Stereoscan 410 Leica electron microscope equipped with a link ISIS EDS apparatus. EDS analyses were performed on the different mineral fibres, either uncolonized or contacted by lichens. The sections were analysed unpolished in order to preserve the possible local surface modification, but preventing the production of fine quantitative analyses. Furthermore, the lichen-derived loss of the original mineral stoichiometries prevented the recalculation of the mineral formula from the EDS analyses. Accordingly, EDS data are reported as atomic weight ratios between main octahedral cations and silicon (Mg/Si for all the minerals, and Ca/Si for diopside and tremolite) in order to highlight the effects of incongruent leaching processes ( Turci et al., 2007).

EDS results were statistically analysed by means of ANOVA with post-hoc Student's $t$-test or Tukey's test. SYSTAT release (version 10; SYSTAT, Evanston, IL, USA) was used to perform the tests ($p < 0.05$).

## 2.2. Mimicking lichen deterioration in the lab

According to Turci et al. (2007), the deterioration by lichen metabolites with acidic and chelating functions was mimicked by incubating the mineral fibres with the commercially available (Merck Pro-analysis) oxalic acid 0.5 mM.

Veins B, C, D which were examined in field conditions as control were used as source of fibres for incubations. In the case of chrysotile from the Balangero mine, control fibres of the Ctl-vein A were not sufficient to be processed and used for incubations, so that two different chrysotile veins deriving from the same mining area were used (Table 1):

Vein E: a relatively pure sample of short fibre chrysotile prepared for commercialization (E-Ctl).

Vein F: a mixed field sample (from the same outcrops analysed in the field) of long-fibre chrysotile (F-Ctl) intergrown with brown, Fe-rich asbestiform balangeroite (F-Blg).

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*Separation and incubation of asbestos fibres*. Small bundles of fibres from each vein type were separated from serpentinite rocks with Teflon pincers and gently crushed in a hand agate mortar in order to further isolate the fibres and favour their good dispersion in the following incubation step. Each sample was suspended in 0.5 mM oxalic acid, at 25 °C, in the dark, using a solid/liquid ratio of 1 mg mL$^{-1}$ (200 mg of fibres within 200 mL of solution). Sodium formaldehyde (1%) was added as antiseptic agent. After 35 days of incubation, the fibres were filtered and dried.

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*Analysis of the solution filtrates*. Inductively coupled plasma atomic emission spectrometry (ICP-AES) analyses were performed on the incubating solution in order to evaluate the leaching of calcium, magnesium and silicon from the fibres. Due to the low solubility of iron hydroxides at pH 4.5 (oxalic acid 0.5 mM), the free iron in solution was not searched. The analyses were performed with an IRIS II Advantage/1000 radial plasma spectrometer by Thermo-Jarrel Ash Corp. The optical system is sealed with inert gas, with no moving parts, high-resolution (ER/S) capable. The Echelle grating and dispersion prism monochromator range is extended between 165 and 800 nm, with an optical resolution of 0.007 nm (at 200 nm). The photo device is a CID (charge injection device) camera frozen to −50 °C. As the result of ICP-AES describes an average process concerning the leaching of a representative amount of starting material (200 mg), a single analysis is reported for each supernatant.

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*Elemental analysis of the solid residuals of the fibres*. Energy dispersive X-ray spectroscopy (EDS) coupled with scanning electron microscopy (SEM) was performed on the unpolished, carbon-coated, incubated fibres using a Stereoscan 410 Leica electron microscope equipped with a link ISIS EDS apparatus. The samples were analysed unpolished in order to preserve the possible local surface modification, but preventing the production of fine quantitative analyses. Furthermore, the leaching-derived loss of the original mineral stoichiometries prevented the recalculation of the mineral formula from the EDS analyses.

Accordingly, EDS data are reported as atomic weight ratios between main octahedral cations and silicon (Mg/Si for all the minerals, and Ca/Si for diopside and tremolite, and Fe/Si for balangeroite) to evaluate the congruence or incongruence of the leaching process ( Turci et al., 2007).

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*Evaluation of the surface-induced ROS production.* The spin trapping agent 5,5'-dimethyl-pyrroline-N-oxide (DMPO), which gives a relatively stable [DMPO-OH]▪adduct, was used to detect the formation of the OH radical in aqueous suspension of the fibrous samples contacted with $H_2O_2$ (Fenton activity). Following a well established technique described in previous studies ( Fubini et al., 1995), the nature and quantity of the stabilized radical was measured by means of Electron Paramagnetic Resonance (EPR) spectroscopy. Fibres were suspended (22 mg mL$^{-1}$) in $H_2O_2$ (0.250 ml, 0.5 M in $H_2O$), DMPO (0.250 ml, 0.05 M) and phosphate buffer (0.500 ml, 1 M, pH 7.4). The radical formation was evaluated by recording at 10', 30', 60' the EPR spectrum of the [DMPO-OH]▪ adduct. All spectra were recorded on a Miniscope MS 100 (Magnettech, Berlin, Germany) EPR spectrometer. The instrument settings were as follows: microwave power, 10 mW; modulation, 1000 mG; scan range, 120 G; centre of field, approx. 3345 G. The number of radicals released is proportional to the intensity of the EPR signal. The signals were double integrated and numeric values were reported, as nanomoles of radicals normalized per milligramme of fibres, in order to quantitatively represent the production of free radicals by mineral fibres. Blanks were performed in the absence of any fibre. All the experiments were repeated at least twice.

Results of EDS and EPR spectroscopies were statistically analysed by means of ANOVA with post-hoc Student's *t*-test or Tukey's test using SYSTAT 10.

## 3. Results

### 3.1. Lichen penetration and deterioration of fibrous veins in the field

All the fibrous vein types examined in the study were penetrated by the lichen *C. vitellina*. The maximum depth of its well-developed hyphal penetration component ranged from few hundreds of microns to several millimetres depending on the fibre orientation, the grain-size, and the occurrence of fractures. Fig. 1 summarizes the wide range of different colonization outcomes found in the field. Chrysotile fibres of vein A were contacted by lichen hyphae down to a depth greater than 2 mm from the rock surface ( Fig. 1a); the polymineralic Ctl-Cst-Dio vein B was generally less penetrated (down to av. 200 µm) by*Candelariella* hyphae ( Fig. 1b and c); the rigid and brittle fibrous antigorite of vein C was completely surrounded by lichen hyphae and the related extracellular polymeric matrices ( Fig. 1d and e); the brittle and powdery tremolite vein D poorly supported the colonization and the outcrop surface was rarely contacted by *Candelariella* thalli. However, below the established thalli a dense hyphal net surrounded the needle-like tremolite fibres ( Fig. 1f and g). An intergranular rather than intragranular penetration was generally observed, with hyphae occupying and/or generating discontinuities around fibre bundles. A hyphal-driven disaggregation of the colonized veins was observed in the order: vein D > veins A, C > vein B. The grain size of fibrous bundles seems to be the key factor in the degree of disaggregation, with the smaller bundles (tremolite vein D) being more disaggregated than bigger ones.
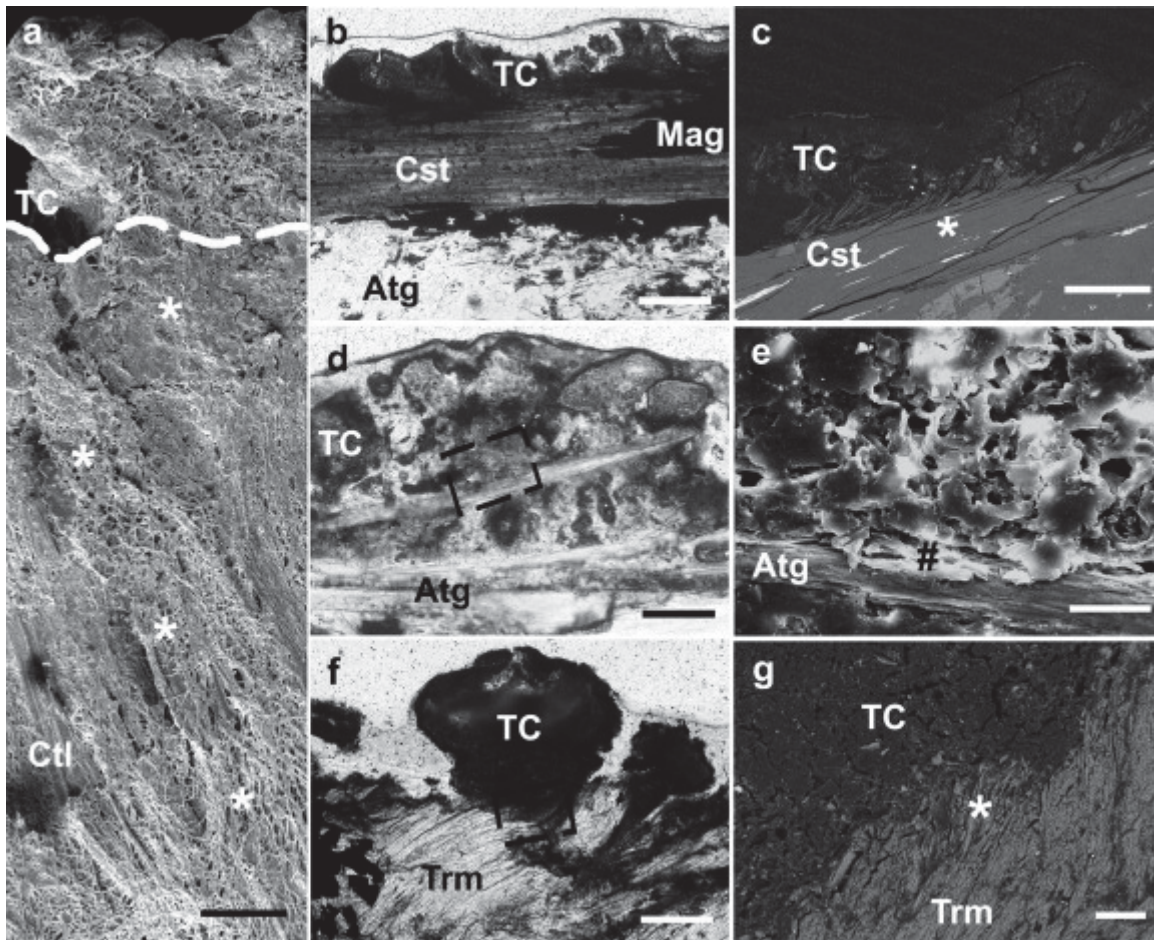
**Fig. 1.**

Lichen growth on fibre-bearing veins in serpentinite rocks. Cross sections of *Candelariella vitellina* on: a, chrysotile vein A; b-c, chrysotile-carlosturanite-diopside vein B; d-e (inset), antigorite vein C; f-g (inset), tremolite vein D. Ctl, chrysotile; Cst, carlosturanite; Atg, antigorite; Mag, magnetite; Trm, tremolite; TC, thalline component; *, hyphal penetration component; #, extracellular polymeric substances; a-e, secondary electron image; c-g, backscattered electron image; b-d-f: thin section under plane polarized light. Relative scale bars: 150 μm (a), 800 μm (b), 500 μm (c), 300 μm (d), 15 μm (e), 300 μm (f), 30 μm (g).

The medullar layer of *Candelariella* thalli did not contain microcrystalline deposits displaying high birefringence between crossed polars which may indicate the possible occurrence of oxalates, ubiquitously detected on serpentinite rocks in the thalli of several other lichen species (see Supplementary Material 3). Accordingly, no crystals having the typical habit of oxalates were observed throughout the SEM observations. On the other hand, yellowish crystals were observed at the cortex layer of the overall examined thalli and identified on the basis of Raman and UV–Vis spectroscopies as pulvinic acid and its derivatives (pulvinic dilactone and calycin), which typically characterize *C. vitellina* and the whole *Candelariella* genus (see Supplementary Material 3).

The effect of lichen colonization induced chemical modification of the fibres to a very different extent. Chrysotile (A-Ctl) from Balangero outcrops was significantly modified, showing a lower Mg/Si ratio with respect to the uncolonized fibre (−24.9%; Fig. 2a); in the complex Ctl-Cst-Dio vein B (Fig. 2d) a lower Mg-depletion was detected in chrysotile (B-Ctl, Mg/Si: −5.6%) with respect to carlosturanite (B-Cst, Mg/Si: −10.0%), while diopside (B-Dio) was unmodified in both its Mg/Si and Ca/Si ratios; antigorite (C-Atg) contacted by lichen hyphae showed a similar Mg/Si to that of uncolonized one (Fig. 2e); tremolite fibres (D-

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Trm) were also not significantly modified by lichens in both Mg/Si and Ca/Si ratios with respect to the control (Fig. 2f).
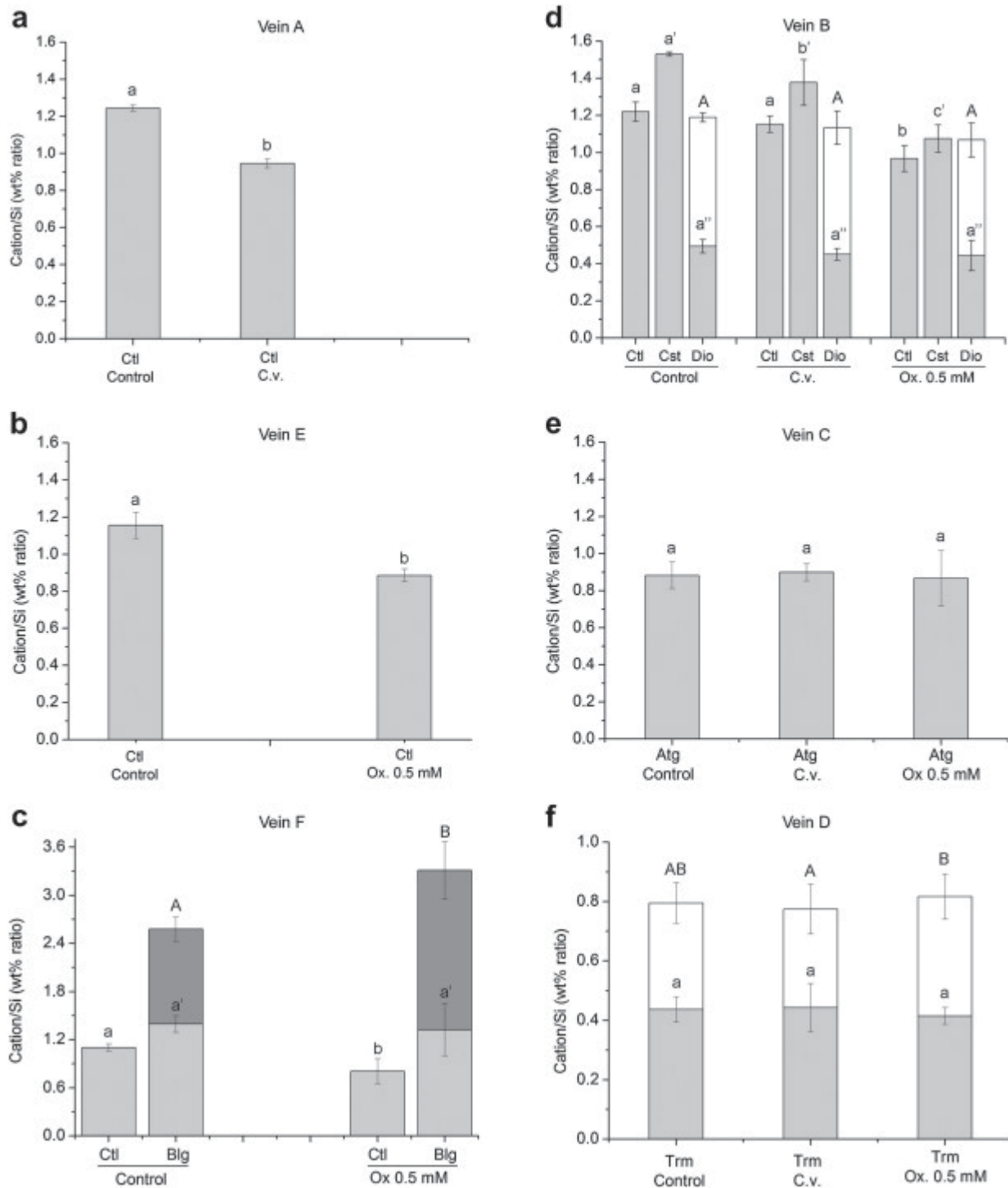


Fig. 2.

Chemical deterioration (variation in the cation/Si wt% ratio) of asbestos and asbestiform minerals from different vein types. a, chrysotile vein A (Ctl); b, pure-chrysotile sample E (Ctl); c, chrysotile (Ctl) - balangeroite (Blg) vein F; d, chrysotile (Ctl)-carlosturanite (Cst)-diopside (Dio) vein B; e, fibrous antigorite (Atg) vein C; f, fibrous tremolite (Trm) vein D. Mg/Si (light grey bars), Fe/Si (dark grey bars) and Ca/Si (empty bars) measured by SEM-EDS on the fibrous minerals exposed at the surface of serpentinite rocks, uncolonized (Control) and colonized by the lichen *Candelariella vitellina* (C.v.) in the field, and after incubation in the laboratory with oxalic acid (Ox. 0.5 mM). Data are reported as average of independent

measures (at least four) ± standard error. For each cation/Si ratio of each mineral of each vein type, columns which do not share at least one letter are statistically different ($p < 0.05$)

## 3.2. Fibre leaching by oxalic acid

In the laboratory, the incubation of the fibrous minerals for 35 days with oxalic acid 0.5 mM determined leaching outcomes mostly similar to those detected in the field below the thalli of *C. vitellina*. In Fig. 2, the "Ox. 0.5 mM" columns report the cations/Si ratios for the laboratory-leached minerals and in Fig. 3 the concentration of the main structural ions found in the supernatant of the leached veins (B–E) is reported.
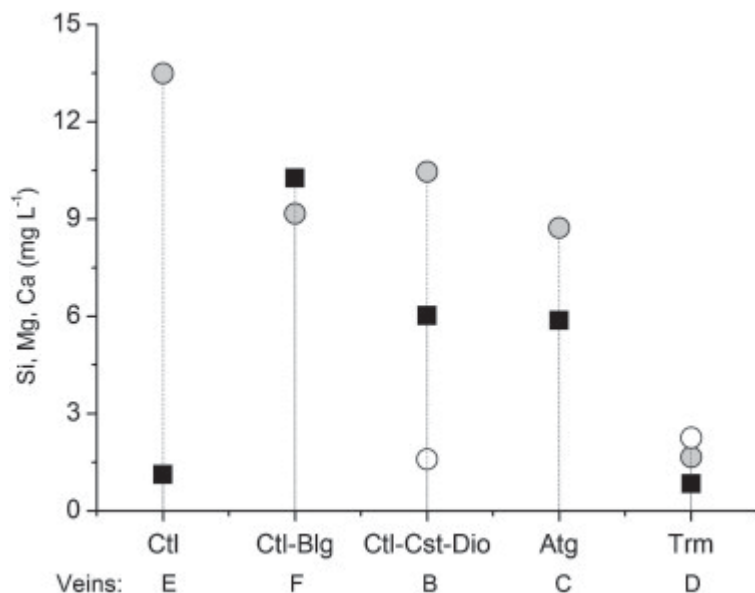


Fig. 3.

Concentration of magnesium (▨), calcium (○) and silicon (■) in the supernatant of fibre suspensions. Chrysotile (Ctl) vein E, chrysotile-balangeroite (Ctl-Blg) vein F, chrysotile-carlosturanite-diopside (Ctl-Cst-Dio) vein B, antigorite (Atg) vein C and tremolite (Trm) vein D after incubation with oxalic acid 0.5 mM for 35 days in the dark.

Chrysotile "lab" samples from Balangero, both the pure (E-Ctl) and the balangeroite-mixed one (F-Ctl), showed a significant decrease of the Mg/Si ratio (−24.4% and −26.9%, with respect to the respective controls; Fig. 2b–c), similarly to what detected below lichens in the field. For balangeroite (F-Blg), the Mg/Si ratio was not modified, while the Fe/Si ratio significantly increased (+67.6%), suggesting a preferential removal of Si (and Mg) with respect to Fe (Fig. 2c). Both chrysotile (B-Ctl) and carlosturanite (B-Cst) in the polymineralic vein B displayed a Mg/Si decrease (−20.7% and −29.8% with respect to the controls), indicating a selective solubilisation of the brucitic layer, in this case significantly higher than that detected below *C. vitellina* ( Fig. 2d). Unmodified cations/Si ratios in diopside (B-Dio), antigorite (C-Atg) and tremolite (D-Trm) were observed with respect to the respective controls ( Fig. 2d–f).

Upon the incubation of pure chrysotile (E-Ctl) a higher concentration of Mg was detected in the supernatant (13.5 mg L$^{-1}$) with respect to silicon (1.1 mg L$^{-1}$) (Fig. 3-Vein E), yielding a Mg/Si ratio of approx. 12 consistent with the incongruent dissolution suggested by EDS measurements. By contrast, a similar release of Mg (9.2 mg L$^{-1}$) and Si (10.3 mg L$^{-1}$) was observed in the case of the mixed vein F, depending on the parallel leaching contributions of chrysotile and balangeroite (Fig. 3-Vein F).

In the case of the polymineralic vein B, the amount of Mg in the supernatant (10.5 mg L$^{-1}$), which relied on the independent leaching contributions of each mineral, was higher than that of Si (6.0 mg L$^{-1}$; Fig. 3-Vein B). Poor Ca occurrence in the supernatant (1.6 mg L$^{-1}$) indicated the slight solubilisation of diopside (B-Dio), which followed the congruent dissolution indicated by EDS.

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Antigorite (Fig. 3-Vein C) also released higher amounts of Mg (8.7 mg $L^{-1}$) with respect to Si (5.9 mg $L^{-1}$), but their ratio in the supernatant (Mg/Si = 1.5) was strongly lower than that detected in the case of pure chrysotile. This datum is consistent with the absence of variation in the Mg/Si ratio in the solid residual indicated by EDS.

Tremolite (Fig. 3-Vein D) leaching determined a Mg amount (1.7 mg $L^{-1}$) in the supernatant much lower than what detected for all the other fibrous samples. Ca was poorly released (2.3 mg $L^{-1}$), similarly to what observed for diopside in the polymineralic vein B. Both Mg and Ca were however higher than Si (0.8 mg $L^{-1}$) yielding a relatively high cations/Si ratio in the supernatant [(Mg + Ca)/Si = 4.7]. This indicates a leaching mechanism incongruent in nature, though only few surface atomic layers were affected, as indicated by the low overall dissolution values and the unaltered bulk EDS analysis.

### 3.3. Effect of fibre deterioration on ROS release

All fibrous samples, including chrysotile-bearing veins, asbestos tremolite and asbestiform antigorite, showed Fenton reactivity, i.e. they released ▪OH radicals in the presence of hydrogen peroxide (Fig. 4). Different absolute values of radical release were detected for the different veins: these variations depend on several factors (including surface area) that complicate the comparison of the specific fibre reactivity and a direct correlation with their toxic potential, the analysis of which goes well beyond the aim of this paper which is limited to the analysis of the deterioration effect on each fibrous mineral or assemblage.
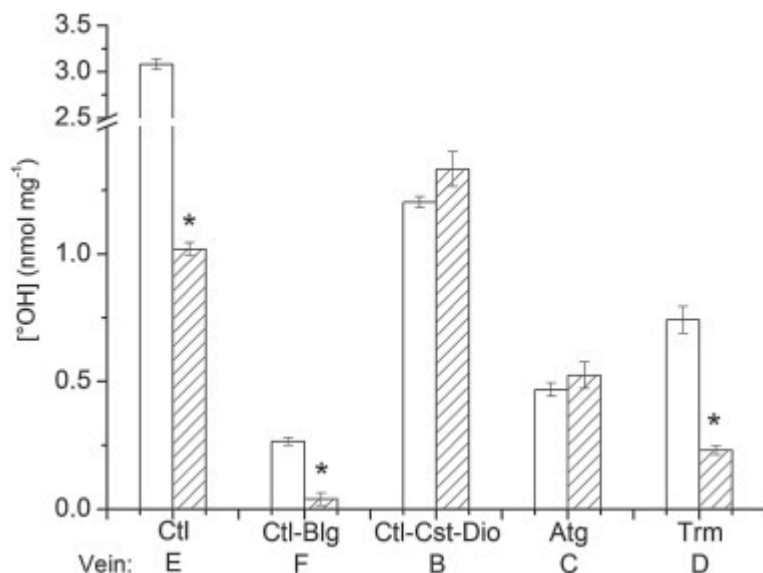


**Fig. 4.**

Fenton-like activity at the surface of chrysotile (Ctl), chrysotile-balangeroite (Ctl-Blg), chrysotile-carlosturanite-diopside (Ctl-Cst-Dio), antigorite (Atg) and tremolite (Trm) fibres. ▪OH concentration measured by EPR/spin trapping technique by suspending in a hydrogen peroxide solution unaltered fibres from the field (control, empty bars) and fibres upon incubation with oxalic acid 0.5 mM (crossed bars). The bars indicate the amount of ▪OH generated as nmol normalized for fibre weight. Data are reported as means ± standard error of at least four independent measures. Asterisks indicate significant differences with respect to the control

The different fibrous samples were differently affected in their surface reactivity after they were chemically modified in the lab in a similar way to what was observed below lichen thalli in the field. Pure chrysotile (E-Ctl), the mixed chrysotile-balangeroite sample (F-Ctl and F-Blg) and fibrous tremolite (D-Trm) showed a significant decrease of radical release upon the incubation with oxalic acid 0.5 mM. By contrast, no significant modification of reactivity was detected in the case of the polymineralic vein B and antigorite vein C.

### 4. Discussion

The hyphal penetration of lichenized- and non-lichenized fungi within a rock and the following metabolite release imply modifications of the chemical and crystallographic features of the contacted minerals (Adamo and Violante,

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2000; Chen et al., 2000; Hoffland et al., 2004; Gadd et al., 2012). In the case of toxic or potentially toxic NOA or asbestiform fibres, such modifications may likely be associated to the modulation of their hazard (Hochella, 1993).

The physical interaction of the epilithic *C. vitellina* with the examined veins is not limited to the rock surface, but extends down to the (sub-) millimetric depth of the hyphal penetration component, consistently with what observed for similar lichens/silicate rocks pairs ( St. Clair and Seaward, 2004; de los Ríos and Ascaso, 2005; Favero-Longo et al., 2005b). The biogeochemical action, recorded in the field and mimicked in the laboratory, affects differently the chemical composition of the various fibrous minerals, thus yielding vein-dependent modifications of the surface reactivity.

The incubation with oxalic acid - a well-known chelator able to leach divalent cations from the octahedral mineral layers (Thomassin et al., 1977; Turci et al., 2007) - yields asbestos and asbestiform fibres similarly modified in the chemical composition to those observed in the field below the lichen thalli. Only in the case of chrysotile and carlosturanite in vein B, the chemical modification obtained in the laboratory is higher than that detected below *C. vitellina*, possibly reflecting the poor hyphal penetration observed within this vein type. However, the absence of oxalate deposits at the *Candelariella*-substrate interface - already reported on solid culture media ( Favero-Longo et al., 2007), on other rock substrates ( Jorge Villar et al., 2005) and on asbestos cement roofs ( Favero-Longo et al., 2009b) - rules out oxalic acid as the major agent of the *Candelariella*-driven dissolution processes. On the other hand, the detected mineral deterioration may be related to the release of secondary metabolites such as pulvinic acid, the precursor of the crystalline deposits observed in the cortex layer and characterizing the *Candelariella* genus ( Culberson, 1979). Pulvinic acid, as oxalic acid, displays acidic and chelating functions that make it active in complexing metals, including $Mg^{2+}$, $Fe^{2+}$ and $Fe^{3+}$, in the alkaline range ( Hauck et al., 2009) which often characterizes water and soils deriving from serpentinized-ultramafic rocks ( Alexander et al., 2007), including asbestos-rich serpentinite sediments ( Schereier et al., 1987). Accordingly, the fibres produced upon incubation for 35 days with oxalic acid not only are similarly modified in their chemical composition to those colonized by *C. vitellina*, but are likely modified through a similar dissolution pathway, thus being suitable for testing the potency to release ROS of lichen-deteriorated fibres. It is worth noting that the very low solubility of *Candelariella* secondary metabolites ( Elix and Stocker-Wörgötter, 2008) prevents to obtain pulvinic acid at a suitable concentration to mimic, in times compatible with laboratory experiments, the mineral dissolution observed in the field after a years- or decades-long lichen colonization. Accordingly, previous experiments showed that leaching effects upon 35 days incubation with saturated pulvinic acid (0.003 mM) are limited to the uppermost atomic layers of the minerals ( Turci et al., 2007).

## 4.1. Chrysotile in pure and mixed veins

Chrysotile, as previously reported with regard to abiotic and biotic leaching systems (Thomassin et al., 1977; Morgan, 1997; Turci et al., 2008; Oze and Solt, 2010), exhibits an incongruent dissolution in all the four studied veins (A, B, E, F). In fact, Mg is leached out more easily than Si, as shown by the decrease of the Mg/Si ratio in the lichen colonized (field) and oxalic acid treated (laboratory) fibres (see Fig. 2a, b, c, d: Ctl columns). Pioneer work by Wilson et al. (1981) reported the complete Mg depletion of chrysotile colonized by *Lecanora atra*, yielding amorphous silica as deterioration product. Incongruent ion depletion was also previously reported for other phyllosilicates, as biotite and muscovite, contacted by lichen thalli ( Ascaso and Wierzchos, 1996; Prieto et al., 1997; Wierzchos and Ascaso, 1998).

The incongruent dissolution determines a high Mg/Si ratio in the supernatant of the pure chrysotile from vein E. This depends on the oxalic acid-driven selective uptake of $Mg^{2+}$ and likely $Fe^{2+/3+}$, its minor substitute in the octahedral brucite-like layer. Iron is indeed chelated by several acidic fungal metabolites, including oxalic acid (Gadd, 1999), and its occurrence in chrysotile accounts for the detrimental surface chemical reactivity (Gazzano et al., 2007). Accordingly, the ROS production is significantly reduced after the leaching of vein E in the laboratory (Fig. 4), suggesting that a similar bioattenuation process, likely driven by the Mg- and Fe-chelating activity of pulvinic acid (Hauck et al., 2007, 2009), may occur in the field in the case of the lichen-modified vein A.

The submicroscopic association of chrysotile with balangeroite or with carlosturanite and diopside may modulate the impact of lichen deterioration on the chemical reactivity in veins F and B, respectively. In the case of the polymineralic

vein F, the decrease of ROS production by the leached fibres with respect to the control (−85%) is even higher than that detected for the pure chrysotile in vein E (−67%). Balangeroite shows an incongruent dissolution in the opposite direction with respect to chrysotile, with silicon being preferentially removed with respect to the divalent structural cations (mainly Fe) (Favero-Longo et al., 2009a), as indicated by the increase of the cations/Si ratio detected by EDS after incubation and the high amount of silicon found in the supernatant. The incongruent dissolutions of both chrysotile and balangeroite leading to a modified surface chemistry may account for the reactivity loss (Turci et al., 2007).

In polymineralic vein B, chrysotile and carlosturanite, which also displays a serpentine-like structure (Ferraris, 1995), show an incongruent dissolution because of the selective Mg-depletion of the brucitic-like layer, which is even higher in the laboratory than in the field. However, no significant changes are shown in terms of ROS production after leaching. The Ca occurrence in the incubation supernatant and the absence of variation in the Mg/Si and Ca/Si in the solid residuals with respect to control account for a congruent dissolution of diopside, likely related to a persistent radical reactivity (see Section 4.3). However, as their close intergrowth prevents the availability of pure samples for the three phases (Groppo and Compagnoni, 2007a), no certainty is possible on both the dissolution mode and extent of carlosturanite and diopside which could account for the definition of the final responsible for the unvaried surface reactivity following the leaching process.

## 4.2. Other fibrous minerals

Antigorite shares the same chemical composition with chrysotile, but maintains its surface reactivity unmodified upon leaching. The different crystal structure of the two serpentine polymorphs accounts for two different dissolution modes, with antigorite being congruently dissolved both in the field below lichens and in the laboratory. The congruent dissolution pattern of antigorite is unveiled by EDS analyses and by the evaluation of Mg and Si amounts in the supernatant which are closer than in the case of chrysotile. This dissolution mode, with a higher Si release, suggests a higher renewal of the exposure of the brucitic-like layers at the surface, including the Fe substituting for Mg, finally accounting for the maintenance of the surface reactivity upon leaching.

Chrysotile displays a cylindrical structure with a continuous tetrahedral silica layer exposing a leacheable brucitic octahedral layer on the outwards of the fibre, whereas antigorite shows an alternating-wave structure in which the silica tetrahedra are tilted periodically below and above the brucitic layer (Wicks and O'Hanley, 1988). The rare fibrous habit depends on peculiar mechanisms of vein formation (Groppo and Compagnoni, 2007b).

When the brucitic layer is removed, the two polymorphs expose a different structured silica layer, with chrysotile exposing a protonable O (Si–O $\rightarrow$ Si–OH), previously shared with Mg, and antigorite exposing a bridge O involved in a siloxane bridge that is forced to break upon protonation at acidic pH (Si–O–Si $\rightarrow$ Si–OH + Si–O$^-$). Such structural features may account for the observed solubility pathways: incongruent for chrysotile and congruent for antigorite).

According to its well-known biopersistence, which accounts for its long durability in human fluids and high toxicity (McDonald and McDonald, 1997), tremolite displays a very low ion release in the supernatant, which justifies the slight variation in the solid residuals and in the field samples below lichen thalli. However, dissolution of cation (Mg, Ca) rich layers, where Fe substitutes for Mg, is significantly higher than that of Si, accounting for a poor, but incongruent dissolution, which justifies the significant decrease in surface reactivity. It is worth noting that another amphibole asbestos, crocidolite which does not occur in Western Alps, but was widely used in asbestos-cement together with chrysotile, also showed a trend towards a poor selective depletion of Mg and Fe with respect to Si below thalli of *Candelariella* (Favero-Longo et al., 2009b). On the other hand, the non-fibrous amphibole ferrohastingsite contacted by lichen hyphae was shown not to change its chemical and structural identity (Barker and Banfield, 1996).

## 4.3. Congruent vs. incongruent dissolution and surface reactivity

The overview of the deterioration processes of asbestos and asbestiform minerals below lichens in the field and in oxalic acid solution in the laboratory highlights that the fibre surface reactivity is modulated by the dissolution mode, rather than by the dissolution extent. The more the dissolution is incongruent, i.e. with the selective cation depletion where reactive iron appears as occasional substitute, the more the surface is modified with respect to the control and becomes less reactive. A congruent dissolution – even in the case of antigorite which shows a significant leaching of both Mg and Si – is not associated with a surface modification, the surface being only renewed and thus reactivity maintained. It is worth noting that pure chrysotile from Central Alps (Val Malenco), showing a higher but more congruent leaching of Mg and Si with respect to pure chrysotile from Balangero, showed a significantly lower reduction of the surface reactivity (Turci et al., 2007).

## 5. Conclusions

The evaluation of the health risk posed by NOA and asbestiform minerals has to take into account surface modifications induced by weathering (Favero-Longo et al., 2009a) and biodeterioration forces on natural outcrops. Present data confirm that the reactivity of fresh fibres cannot be used to assess the hazard of NOA. Lichen deterioration driven by metabolites with acidic and chelating functions, mimicked in the laboratory using the commercially available oxalic acid, modifies chemistry and surface reactivity of fibre-bearing veins to a different extent. The veins exclusively bearing minerals subjected to incongruent dissolution (Veins E, F, D) show a significant (even if not complete) inactivation of the surface, while the veins bearing minerals subjected to congruent dissolution (Veins B, C) maintain their reactivity. Lichens incongruently leach chrysotile both in monomineralic and polymineralic veins, but in the latter ones the surface reactivity is maintained or diminished according to the associated minerals and their dissolution mode. Although a bioattenuation role of lichens is envisaged for some veins bearing chrysotile and, surprisingly, tremolite, other veins result unaffected, suggesting that this positive attenuating effect of lichens cannot be generalized for all colonized NOA and has to be evaluated case by case.

## References

Adamo and Violante, 2000 P. Adamo, P. Violante **Weathering of rocks and neogenesis of minerals associated with lichen activity** Applied Clay Science, 16 (2000), pp. 229–256

Alexander et al., 2007 E.B. Alexander, R.G. Coleman, T. Keeler-Wolf, S.P. Harrison **Serpentine Geoecology of Western North America** Oxford University Press, New York (2007)

Ascaso and Wierzchos, 1996 C. Ascaso, J. Wierzchos **Morphological and chemical features of bioweathered granitic biotite induced by lichen activity** Clays and Clay Minerals, 44 (1996), pp. 652–657

Ballirano et al., 2008 B. Ballirano, G. Andreozzi, G. Belardi **Crystal chemical and structural characterization of fibrous tremolite from Susa Valley, Italy, with comments on potential harmful effects on human health** American Mineralogist, 93 (2008), pp. 1349–1355

Baris et al., 1987 I. Baris, L. Simonato, M. Artvinlii, F. Pooley, R. Saracci, J. Skidmore, C. Wagner **Epidemiological and environmental evidence of the health effects of exposure to erionite fibres: a four-year study in the Cappadocian region of Turkey** International Journal of Cancer, 39 (1987), pp. 7–10

Barker and Banfield, 1996 W.W. Barker, J.F. Banfield **Biologically versus inorganically mediated weathering reactions: relationships between minerals and extracellular microbial polymers in lithobiontic communities** Chemical Geology, 132 (1996), pp. 55–69

Chen et al., 2000 J. Chen, H.P. Blume, L. Beyer **Weathering of rocks induced by lichen colonization – A review** Catena, 39 (2000), pp. 121–146

Comba et al., 2003 A. Comba, P. Gianfagna, L. Paoletti **Pleural mesothelioma cases in Biancavilla are related to a new fluoro-edenite fibrous amphibole** Archives of Environmental & Occupational Health, 58 (2003), pp. 229–232

Compagnoni et al., 1983 R. Compagnoni, G. Ferraris, L. Fiora **Balangeroite, a new fibrous silicate related to gageite from Balangero, Italy** American Mineralogist, 68 (1983), pp. 214–219

Compagnoni et al., 1985 R. Compagnoni, G. Ferraris, L. Mellini **Carlosturanite, a new asbestiform rock-forming silicate from Val Varaita, Italy** American Mineralogist, 70 (1985), pp. 767–772

Culberson, 1979 C.F. Culberson **Chemical and Botanical Guide to Lichen Products (Reprint)** Otto Koeltz Science Publishers, Koenigstein (1979)

Culley et al., 2010 M.R. Culley, J. Zorland, K. Freire **Community responses to naturally occurring asbestos: implications for public health practice** Health Education Research, 25 (2010), pp. 877–891

Daghino et al., 2008 S. Daghino, E. Martino, E. Vurro, M. Tomatis, M. Girlanda, B. Fubini, S. Perotto **Bioweathering of chrysotile by fungi isolated in ophiolitic sites** FEMS Microbiological Letters, 285 (2008), pp. 242–249

de los Ríos and Ascaso, 2005 A. de los Ríos, C. Ascaso **Contributions of in situ microscopy to the current understanding of stone biodeterioration** International Microbiology, 8 (2005), pp. 181–188

Elix and Stocker-Wörgötter, 2008 J.A. Elix, E. Stocker-Wörgötter **Biochemistry and secondary metabolites** T.H. Nash III. (Ed.), Lichen Biology (second ed.), Cambridge University Press, Cambridge (2008), pp. 104–133

Favero-Longo et al., 2004 S.E. Favero-Longo, D. Isocrono, R. Piervittori **Lichens and ultramafic rocks: a review** Lichenologist, 36 (2004), pp. 391–404

Favero-Longo et al., 2005a S.E. Favero-Longo, F. Turci, M. Tomatis, D. Castelli, P. Bonfante, M.F. Hochella, R. Piervittori, B. Fubini **Chrysotile asbestos is progressively converted into a non-fibrous amorphous material by the chelating action of lichen metabolites** Journal of Environmental Monitoring, 7 (2005), pp. 764–766

Favero Longo et al., 2005b S.E. Favero Longo, D. Castelli, O. Salvadori, E. Belluso, R. Piervittori **Pedogenetic action of *Lecidea atrobrunnea*, *Rhizocarpon geographicum* gr. and *Sporastatia testudinea* on serpentinized ultramafic rocks in an alpine environment** International Biodeterioration & Biodegradation, 56 (2005), pp. 17–27

Favero-Longo et al., 2006 S.E. Favero-Longo, C. Siniscalco, R. Piervittori **Plant and lichen colonization in an asbestos mine: spontaneous bio-attenuation limits air dispersion of fibres** Plant Biosystems, 140 (2006), pp. 1–17

Favero-Longo et al., 2007 S.E. Favero-Longo, M. Girlanda, R. Honegger, B. Fubini, R. Piervittori **Interactions of sterile-cultured lichen-forming ascomycetes with asbestos fibres** Mycological Research, 111 (2007), pp. 473–481

Favero-Longo et al., 2009a S.E. Favero-Longo, F. Turci, M. Tomatis, R. Compagnoni, R. Piervittori, B. Fubini **The effect of weathering on ecopersistence, reactivity and potential toxicity of naturally occurring asbestos and asbestiform minerals** Journal of Toxicology and Environmental Health, Part A, 72 (2009), pp. 305–314

Favero-Longo et al., 2009b S.E. Favero-Longo, D. Castelli, B. Fubini, R. Piervittori **Lichens on asbestos cement roofs: bioweathering and biocovering effects** Journal of Hazardous Materials, 162 (2009), pp. 1300–1308

Ferraris, 1995 G. Ferraris **Recent contributions to the characterization of minerals from metamorphic rocks of Western Alps** in: B. Lombardo (Ed.), Studies on Metamorphic Rocks and Minerals of the Western Alps. A Volume in Memory of Ugo Pognante, Bollettino del Museo Regionale Scienze naturali, vol. 13, supplemento 2, Museo Regionale di Scienze Naturali, Torino (1995), pp. 55–74

Fubini and Fenoglio, 2007 B. Fubini, I. Fenoglio **Toxic potential of mineral dusts** Elements, 3 (2007), pp. 407–414

Fubini et al., 1995 B. Fubini, L. Mollo, E. Giamello **Free radical generation at the solid/liquid interface in iron containing minerals** Free Radical Research, 23 (1995), pp. 593–614

Gadd, 1999 G.M. Gadd **Fungal production of citric and oxalic acid: importance in metal speciation, physiology and biogeochemical processes** Advances in Microbial Physiology, 41 (1999), pp. 47–92

Gadd et al., 2012 G.M. Gadd, Y.J. Rhee, K. Stephenson, Z. Wei **Geomycology: metals, actinides and biominerals** Environmental Microbiology Reports, 4 (2012), pp. 270–296

Gazzano et al., 2007 E. Gazzano, F. Turci, E. Foresti, M.G. Putzu, E. Aldieri, F. Silvagno, I.G. Lesci, M. Tomatis, C. Riganti, C. Romano, B. Fubini, N. Roveri, D. Ghigo **Iron-loaded synthetic chrysotile: a new model solid for studying the role of iron in asbestos toxicity** Chemical Research in Toxicology, 20 (2007), pp. 380–387

Groppo et al., 2006 C. Groppo, C. Rinaudo, S. Cairo, D. Gastaldi, R. Compagnoni **Micro-Raman spectroscopy for a quick and reliable identification of serpentine minerals from ultramafics** European Journal of Mineralogy, 18 (2006), pp. 319–329

Groppo and Compagnoni, 2007a C. Groppo, R. Compagnoni **Metamorphic veins from the serpentinites of the Piemonte Zone, western Alps, Italy: a review** Periodico di Mineralogia, 76 (2007), pp. 127–153 (Special Issue: In honour of Ezio Callegari on his retirement)

Groppo and Compagnoni, 2007b C. Groppo, R. Compagnoni **Ubiquitous fibrous antigorite veins from the Lanzo Ultramafic Massif, Internal Western Alps (Italy): characterisation and genetic conditions** Periodico di Mineralogia, 76 (2007), pp. 169–181 (Special Issue: In honour of Ezio Callegari on his retirement)

Hauck et al., 2007 M. Hauck, S. Huneck, J.A. Elix, A. Paul **Does secondary chemistry enable lichens to grow on iron-rich substrates?** Flora, 202 (2007), pp. 471–478

Hauck et al., 2009 M. Hauck, S.R. Jürgens, K. Willenbruch, S. Huneck, C. Leuschner **Dissociation and metal-binding characteristics of yellow lichen substances suggest a relationship with the site preferences of lichens** Annals of Botany, 183 (2009), pp. 13–22

Hochella, 1993 M.F. Hochella **Surface chemistry, structure, and reactivity of hazardous mineral dust**,in: G.D. Guthrie, B.T. Mossman (Eds.), Health Effects of Mineral Dusts, Reviews in Mineralogy, vol. 28, Book Crafters, Inc., Chelsea, Michigan (1993), pp. 275–308

Hoffland et al., 2004 E. Hoffland, T.Y. Kuyper, H. Wallander, C. Plassard, A.A. Gorbushina, K. Haselwndter, S. Holmström, R. Landweert, U.S. Lundström, A. Rosling, R. Sen, M.M. Smits, P.A.W. van Hees, N. van Breemen **The role of fungi in weathering** Frontiers in Ecology and the Environment, 2 (2004), pp. 258–264

International Ban Asbestos Secretariat, 2011 International Ban Asbestos Secretariat **Chronology of National Asbestos Ban** (2011) http://ibasecretariat.org/chron_ban_list.php (last accessed on July 2012)

Jorge Villar et al., 2005 S.E. Jorge Villar, H.G.M. Edwards, M.R.D. Seaward **Raman spectroscopy of hot desert, high altitude epilithic lichens** Analyst, 130 (2005), pp. 730–737

Kane, 1996 A.B. Kane **Mechanisms of mineral fibre carcinogenesis** A.B. Kane, P. Boffetta, R. Saracci, J.D. Wilbourn (Eds.), Mechanisms of Fibre Carcinogenesis, IARC – WHO, Lyon (1996), pp. 11–34

Lee et al., 2008 R.J. Lee, B.R. Strohmeier, K.L. Bunker, D.R. Van Orden **Naturally occurring asbestos: a recurring public policy challenge** Journal of Hazardous Materials, 153 (2008), pp. 1–21

McDonald et al., 2004 J.C. McDonald, J. Harris, B. Armstrong **Mortality in a cohort of vermiculite miners exposed to fibrous amphibole in Libby, Montana** Occupational and Environmental Medicine, 61 (2004), pp. 363–366

McDonald and McDonald, 1997 J.C. McDonald, A.D. McDonald **Chrysotile, tremolite and carcinogenicity** The Annals of Occupational Hygiene, 41 (1997), pp. 699–705

Morgan, 1997 A. Morgan **Acid leaching studies of chrysotile asbestos from mines in the Coalingia region of California and from Quebec and British Columbia** Annals of Occupational Hygiene, 41 (1997), pp. 249–268

O'Hanley, 1996 D. O'Hanley **Serpentinites. Records of Tectonics and Petrological History** Oxford Monographs on Geology and Geophysics, vol. 34Oxford University press, Oxford (1996)

Oze and Solt, 2010 C. Oze, K.L. Solt **Biodurability of chrysotile and tremolite asbestos in simulated lung and gastric fluids** American Mineralogist, 95 (2010), pp. 825–831

Piervittori et al., 1991 R. Piervittori, A. Laccisaglia, L.M. Gallo, L. Appolonia **Aspetti floristico-vegetazionali e metodologici relativi ai licheni su materiali lapidei in Valle d'Aosta** Revue Valdôtaine d'Histoire Naturelle, 45 (1991), pp. 53–86

Prieto et al., 1997 B. Prieto, B. Silva, T. Rivas, J. Wierzchos, C. Ascaso **Mineralogical transformation and neoformation in granite caused by the lichens *Tephromela atra* and *Ochrolechia porella*** International Biodeterioration & Biodegradation, 40 (1997), pp. 191–199

Pugnaloni et al., 2010 A. Pugnaloni, F. Giantomassi, G. Lucarini, S. Capella, M. Mattioli Belmonte, M. Orciani, E. Belluso **Effects of asbestiform antigorite on human alveolar epithelial A549 cells: a morphological and immunohistochemical study** Acta Histochemica, 112 (2010), pp. 133–146

Ross and Nolan, 2003 M. Ross, R.P. Nolan **History of asbestos discovery and use and asbestos-related disease in context with the occurrence of asbestos within ophiolite complexes** Special Paper 373 Y. Dilek, S. Newcomb (Eds.), Ophiolite Concept and the Evolution of Geological Thought, Geological Society of America, Boulder (Colorado) (2003), pp. 447–470

Schreier et al., 1987 H. Schreier, J.A. Omueti, L.M. Lavkulich **Weathering processes of asbestos-rich serpentinitic sediments** Soil Science Society of America Journal, 51 (1987), pp. 993–999

St. Clair and Seaward, 2004 L.L. St. Clair, M.R.D. Seaward **Biodeterioration of Stone Surfaces: Lichens and Biofilms as Weathering Agents of Rocks and Cultural Heritage** Kluwer Academic Publishers, Dordrecht (2004)

Thomassin et al., 1977 J.H. Thomassin, J. Goni, P. Baillif, J.C. Touray, M.C. Jaurand **An XPS study of the dissolution kinetics of chrysotile in 0.1 N oxalic acid at different temperatures** Physics and Chemistry of Minerals, 1 (1977), pp. 385–398

Turci et al., 2007 F. Turci, S.E. Favero-Longo, M. Tomatis, G. Martra, D. Castelli, R. Piervittori, B. Fubini **A biomimetic approach to the chemical inactivation of chrysotile fibres by lichen metabolites** Chemistry – A European Journal, 13 (2007), pp. 4081–4093

Turci et al., 2008 F. Turci, M. Tomatis, S. Mantegna, G. Cravotto, B. Fubini **A new approach to the decontamination of asbestos-polluted waters by treatment with oxalic acid under power ultrasound** Ultrasonics Sonochemistry, 15 (2008), pp. 420–427

Turci et al., 2009 F. Turci, M. Tomatis, R. Compagnoni, B. Fubini **Role of associated mineral fibre in chrysotile asbestos health effects: the case of balangeroite** Annals of Occupational Hygiene, 53 (2009), pp. 491–497

US-EPA, 2011 US-EPA **Naturally Occurring Asbestos** (2011) http://www.epa.gov/asbestos/pubs/clean.html (Last accessed on July 2012)

Virta, 2005 R.L. Virta **Asbestos** U.S. Geological Survey Mineral Yearbook, U.S. Geological Survey, Denver Federal Centre, Denver, CT, USA (2005), pp. 8.1–8.6 http://minerals.usgs.gov/minerals/pubs/commodity/asbestos/ (Last accessed on July 2012)

Warscheid and Braams, 2000 Th. Warscheid, J. Braams **Biodeterioration of stone: a review** International Biodeterioration & Biodegradation, 46 (2000), pp. 343–368

Wicks and O'Hanley, 1988 F.J. Wicks, D.S. O'Hanley **Serpentine minerals: structures and petrology** Reviews in Mineralogy and Geochemistry, 19 (1988), pp. 91–167

Wierzchos and Ascaso, 1998 J. Wierzchos, C. Ascaso **Mineralogical transformation of bioweathered granitic biotite, studied by HRTEM: evidence for a new pathway in lichen activity** Clays and Clay Minerals, 46 (1998), pp. 446–452

Wilson et al., 1981 M.J. Wilson, D. Jones, W.J. McHardy **The weathering of serpentinite by *Lecanora atra*** Lichenologist, 13 (1981), pp. 167–176