Journal of Hazardous Materials 162 (2009) 1300–1308

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Lichens on asbestos–cement roofs: Bioweathering and biocovering effects

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ARTICLE INFO

Article history:
Received 13 December 2007
Received in revised form 30 April 2008
Accepted 9 June 2008
Available online 24 June 2008

Keywords:
Asbestos–cement
Biocovering
Bioweathering
Image analysis
Lichen

ABSTRACT

Asbestos–cement roofs, the most widespread sources of airborne, toxic and carcinogenic asbestos fibres, are often colonized by lichens. Since these latter are physical and chemical weathering agents, they have been often considered as significant responsible of disaggregation processes increasing fibre dispersion. Consequently, official guidelines for the management of asbestos often suggest their removal. Weathering and/or covering effects of lichens on asbestos–cement, however, have never been deeply investigated and available procedures to evaluate asbestos–cement aging do not take the biological colonization into account. In this study we show that a 25% lichen cover modifies physical and chemical properties of asbestos–cement sheets containing chrysotile and crocidolite fibres. By innovatively coupling pull up tests and image analysis of linear structures, we show that fibre loss is significantly lower (~30%) where lichens develop and offer a physical barrier to the fibre detachment. Below the most covering lichens (*Acarospora cervina*, *Candelariella* ssp.), chrysotile and crocidolite undergo a partial incongruent dissolution, which in laboratory assays generally determined a reduction of their surface reactivity. Because of their biocovering and bioweathering effects, lichens on asbestos–cement play a role which differs from the current public opinion and the assumptions of some official regulations, acting as effective spontaneous bioattenuation agents.

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1. Introduction

Asbestos–cement is a composite material that consists of Portland cement reinforced with asbestos fibres, mainly serpentine (chrysotile) and occasionally amphiboles (crocidolite, amosite) [1]. Because of its easy and inexpensive processing as well as of high physical and chemical resistance, during the last century it was used as building material with various forms and styles to suit different needs. In the US, asbestos–cement shingles and corrugated sheets were recommended after the 1920s as roofing materials to avoid hazards associated with wood and they were widely used for lightweight housing and industrial buildings [2], thus rising above 1450 thousand metric tonnes of production from 1965 to 2000 [3]. The findings of health diseases due to asbestos determined from the late 1960s a progressive reduction of fibre uses and led to the promulgation of laws which restricted or banned asbestos exploitation in most western countries from the beginning of 1990s [4–6]. Nevertheless asbestos–cement is still used in many countries [7,8] and million tonnes of this material till need to be disposed in those countries which have already banned its utilization [9].

Since pathogenic effect strictly depends on the inhalation of the fibrous minerals [5], asbestos–cement does not constitute, and is usually not considered, a health hazard until fibres are bound in the hard cement mass, e.g. [10–16]. Even if asbestos–cement largely gained a reputation of durability and indestructibility, it is now accepted that weathering processes after many years (2–4 decades) commonly develop a loose and brittle surface layer which allows the exposure of the fibres, their loss and their consequent air dispersion [17]. This aging of the asbestos–cement surfaces is known to be often associated with a colonization of cyanobacteria, algae, fungi, lichens and mosses which benefit of the water-absorbing capacity and of a favorable texture for the lodgments of propagules and the attachment of young thalli (the definitions of lichenological terms used in this article are supplied in Glossary) [18]. Floristic and ecological investigations about lichen colonization on aged asbestos–cement were already performed, which showed variable cover values from ca. 10 to 70% [18–21].

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0304-3894/$ – see front matter © 2008 Published by Elsevier B.V.
doi:10.1016/j.jhazmat.2008.06.060

Since lichen occurrence is generally accepted to make ineffective the application of surface coatings, their removal by high-pressure water jetting, remote cleaning and cleaning with biocides is commonly suggested by public administrations, e.g. [10,13,14]. Different studies, moreover, focused on the physico-chemical techniques for the removal of any lichen cover from asbestos-rich roofs [20].

Lichen effects on the durability of this material, however, are still debated, also through the official guidelines for the asbestos management. Sometimes lichens are considered as responsible of the softening of the asbestos surface [13,17]: secreting oxalic acid, they attack the alkaline cement matrix over time leading to accelerated deterioration [11]; their dark color causes a substantial increase in roof cavity temperature [22]. In other case, the lichen growth, although visually unattractive, is retained to have no significant effect on the strength, durability or lifetime of asbestos–cement [14]. However, it is worth noting that the evaluation of the aging, in terms of disaggregation, of asbestos–cement roofs is currently performed by tests, as pull up test, e.g. [23], which do not take the biological colonization into account.

In recent papers we have reported that lichens colonize and weather chrysotile-asbestos bearing rocks [24,25] and that their metabolites modify the surface activity of the fibres, suggesting a role for lichens in environmental-friendly decontamination procedures [26,27]. The present study first evaluates how the biogeophysical and biogeochemical action of lichens affects the structural and chemical preservation of asbestos–cement corrugated-sheets, which ultimately modulates the environmental health hazard [17]. The extent of fibre loss from the substrate, both in presence and absence of lichen cover, is evaluated by innovatively coupling pull up tests and image analysis. Chemical composition of both chrysotile and crocidolite asbestos, colonized or not by lichens, is examined. Minerals at the interface between lichens and asbestos–cement are analyzed in order to infer mechanisms and compounds potentially driving lichen weathering processes. Weathering and covering roles of lichens are finally discussed in order to evaluate the environmental-safety significance of their occurrence on asbestos–cement.

2. Materials and methods

2.1. Sampling design

Lichen colonization was examined on the corrugated asbestos–cement roofs of industrial sheds in the mining area of Balangero (Torino, Italy), the largest asbestos mine of western Europe, abandoned from the beginning of the 1990s. Asbestos–cement was produced by Eternit (at Casale Monferrato, Alessandria, Italy), just using chrysotile fibres [$Mg_3Si_2O_5(OH)_4$, white serpentine asbestos] which were produced in the mine, and therefore were largely used for the roofs of all the buildings of this industrial area (i.e. milling and sacking plants, asbestos-sack deposits, garages, houses of miners, offices). The material also contains crocidolite fibres [$Na_2(Fe^{2+},Mg)_3Fe_2{}^{3+}[Si_8O_{22}](OH)_2$, blue amphibole asbestos], originating from Southern Africa, which were often mixed with chrysotile in order to increase the technical properties.

Six relevés of the lichen cover were carried out on the roofs of the garages, which go back to the 1960s. These were built with Eternit corrugated slabs which show a height of the waves of 60 mm and a length of 20 cm. Sampling area for each relevé was 100 cm^2 (5 cm × 20 cm, width × length) and was oriented perpendicularly to the axis of the waves: it included one complete wave (one ridge and one vale).

Lichens were identified following Clauzade and Roux [28], Purvis et al. [29], and Wirth [30]. Nomenclature of taxa follows Nimis and Martellos [31]. Dominant cyanobacterial species were identified following Anagnostidis and Komárek [32].

2.2. Image analysis of lichen covering and fibre loss from asbestos–cement

In the pull up test, used to evaluate the disaggregation of asbestos cement, an adhesive is applied on the substrate and the snatched material undergoes a gravimetric analysis [23], without discriminating the contribution of fibres, with respect to other lithic components and/or biological colonizators, as lichens.

In this paper, the extent of lichen covering and its effects on the loss of fibres from asbestos–cement was evaluated according to an original protocol which innovatively couples the pull up test and image analysis of linear structures by WinRHIZO™ (2004). Automated counting systems using image analysis were previously developed in order to avoid high bias and variability in measurements of airborne fibres due to human analysts [33–35]. Although the counting system was able to give reasonable agreement with human counters for certain types of samples, several problems depending on the complexity of fibre shapes (e.g. bundles, agglomerates, split fibres) and on the poor contrast between fibres and background, did a significant fraction of the fibres misidentified as multiple fibres or not detected at all [36]. Differing from previous image analysis systems, which counted the fibres, WinRHIZO™ software measures the lengths of the linear structures, selected on the basis of diameter classes, thus including fibre bundles showing a diameter around or higher than 20 μm and excluding the non-fibrous component. Even if fibre bundles exceeding the diameter of 5 μm do not correspond to the fibre fraction which is retained to be directly inhalable [37], their total length appears as an estimate of the released fibre fraction which more directly affects the environmental safety.

2.2.1. Protocol

After performing a photographic strip (digital 3.34 megapixel camera Nikon Coolpix 995), a polyester adhesive tape (3M 396, 3M®, Italy), 5.1 cm wide, 0.1 mm thick, 20 cm long, was applied on each sampling area. The adhesive tape was then pulled up and applied on a polyester film (Ink Jet Films, Folex Imaging®).

The photographic strips were composed using Adobe® Photoshop 5.5 and were then loaded in WinRHIZO™ (Régent Instrument, version 2004a). Through image analysis of the photographic documentation, the colonized and uncolonized areas were measured. According to Christensen [21], lichen colonization was considered as percentage cover for each species.

Also the adhesive fragments were processed through an image analysis using WinRHIZO™. The images of the polyester strips were acquired using a scanner EPSON Expression 1680. An evaluation of the total length of the fibre bundles, which were snatched from the roof (cm^{-1}: centimetric length of fibre bundles released from cm^2 of substrate), was performed by selectively measuring the linear structures, adopting a 1:3 ratio as width/length limit. In this way, the software selected and measured the length of fibre bundles showing a diameter between ca. 20 and 500 μm; contributes by non-fibrous grains were mostly discarded from the computation (Fig. 1). Average percent contributions to the calculated lengths of different diameter classes of fibre bundles (up to 100, 101–200, 201–300, 301–400, 401–500 μm) were also calculated. Linear structures showing a diameter higher than 500 μm mainly consisted of heterogeneous aggregates and were excluded from the computation.

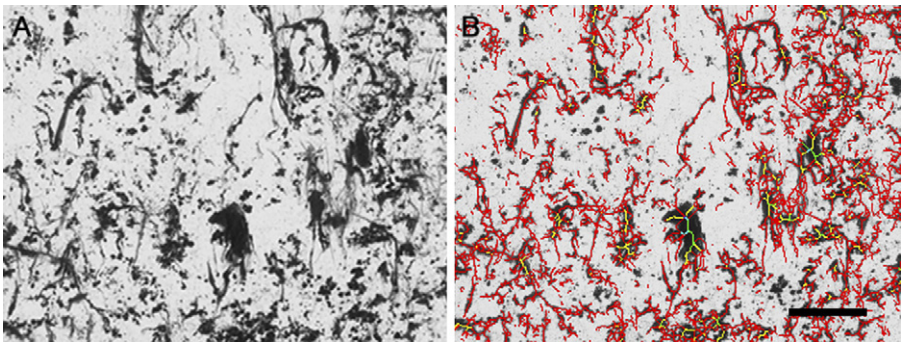

Fig. 1. Scanned image (A) and image analysis (B) of a polyester adhesive tape applied to an asbestos–cement corrugate and then pulled up. Red lines mark linear structures having a diameter lower than 500 μm, while differently colored lines mark linear structures having a higher diameter. In order to mostly exclude granular, non-fibrous particles and aggregates from the measure of the snatched fibres, only linear structures having 1:3 as width/length ratio and a diameter lower than 500 μm were considered in the performed elaboration. Bar = 5 mm.

By overlapping the photographic strips and adhesive analysis, the evaluation of the fibre bundles was related to colonized and uncolonized areas; the effects of the different identified species were thereafter evaluated. The results were statistically analyzed by *t*-test and Tukey's tests (ANOVA; $P < 0.05$ considered significant), using Systat 10.2 (Systat Software, Inc., 2002).

2.3. Analysis of lichen weathering

The penetration of lichen hyphae and their biogeochemical action were evaluated according to Favero-Longo et al. [26,38], focusing on the two most significant taxa checked in the relevés, mostly occurring on the ridges of the sheets: *Candelariella* ssp. and *Acarospora cervina*.

The examination of the lichen penetration was performed under reflected light with the polarizing microscope as well as with scanning electron microscopy (SEM). Lichen chemical effects on the fibre composition were examined by coupling SEM with Energy Dispersion Spectroscopy (SEM-EDS), while mineral phases at the lichen–substratum interface were also investigated by X-Ray Powder Diffraction (XRPD).

2.3.1. Light microscopy

The observations under reflected light microscopy (Leitz mod. Orthoplan) were carried out on 6 polished cross-sections (20 mm × 20 mm × 5 mm) for each species, obtained after including samples in polyester resin. These sections were stained using the Periodic Acid Schiff's (PAS) method [39] in order to highlight the hyphal penetration component (HPC, *sensu* Favero-Longo et al. [38]).

2.3.2. SEM-EDS

SEM observations and chemical analyses of chrysotile and crocidolite fibres were performed on carbon-coated thin cross-sections, using a Stereoscan S360 Cambridge electron microscope equipped with an Energy 200 Oxford Instruments EDS apparatus. The accelerating voltage was 15 kV and the counting time for EDS analyses was 50 s; pure oxides were used as standards. EDS analyses were acquired and processed using a Microanalysis Suite Issue 12, INCA, Suite, version 4.01. Analyses (as weight % of the oxides of the elemental constituents) were normalized under anhydrous conditions, thus allowing for the relative variations of the chemical constituents, which usually characterize the incongruent dissolution of asbestos minerals (details on the normalization procedure are given in the Supplementary Materials of Favero-Longo et al. [26]). The SiO_2, Al_2O_3, FeO_{tot}, MgO wt% contents and SiO_2:MgO ratios were compared in chrysotile fibres in contact with lichen hyphae (10 and 8 fibres were analyzed for *Candelariella* and *Acarospora*, respectively) and in chrysotile fibres exposed on the roof surface, in absence of lichen colonization (10 fibres as control). The SiO_2, Na_2O, FeO_{tot}, MgO wt% contents and SiO_2:FeO_{tot} ratios were compared in crocidolite fibres in contact with lichen hyphae (7 and 9 fibres were analyzed for *Candelariella* and *Acarospora*, respectively) and in crocidolite fibres exposed on the roof surface, in absence of lichen colonization (5 fibres as control). The results were statistically analyzed by Tukey's tests (ANOVA; $P < 0.05$ considered significant), using Systat 10.2 (Systat Software, Inc., 2002).

2.3.3. XRPD

Mineral phases at the lichen–substrate interface were recognized using X-ray powder diffractometry technique with copper anticathode X-ray tube (Siemens D5000, with θ–2θ geometry). The spectra obtained were identified by comparison with those contained in the J.C.P.O.D.S. (Joint Committee of Powder Diffraction Standard) archives. Analyses were performed on two colonized samples for each considered taxon and on two uncolonized samples as control.

3. Results and discussion

3.1. Image analysis of lichen covering

The results of the lichen-cover analysis are presented in Table 1; photographic strips of representative relevés are shown in Fig. 2A and C.

Lichens show an average cover over 25%, while the cyanobacterial cover [mainly *Gloeocapsa* cfr. *sanguinea* (Ag.) Kütz.] is down to 8%; no mosses occur. The crustose *Acarospora cervina,* which was already signaled on asbestos–cement by Martin et al. [20], shows the highest average cover (24.3%), mainly occurring on the ridge of the corrugates. *Candelariella aurella* and *C. vitellina*, very common on asbestos–cement [20,21], show the highest number of thalli, but their scattered morphology determines average cover values around 3%; they develop on both ridges and vales, though on vales they often grow on the cyanobacterial layer in agreement with Turian [40]. Other crustose species as *Caloplaca holocarpa* and *Lecanora dispersa*, characterized by an inconspicuous thallus, do not develop significant average covers (<1.0%). *Verrucaria nigrescens* and *Caloplaca teicholyta* sporadically occur. The presence of foliose species is limited to young thalli of the nitrophytic *Physcia dubia*.

Table 1
Analysis of the biological colonization on asbestos–cement corrugates in the Balangero mine (Torino, Italy)

Relevés[a]	GF[b]	1	2	3	4	5	6	Av.
Lichen cover (%)		59.1	22.0	24.4	22.8	23.4	20.2	**28.6**
Acarospora cervina (Ach.) Massal.	cc	46.1	21.8	22.3	15.2	23.4	17.0	**24.3**
Caloplaca holocarpa (Ach.) A. E. Wade	sc	+	+	–	+	+	+	**+**
Caloplaca teicholyta (Ach.) J. Steiner	cc	–	+	–	–	–	–	**+**
Candelariella aurella (Hoffm.) Zahlbr.	sc	13.0	+	+	7.6	–	1.5	**3.7**
Candelariella vitellina (Hoffm.) Müll. Arg.	sc	–	–	2.0	+	–	1.6	**0.6**
Lecanora dispersa (Pers.) Sommerf.	sc	–	+	–	–	–	–	**+**
Physcia dubia (Hoffm.) Lettau	f	–	+	–	–	–	–	**+**
Verrucaria nigrescens Pers.	cc	–	+	–	–	–	+	**+**
Cyanobacterial cover (%)		8.0	8.0	6.0	6.4	7.6	12.0	**8.0**

[a] Relevés 1–6: altitude 580 m a.s.l., area 100 cm^2, slope of the corrugates 10°, axis of the waves oriented from E to W. Data are expressed as specific % cover; +: presence of the species (cover <0.5%).
[b] GF: growth form (cc, crustose species with contiguous areolae; f, foliose species; sc, crustose species with scattered inconspicuous areolae or apothecia).

3.2. Image analysis of the fibre loss from asbestos–cement

The results of image analyses performed on the polyester adhesive snatched from lichen relevés are illustrated in Fig. 2B and D, where submillimetric and millimetric particles removed from the surface (red dots and lines) mostly correspond to bundles of fibres from few hundreds μm up to 0.3 cm long. Within the diameter class of fibre bundles between 20 and 500 μm, average contributions of diameter subclasses appear strongly constant, possibly reflecting the initial composition of the examined material. Lower diameter classes of fibre bundles, i.e. from 20 to 100 μm (30 ± 2%), from 101 to 200 μm (27 ± 1%) and from 201 to 300 μm (27 ± 1%), strongly overcome those of the other two subclasses, from 301 to 400 μm (10 ± 1%) and from 401 to 500 μm (10 ± 1%).

A comparison between the photographic strips and the polyester adhesive images shows that particle removal is lower where lichens occur, in particular *Acarospora*. Average data on particle removal from colonized and uncolonized areas are summarized

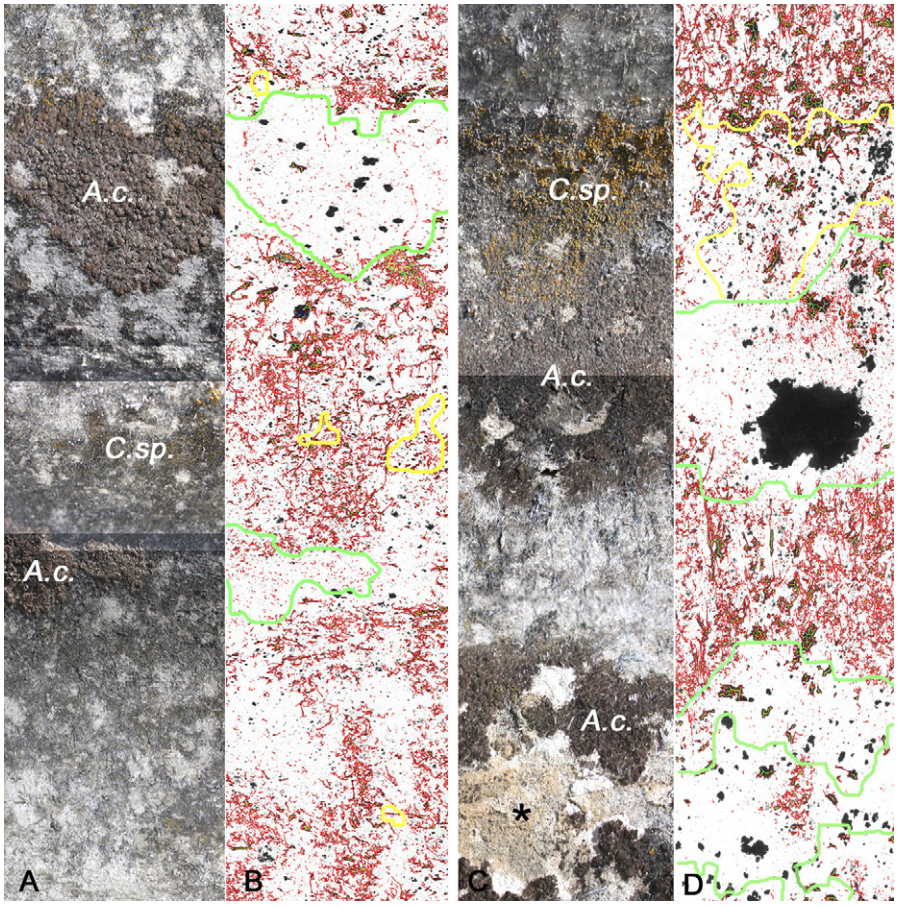

Fig. 2. Lichen relevés on asbestos–cement corrugates (A and C) and image analysis of the related adhesive tapes (B and D) used in pull up tests. Relevé area: 20 cm × 5 cm. Photographic strips of relevés 3 (A) and 1 (C), colonized by thalli of *Acarospora cervina* (*A.c.*), *Candelariella aurella* and *C. vitellina* (*C.* sp.). * indicates an area where the decay of the old central part of a thallus of *A. cervina* has determined a breach in its center, thus secondary exposing the substrate. Image analysis of adhesive tapes applied and pulled up from relevés 3 (B) and 1 (D). The bounds of the thalli of *Acarospora cervina* and *Candelariella* spp. are marked with green and yellow continuous lines, respectively. Red lines correspond to linear structures, mainly bundles of fibres, snatched from the substrate.

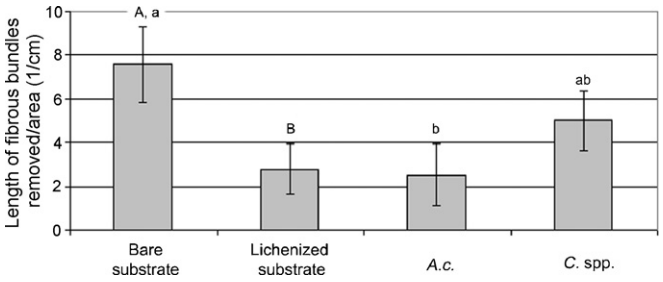

Fig. 3. Summary of the loss of fibre bundles from asbestos–cement consequent to pull up tests. Average values of fibres snatched (cm^{-1}) with the adhesive tapes from colonized and uncolonized areas are reported (first and second columns on the left). Fibre loss from colonized areas is further discriminated on the basis of the covering lichen species: *Acarospora cervina* (A.c.), *Candelariella aurella* and *C. vitellina* (*C.* spp.). (third and fourth columns on the right). The columns which do not share at least one letter are statistically different (capital letters: difference between bare substrate and lichen-colonized substrate according to *t*-Student test; lower-case letters: differences between bare substrate and areas colonized by *A. cervina* and *Candelariella* spp. according to Tukey's test). Significant differences: bare substrate versus lichen-colonized substrate: $P < 0.000$; bare substrate versus *A. cervina*-colonized substrate: $P < 0.000$.

in Fig. 3: lichen cover determines a significant reduction in fibre removal of about 70%, from 7.5 to 2.8 cm^{-1} (centimetric length of fibre bundles released from cm^2 of substrate). Uncolonized areas are indeed completely exposed to the solubilization of the carbonatic component, progressively exposing the less soluble fibre component to the surface, which is directly contacted by the adhesive and snatched from the surface. On the contrary, lichen thalli afford a physical barrier to the adhesive, which simulates the external physical agents, and fibres cannot be snatched. A protective role against external weathering agents was already reported for lichens on Cultural Heritage stones [41]. Experimental data showed that lichen thalli of *Aspicilia calcarea* (L.) Mudd significantly reduce the solvent effect of rain falling on gypsum, limiting the loss of lithic material due to the surface runoff [42].

Fibre loss is also reduced where the old, central parts of the thalli begin to decay, showing a central breach where the substrate is exposed: the previous occurrence of the thallus could have acted as a protective umbrella which largely limited the solubilization of the carbonate matrix, thus preserving its cohesive action [42]. However, a lithic loss contemporary to the thallus center decay could not be completely ruled out.

Different morphology of lichen species also affects the protective action of the lichen cover. Observations on polished cross sections show that a strong cohesion between the contiguous areolae characterizes the centimetric thalli of the nitrophilous *Acarospora* (Fig. 4A), which determine a decrease of the fibre loss up to 70%. By contrast, thalli of the nitrophilous and xerophytic *Candelariella* spp., which mainly consist of scattered areolae and apothecia (Fig. 4B), do not offer a significant protective shield to the adhesive snatch.

3.3. Light microscopy observation of lichen penetration into asbestos–cement

Lichen penetration into asbestos–cement is here firstly analyzed. Observations on the polished cross sections after staining

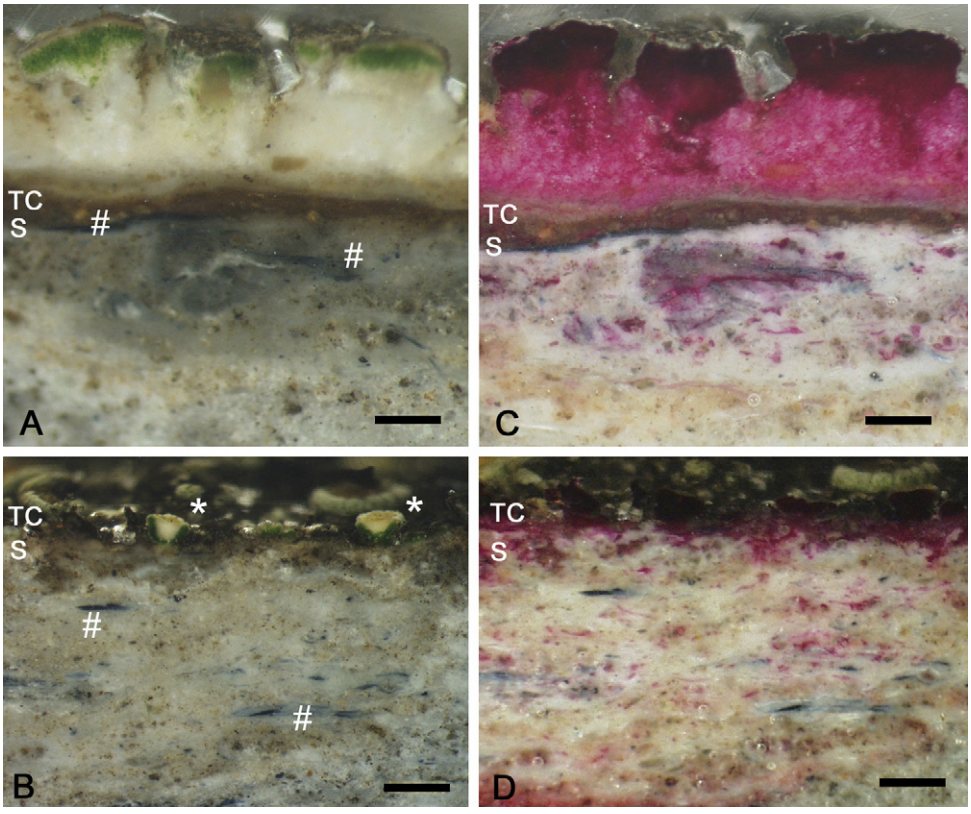

Fig. 4. Lichen colonization of asbestos–cement corrugates. Polished cross sections before (A and B) and after (B and C) staining with PAS. (A) The well developed thalline component (TC) of *Acarospora cervina* shows a strong cohesion between the contiguous areolae, completely covering the asbestos–cement substrate (S). Bar = 0.15 mm. (B) Scattered areolae and apothecia (*) of *Candelariella vitellina* only partially cover the asbestos–cement substrate (S). Crocidolite bundles are well recognizable because of their blue color (#), while white chrysotile does not stand out against the calcareous matrix. Bar = 1.0 mm. (C) The hyphal penetration component (HPC) of *A. cervina* poorly develops in the substrate, only colonizing the early hundreds of μm beneath the surface. Bar = 1.0 mm. (D) A scattered, but well developed hyphal penetration component deeply penetrates beneath the thallus of *C. vitellina*. Bar = 1.0 mm.

with PAS show that, beyond the thalline component (TC, *sensu* Favero-Longo et al. [38]), a well developed hyphal penetration component (HPC, *sensu* Favero-Longo et al. [38]) also characterizes the different lichen species. The HPC of *Acarospora cervina* slightly penetrates down to few hundreds of μm (Fig. 4C). Beneath the TC of *Candelariella* spp., hyphae are detected down to 2–3 mm, showing a scattered distribution which depends on the high, but discontinuous porosity of the examined material (Fig. 4D). This significant difference is consistent with observation of different ability of lichen species in penetrating lithic substrates (e.g. limestone [43], serpentinite [38]). Values up to 6 mm depth of the HPC of *Candelariella* spp. are consistent with those of other species into limestone and sandstone (penetration in limestone up to 16 mm [44]); values of few hundreds of micrometers, observed for *Acarospora*, are consistent with what was observed for several species on limestone (penetration up to 0.3 mm [43]) and silicate rocks (e.g. penetration in gabbros up to 0.5 mm [45]). Lichen effects on the asbestos–cement preservation thus spread beneath the surface and also involve the minerals of the deeper layers.

3.4. SEM-EDS analysis of fibre weathering by lichens

SEM observations on thin section show that chrysotile and crocidolite fibres are locally enveloped by lichen hyphae both at the level of HPC and within the medulla (Fig. 5). EDS analyses highlight that fibres which are contacted by lichen hyphae differ in their chemical composition with respect to those of the uncolonized areas.

Chrysotile fibres (Fig. 6A) show a significant depletion of MgO, its content decreasing from 45 wt% down to 33 and 27% below *Candelariella* and *Acarospora* respectively, and a significant relative increase of SiO_2 from 48 wt% up to 54 and 56%. FeO_{tot} content significantly increases in the fibres contacted by *Acarospora* (11 wt%) with respect to those below *Candelariella* (4%) and the uncolonized control (2%). MgO depletion of chrysotile in lichen-colonized asbestos–cement is consistent to what we observed for chrysotile in lichen-colonized asbestos-rich rocks [24]. An incongruent dissolution process due to a selective extraction of magnesium was previously reported as the prominent feature of chrysotile leaching during incubation in acidic and chelating solutions of lichen metabolites, as oxalic acid, eventually transforming fibres into amorphous silica debris when used in high concentrations [27,46]. Our analyses account for a similar leaching process in asbestos–cement driven by lichen metabolites, whose occurrence at the lichen–substrate interface is supported by XRPD results showing biomineralization of oxalates (see Section 3.5). In order to account for the apparent enrichment in the SiO_2 content, it should be noted that when comparing the concentrations of element oxides in weathered mineral samples and controls, changes in one constituent cause apparent changes in all other constituents. For example, if a mineral loses a significant amount of a major element as a result of chemical weathering, the percentage concentrations of all other elements increase accordingly, thereby erroneously suggesting additions of these elements to the mineral [26,47].

Crocidolite fibres, well known for their higher biopersistence in human lungs with respect to chrysotile [48], also exhibit relevant chemical modifications, *Acarospora* being more effective in weathering than *Candelariella*. Particularly, fibres contacted by *Acarospora* hyphae are significantly depleted in the Na_2O content, also exhibiting a significant relative increase in SiO_2, from 54 to 61 wt%. Since only some fibres show a decrease in their FeO_{tot} content, whereas others do not exhibit any modification, iron variation is not significant, but a general trend towards an increase in the SiO_2:FeO_{tot} ratio can be argued (Fig. 6B). Crocidolite fibres contacted by *Candelariella* hyphae also show a similar trend, but lower general variations with respect to fibres contacted by *A. cervina* make this insignificant on the basis of Tukey's test. If only data about the uncolonized fibres and those contacted by *C. vitellina* are analyzed with *t*-test, significant depletion of MgO ($P < 0.002$) and relative increase of SiO_2 ($P < 0.02$) are detected. The variation in the SiO_2 content suggests that also the amphibole encounters an incongruent dissolution process, possibly driven by the leaching action of lichen metabolites. Chemical modifications in crocidolite were not previously detected in crocidolite fibres inhaled by rats after 365 days [49]. By contrast, a significant release of Fe in culturing solutions was detected when fibres were incubated with soil fungi in laboratory experiments, thus suggesting an iron extraction due to siderophore-like molecules or acidic metabolites [50–52]. The depletion of Na, which occupies exposed sites along the 110 mineral cleavage plain [53], and the trend towards a decrease in the FeO_{tot} content which are determined by *Acarospora* may follow a similar mechanism driven by lichen metabolites.

3.5. XRPD analysis of the mineral composition at the lichen–substrate interface

XRPD analyses allow to recognize the mineral phases occurring in the bare and colonized tiles (Fig. 7). In addition to the dominant calcite, chrysotile is always detected, while crocidolite sporadically occurs, due to the different amounts of white and blue fibres which were used in the production of asbestos–cement. Ubiquitous quartz, most likely due to airborne transport [38], also occurs. On the uncolonized tiles, crystallized magnesium-chloride derivatives were detected, which are commonly found as reaction products of strength development processes in magnesium oxychloride cements [54,55].

Calcium oxalate monohydrate was detected at the *Acarospora*-substrate interface, accounting for a biomineralization process related to the lichen weathering action (see Section 3.4) [44,56]. Oxalate occurrence, in particular, suggests that the mycobiont of this species abundantly secretes the well known chelant oxalic acid, which may be the main responsible of the depletion of MgO from chrysotile and that of Na_2O and FeO_{tot} from crocidolite, according to Favero-Longo et al. [24]. Higher solubility of magnesium, iron and disodic oxalates with respect to calcium oxalates accounts for their absence at the lichen–rock interface [24,27,38,57]. On the contrary, no oxalates, but minor iron oxy-hydroxides, often reported at the lichen–substrate interface [24,44,56], occur below *Cande-*

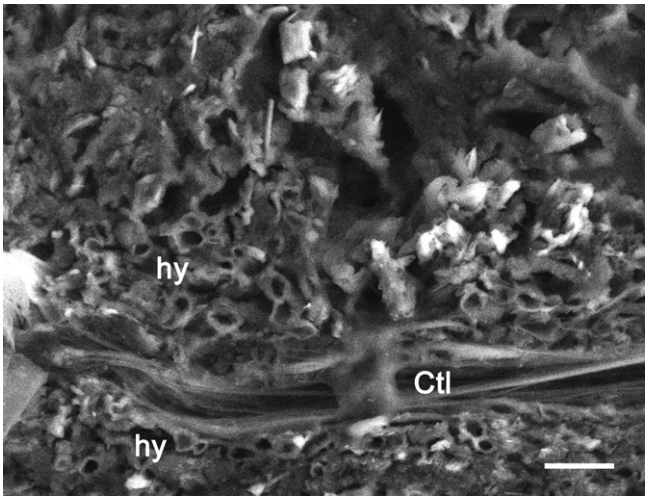

Fig. 5. Hyphae-fibres interaction at lichen-substrate interface. A bundle of chrysotile fibres (Ctl) is enveloped by hyphae (hy, in cross section) at the base of the medulla of *Candelariella vitellina*. Bar = 10 μm.

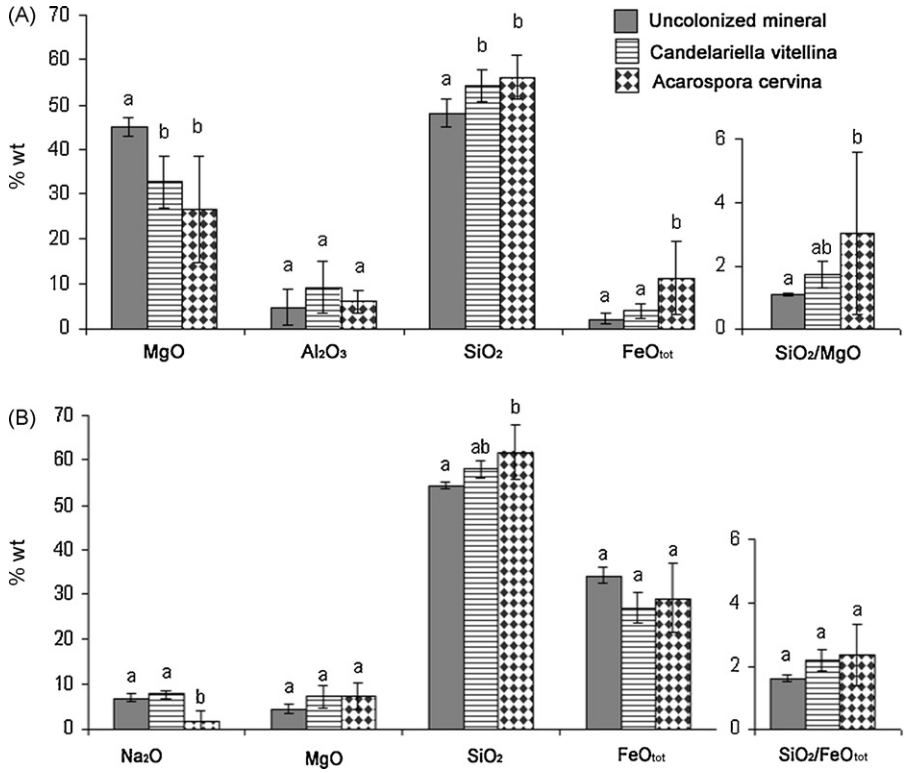

Fig. 6. Chemical composition of (A) chrysotile [$Mg_3Si_2O_5(OH)_4$] and (B) crocidolite [$Na_2(Fe^{2+},Mg)_3Fe_2^{3+}[Si_8O_{22}](OH)_2$] fibres uncolonized (control) and contacted by lichen hyphae of *Candelariella vitellina* and *Acarospora cervina* on asbestos–cement corrugates. Data are presented as means ± standard deviations of independent EDS analyses. With regard to each analyzed oxide, columns marked with different letters differ according to Tukey's test. (A) Chrysotile—significant differences: control versus *C. vitellina*: MgO $P < 0.009$, SiO_2 $P < 0.009$; control versus *A. cervina*: MgO $P < 0.000$, SiO_2 $P < 0.000$, FeO_{tot} $P < 0.001$, SiO_2/MgO $P < 0.025$; *A. cervina* versus *C. vitellina*: FeO_{tot} $P < 0.013$. (B) Crocidolite—significant differences: control versus *A. cervina*: Na_2O $P < 0.000$, SiO_2 $P < 0.015$; *A. cervina* versus *C. vitellina*: Na_2O $P < 0.000$.

lariella thalli, confirming that other chemicals should drive the leaching action of this species [26]. Secondary metabolites of *Candelariella* (pulvinic acid derivatives) recently turned out to weakly modify the surface chemistry of chrysotile after an incubation of five weeks, producing a trend towards the typical incongruent dissolution of chrysotile and determining the precipitation of iron oxy-hydroxides [27]. The low biogeochemical depletion in FeO_{tot} of crocidolite driven by *Candelariella* could also be explained by a similar mechanism, just considering the decades-long persistence of lichens colonization. The absence of variation in the Na_2O content, however, suggests that leaching mechanisms determined by the *Candelariella* metabolites are different with respect to those due to the chelating action of oxalic acid.

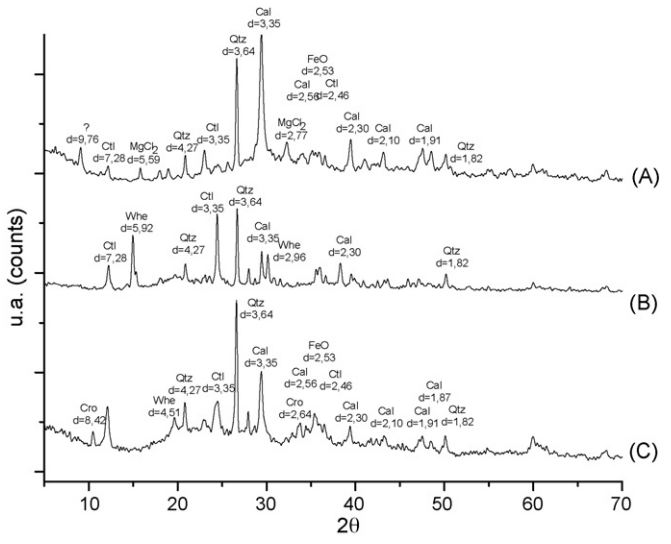

Fig. 7. X-ray powder diffraction patterns of asbestos–cement: (A) uncolonized by lichens, (B) at *Acarospora cervina* interface, (C) at *Candelariella vitellina* interface. On pattern C, neoformation of calcium oxalate monohydrate (whewellite) is observed. Only the main peaks are marked and d_{hkl} reported. Cal, calcite; Ctl, chrysotile; Cro, crocidolite; FeO, iron oxy-hydroxide s.l.; MgCl, magnesium chloride derivatives; Qtz, quartz; Whe, whewellite.

4. Conclusions

Biocovering and bioweathering effects of lichen colonization coexist on asbestos–cement roofs. Differing from the public opinion, lichen cover exerts a positive action in limiting the detach of asbestos from the carbonatic matrix and its loss and consequent dispersion in the environment. Moreover, lichens, by physically contacting the fibres, chemically modify their composition. Laboratory experiments showed that modifications in the chemical composition of chrysotile and crocidolite, similar to those we detected below lichens on asbestos–cement, affect their surface chemistry, determining a decrease of their Fenton activity [24,27,51,52], i.e. one of the main factors currently related to asbestos toxicity [5,6,37]. Consequently, our findings suggest that the lichen colonization and weathering of asbestos–cement roofs could contribute to a partial inactivation of the fibres.

Lichen colonization, however, rarely affect the whole roof surface and cover values higher than 25% are sporadically reported in literature (70% in ref. [20]). Since lichen colonization is more developed on older substrata, higher cover values are associated with a higher aging of the uncolonized areas, which could probably show

higher deterioration. Only a complete evaluation of (i) the lichen cover extent, potentially adopting the image analysis growing technology, (ii) the specific composition of the lichen communities and (iii) the conservation of the uncolonized areas could be definitely accepted as a reliable approach in defining the priority principles and the management techniques for asbestos–cement decontamination.

Even if a lichen cover around 100% could completely limit the fibre release, unfortunately no effective techniques are currently available for increasing lichen colonization on rock-like substrates [58,59], preventing the possibility of a lichen bioremediation of asbestos–cement roofs. Since lichen cover acts as a partial, but effective spontaneous bioattenuation factor, removal or chemical killing of thalli [20] appear as counterproductive approaches in the management of asbestos–cement which cannot be completely substituted with other materials.

Acknowledgements

The authors wish to express their gratitude to the R.S.A. s.r.l. staff of the Balangero mine for the assistance during the field work and to Prof. Consolata Siniscalco (University of Torino) for WinRHIZO software. This research is supported by funds from Regione Piemonte “Direzione regionale 22: Tutela e Risanamento Ambientale, Programmazione, Gestione Rifiuti” and from CEBIOVEM (The Centre of Excellence for Plant and Microbial Biosensing) in the context of a multidisciplinary project “Asbestos hazard in Western Alps”.

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Glossary

Modified from Purvis et al. [29] and Hawksworth et al. [60].

Apothecium (pl. apothecia): a cup or saucer-like fruiting-body producing sexual reproductive structures (spores)
Areola (pl. areolae): an island portion of a crustose thallus separated from adjacent areolae (crazy-paving-like)
Crustose: crust-like thallus, stretching over and firmly fixed to the substratum
Cyanobacterium (pl. cyanobacteria): a phylum of bacteria that obtain their energy from photosynthesis, also known as blue-green algae.
Foliose: leaf-like thallus
Hypha (pl. hyphae): one of the fungal filaments forming the thallus
Lichen: a stable-self supporting association of a fungus (mycobiont) and an alga or cyanobacterium (photobiont)
Lichen-cover: covering of a surface due to lichen thalli
Medulla: the loose layer of hyphae forming the lower part of a thallus
Mycobiont: see Lichen
Nitrophytic: having a preference for habitat rich in nitrogen
Propagule: asexual and sexual reproductive structure
ssp.: all the occurring species included in the genus
Thallus: the vegetative body
Xerophytic: having a preference for dry habitats