Title

Siderophore-mediated iron removal from chrysotile: Implications for asbestos toxicity reduction and bioremediation

Authors

Mohanty, Sanjay K
Gonneau, Cedric
Salamatipour, Ashkan
et al.

Publication Date

2018

DOI

10.1016/j.jhazmat.2017.07.033

Peer reviewed

HHS Public Access

Author manuscript

J Hazard Mater. Author manuscript; available in PMC 2019 January 05.

Published in final edited form as:

J Hazard Mater. 2018 January 05; 341: 290–296. doi:10.1016/j.jhazmat.2017.07.033.

Siderophore-mediated iron removal from chrysotile: Implications for asbestos toxicity reduction and bioremediation

Sanjay K. Mohanty[†,1], **Cedric Gonneau**[‡], **Ashkan Salamatipour**[§], **Ralph A. Pietrofesa**[§], **Brenda Casper**[‡], **Melpo Christofidou-Solomidou**[§], and **Jane W. Willenbring**[†,2]

[†]Department of Earth and Environmental Science, University of Pennsylvania, Philadelphia, Pennsylvania, 19104

[‡]Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania, 19104

[§]Division of Pulmonary, Allergy, and Critical Care Medicine and the Department of Medicine, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, 19104

Abstract

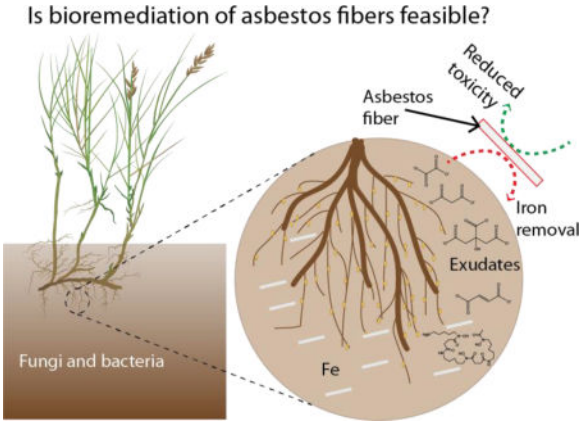
Asbestos fibers are highly toxic (Group 1 carcinogen) due to their high aspect ratio, durability, and the presence of iron. In nature, plants, fungi, and microorganisms release exudates, which can alter the physical and chemical properties of soil minerals including asbestos minerals. We examined whether exudates from bacteria and fungi at environmentally relevant concentrations can alter chrysotile, the most widely used asbestos mineral, and lower its toxicity. We monitored the release of iron from chrysotile in the presence of organic acid ligands and iron-specific siderophores derived from bacteria and fungi and measured any change in fiber toxicity toward peritoneal macrophages harvested from mice. Both fungal and bacterial siderophores increased the removal of iron from asbestos fibers. In contrast, organic acid ligands at environmentally relevant concentrations neither released iron from fibers nor helped in siderophore-mediated iron removal. Removal of plant-available or exchangeable iron did not diminish iron dissolution by both types of siderophores, which indicates that siderophores can effectively remove structural iron from chrysotile fibers. Removal of iron by siderophore lowered the fiber toxicity; fungal siderophore appears to be more effective than bacterial siderophore in lowering the toxicity. These results indicate that prolonged exposure to siderophores, not organic acids, in the soil environment decreases asbestos fiber toxicity and possibly lowers the health risks. Thus, bioremediation should be explored as a viable strategy to manage asbestos-contaminated sites such as Brownfield sites, which are currently left untreated despite dangers to surrounding communities.

Graphical abstract

Correspondence to: Jane W. Willenbring.

[1]Present and permanent address: Department of Civil and Environmental Engineering, University of California, Los Angeles, 90095

[2]Present and permanent address: Scripps Institution of Oceanography, University of California, San Diego, 92093



Keywords

Chrysotile; bioremediation; Brownfield; asbestos toxicity; iron removal

1. Introduction

Asbestos is a group of six naturally occurring fibrous minerals that belong to serpentine and amphibole mineral families. Among them, chrysotile, a serpentine mineral, has been mined in many places around the world and was most commonly used in many commercial products over the last century owing to its unique properties such as resistance to abrasion and fire [1]. However, exposure to asbestos fibers can cause serious health conditions such as asbestosis, and stomach and lung cancers [2–5]. Thus, asbestos use is banned in many developed countries, although many developing countries including China and India continue large-scale asbestos use [6]. In the U.S., nearly a thousand sites are either contaminated with asbestos-containing materials or naturally occurring asbestos minerals; many are Brownfield sites that are typically left untreated [5], despite imminent health risks to surrounding communities. As the recommended remediation method—soil capping—is cost prohibitive, the potential for alternative cost-effective remediation strategies, including bioremediation, has been explored [7–13]. However, the bioremediation mechanism and its feasibility in environmental relevant condition is lacking.

The primary goal of any remediation strategy is to minimize exposure by either removal or containment of the contaminants and/or their transformation to non-toxic byproducts. Active removal of fibers or reduction of their toxicity in soil becomes necessary because of their potential to contaminate nearby stream [14] and increase fiber exposure to community via irrigation of contaminated water [15]. The toxicity of asbestos fibers is typically attributed to its long aspect ratio [16], which makes it harder for macrophages to remove them from the lung [17]. As a defense mechanism, macrophages or other immune cells in lungs destroy or remove foreign materials, such as bacteria, particles or asbestos, by two mechanisms: phagocytosing or engulfing particles and production of reactive oxygen species (ROS). As asbestos fibers are often too large to be engulfed by macrophages and have high resistance to chemical attack, macrophages produce excessive ROS and cause inflammation and DNA damage [18–20]—a precursor for tumor development [21]. More recently, fiber surface

properties, such as iron content, are attributed to asbestos toxicity [22, 23]. Iron can be present as part of the mineral stoichiometry in amphibole or as an impurity in chrysotile [24]. In chrysotile, magnesium in the outer layer can be replaced by both ferrous and ferric iron whereas silicon in the inner silica layer can be replaced by ferric iron, although the greatest fraction of iron is found to be present in the outer layer [25]. Pascolo, et al. [22] showed that the presence of iron can increase oxidative stress in macrophages and increase the production of ROS. Using Fe-doped synthetic chrysotile fibers, Gazzano, et al. [26] showed that iron ions at specific sites in chrysotile can catalyze generation of ROS. Thus, removal of iron is considered a first-order mechanism to reduce the fiber toxicity [27, 28].

Iron is also an essential nutrient for all living organisms including plants, fungi, and bacteria. Because of its low solubility, iron is often limiting in the soil-water environment [29]. To solubilize iron from soil minerals and make it bioavailable, plant, fungi, and bacteria release exudates such as siderophores, which are high-affinity iron-chelating compounds or iron-specific ligands, and organic acids, which are non-specific ligands [13, 30–33]. The same processes could also release iron from asbestos and potentially lower its toxicity. Numerous studies demonstrated that bacteria, lichen, and fungi can degrade asbestos [7–9, 11–13, 32, 34, 35] and attributed the degradation to exudates such as organic acids and siderophores. These organic exudates and siderophores can bind ferric and ferrous iron although the exudates have a stronger affinity to ferric iron than ferrous iron [36]. Although numerous studies have examined the mechanism of iron removal from iron oxides by organic acids and siderophores [36–39], only one study [40] has examined iron dissolution from asbestos minerals, and it used exudates at unrealistically high concentrations, orders of magnitude higher than that expected in the soil environment [29]. The iron removal efficiency of exudates at environmentally relevant levels is unknown. More importantly, it is unclear if exposure to exudates can ultimately lower asbestos toxicity, as the toxicity of exudate-treated asbestos fibers was rarely measured [40]. Thus, the feasibility of bioremediation of asbestos-contaminated sites using siderophores remains poorly understood.

We aimed to examine the removal of iron by exudates at environmentally relevant concentrations. To accomplish this, we exposed ground chrysotile fibers to three organic acid exudates, a fungal siderophore, and a bacterial siderophore. The treated and untreated fibers were exposed to peritoneal macrophages, the immune cells that play a critical role in asbestos-inflammation and ultimate tumor development, and the reactive oxygen species concentrations generated by the exposed macrophages compared. Our findings provide a better understanding of the mechanism by which iron is removed by exudates at environmentally relevant concentrations and help address the pertinent question: Is bioremediation of asbestos fiber feasible using a siderophore approach?

2. Experimental

2.1. Asbestos suspension preparation

Chrysotile ore (Glove, Arizona) was hammered to separate fibrous bundles from other rock impurities. The handpicked fiber bundles were ground in dry condition for 15 minutes in a high-energy vibratory ball mill (Model 8000, SPEX Industries, Inc.). The fiber was ground in dry conditions because we previously showed that wet-grinding (in water) can remove a

fraction of iron from chrysotile and reduce the fiber toxicity [41]. Using energy dispersive x-ray spectroscopy (EDS) in conjunction with scanning electron microscopy [41], we measured the iron content of the chrysotile to be 2.3% by weight. This method measured the concentration of iron on fiber surface, which is most susceptible for dissolution via exudates. Chrysotile fibers typically contain brucite as an impurity, which increases the pH of the fiber suspension due to dissolution of OH^- along with Mg^{2+}. Although pure brucite and the brucite-like outer layer of chrysotile or other phyllosillicates have an identical chemical formula, that is $Mg(OH)_2$, the magnesium atom in chrysotile shares an oxygen atom with silicon, which significantly decreases Mg^{2+} dissolution from chrysotile compared to that from brucite [42]. To examine the dissolution of chrysotile, we first removed brucite impurities from chrysotile mixture following the hydrothermal method outlined elsewhere [43]. Briefly, 4 g of dry fibers was mixed with 400 mL of concentrated NH_4Cl (393 g/L) at 110°C until it formed a slurry. The slurry was then washed with deionized (DI) water and centrifuged at least five times to separate purified fibers. Magnetite impurities were removed by using a magnetic stirrer and discarding solid residues that settled within 5 min in the suspension. The purified fibers were resuspended in 1 mM MOPS buffer and 10 mM NaCl to achieve an initial concentration between 250–300 mg/L. If needed, the pH was adjusted to the desired value by adding a small volume of concentrated (10 N) HCl or NaOH. Unless otherwise mentioned, the pH of fiber suspension was adjusted to 7.

2.2. Dissolution of iron from chrysotile fibers

A bacterial siderophore (Desferrioxamine B; DFO; Sigma-Aldrich) was spiked into 200 mL of fiber suspension (250–300 mg/L) in plastic amber bottles to achieve a final siderophore concentration of 1 or 10 μM. The suspension was mixed on a roller at 60 rpm for a maximum of 16 days, and 7 mL of suspension was extracted at different time intervals: 2h, 4h, 8h, 1d, 2d, 4d, 8d, 12 d and 16 d. The suspension was filtered using 0.2 μm syringe filters (Nylon) and the solute was analyzed for iron concentration using inductively coupled plasma optical emission spectrometry (ICP-OES). This method measures total dissolved iron without distinguishing between ferrous and ferric iron.

To examine whether exposure to oxalic acid ligand—a common organic acid exudate—would change siderophore-mediated iron dissolution, the experiment was repeated using 10 μM DFO with or without oxalic acid (100 μM). Organic acid ligands could help dissolve magnesium and other coatings or impurities from asbestos fibers, thereby exposing more iron to be removed by siderophores [44]. To examine the effect of pre-exposure to organic acid ligands, fiber suspension was exposed to 100 μM of oxalic, malonic, or citric acid for 48 h before exposure to 1 μM of DFO for the next 48 h. The results were compared with the suspension exposed to 1 μM of DFO and 100 μM of each organic acid for 96 h.

Because adsorption of siderophores on iron-containing minerals is a prerequisite for dissolution of iron [39], siderophores are more likely to remove irons from fiber surface, most of which are exchangeable or readily available for plant or microbial acquisition. Can siderophores access and dissolve structural iron after the removal of exchangeable iron? To examine whether removal of plant-available, exchangeable iron diminishes the ability of the siderophore to remove iron, fibers were first pretreated with diethylene triamine penta-acetic

acid (DTPA) solution for 24 h. The DTPA solution was prepared by mixing 0.005M DTPA, 0.01M calcium chloride, and 0.1M triethanolamine and adjusting the pH to 7.3 with 12M HCl [45]. For control, fibers were treated with 0.01M calcium chloride, which represent fibers with plant-available iron. After 24 h of mixing at 60 rpm, the fiber suspension was centrifuged at 5000 rpm for 10 min to separate fibers from the supernatant. To remove residual DTPA solution, the fibers were washed in DI water and centrifuged to discard the supernatant; this step was repeated five times. The fibers with and without plant-available iron were exposed to 1 μM of DFO or a fungal siderophore (Iron-free Ferrichrome; FC; Sigma-Aldrich) for 14 d. 7 mL of suspension was pipetted out from each batch at different time intervals to analyze iron concentration in the water. The results were compared with those where plant available iron was not removed from fibers prior to their exposure to the siderophores. The dissolution experiment was conducted in duplicate batches.

2.3. Quantification of asbestos-induced ROS in elicited murine peritoneal macrophages

After siderophore treatment of fibers with and without plant available iron, the fibers were separated by centrifugation and stored in phosphate-buffered saline (PBS) solution for toxicity measurement. Fibers from duplicate batches per condition were mixed to create a composite sample. The fibers were isolated from suspension by centrifugation and washed in DI water before suspending in PBS solution to make the final fiber concentration 1 μg/mL. We measured the fiber toxicity based on asbestos-induced ROS generated by peritoneal macrophages (MF) [46]. The details of this method were described in our previous study [41]. Briefly, the macrophages from mice were harvested from the peritoneum following elicitation using thioglycollate broth and exposed to asbestos fibers. Plated MF cells were kept in PBS solution with or without the selected ground fibers at a concentration of 20 $\mu g/cm^2$, and the level of oxidative stress in the cells was estimated using a fluorogenic probe (CellROX® Green Reagent, Molecular Probes by Life Technologies, Eugene, Oregon, USA), which produces a green photostable fluorescent signal upon reaction with ROS. At 6 hours post-asbestos exposure, cells were stained with 5 μM CGR Reagent (and DAPI for fluorescent imaging) by adding the probe(s) to complete media and incubating at 37° C for 30 minutes. Cells were washed 3 times with PBS and the fluorescence intensity then measured using a SpectraMax® i3 Multi-Mode Microplate Detection Platform (Molecular Devices, Sunnyvale, CA, USA) using an excitation wavelength of 485 nm, with fluorescence emission detection at 520 nm. Data are presented as mean ± standard error of the mean. Fluorescence microscopy was also performed on stained cells and images were captured on an Eclipse TE2000-U microscope (Nikon, Japan) equipped with a digital camera (Retiga 2000R, QImaging, Surrey, BC, Canada) using 20× magnification.

2.4. Statistical analysis

Differences of ROS levels due to exposure to $CaCl_2$/DTPA and siderophore treatments were determined using a two-way ANOVA (R v3.3.3) followed by a Tukey post-hoc analysis. Asterisks shown in figures indicate significant differences between groups (* = $p<0.05$).

3. Results

3.1. Dissolution of iron from chrysotile by DFO

DFO released iron from fibers into the solution, and the iron concentration increased with an increase in the concentration of DFO from 1 μM to 10 μM (Figure 1). Upon exposure to 1 μM DFO, iron was released from fibers within 24 h, and any further exposure time did not change the concentration of iron in the solution, suggesting dissolution equilibrium was achieved within 24 h. However, when the concentration of DFO was increased to 10 μM, equilibrium was delayed to at least 120 h. The amount of iron released per gram of fiber per μM exposure to DFO did not proportionally increase when the concentration of DFO was increased 10 times.

In contrast to DFO, oxalic acid (100 μM) had no effect on iron release (Figure 1). The concentration of iron in the suspension with 100 μM of oxalic acid was below the analytical detection limit (1 μg/L). The amount of iron released by 10 μM DFO was similar to the amount of iron released by 10 μM DFO plus 100 μM of oxalic acid, indicating oxalic acid presence did not have any significant effect on the siderophore-mediated iron release.

3.2. Effect of pre-exposure to organic acid exudates on siderophore-mediated iron release

The presence of oxalic acid and malonic acid (100 μM) appears to have no effect on siderophore-mediated iron release from asbestos fibers (Figure 2). In contrast, citric acid increased the concentration of iron released with exposure to 1 μM DFO. Similarly, pre-exposure to organic acid exudates did not change the amount of iron released with 1 μM of DFO, suggesting the limiting factor for iron release from asbestos is the presence of siderophore, not organic acids.

3.3. Comparison of iron release by bacterial and fungal siderophore

The fungal and bacterial siderophores released similar amounts of iron from chrysotile fibers: 17±2 μM of Fe per g of fiber per μM of siderophore (Figure 3). Removal of plant-available iron did not diminish the ability of either siderophore to remove iron from the fibers. Without a siderophore, dissolved iron concentrations in the samples containing fibers with plant-available iron were below the detection limit, but iron concentrations in the samples with DTPA-treated fibers were above the detection limit, which indicates that the adsorbed DTPA could have contributed to a small amount of iron being released. Nevertheless, the concentration of iron released by either siderophore was well above (nearly 10 times) the concentration of iron in the control treatment for the DTPA-treated fibers. Therefore, the two siderophores (DFO and FC) were apparently equally effective in releasing iron from fibers despite pre-removal of plant-available iron from fibers.

3.4. Effect of treatments on the fiber-induced ROS generation

To assess the toxicity of fibers treated by DTPA and/or both types of siderophores, we determined levels of asbestos-induced ROS in elicited murine peritoneal macrophages at 6 hours after asbestos exposure. In control samples without a siderophore, the DTPA-treatment, which removes plant-available iron, lowered the generation of ROS significantly ($p<0.05$) (Figure 4). For the fibers with plant-available iron, treatment by either siderophore

reduced ROS generation compared to control. However, for the fibers without plant-available iron (DTPA-treated fibers), the fungal siderophore (FC), but not the bacterial siderophore (DFO), significantly lowered ROS generation compared to the control (Figure 4).

4. Discussion

4.1. Siderophores remove iron from asbestos fibers

Our studies confirmed that when used at environmentally relevant concentrations (1–10 μM), both the fungal (FC) and bacterial (DFO) siderophores successfully removed substantial amounts of iron from chrysotile. Increasing DFO concentrations did not increase the release iron proportionally, which indicates that the dissolution is limited by available iron. Chrysotile [$Mg_3Si_2O_5(OH)_4$] typically contains 1–6% iron, where iron isomorphously replaces magnesium or silicon in outer Mg-hydroxide octahedral or inner silica tetrahedral, respectively [47]. Thus, the efficiency of siderophore-mediated removal would depend on the amount of iron accessible to the siderophore, particularly in the outer layer. Studies examining siderophore-mediated iron removal from chrysotile are lacking, although many studies have examined the mechanism by which siderophores remove iron from other iron-containing minerals [38–40, 48–50]. Based on these studies, high affinity of siderophore for iron binding sites or adsorption of siderophore is a prerequisite for iron dissolution [39]. At low concentrations, most of those adsorbed on asbestos fibers could effectively remove iron from the surface. Increasing the siderophore concentration would increase surface coverage (if asbestos surface area is not limiting) and increase the iron concentration.

The fact that the time to reach iron dissolution equilibrium increased with the concentration of DFO may relate to how iron is bound within the chrysotile fiber. Iron present in the brucite-like outer layer could be readily released by DFO, whereas iron present in the silica tetrahedra could be slow to release. At low concentrations of DFO, most of the iron was released from surface sites. With increases in concentration of DFO, dissolution could have occurred from octahedral sites due to exhaustion of surface sites. Dissolution of iron by both types of siderophore even after the removal of plant-available iron (or exchangeable sites) confirmed this hypothesis. DTPA has been used to remove exchangeable ions from soil minerals. Treating chrysotile fibers with DTPA removed a small amount of iron (plant-available) from surface sites, but the removal of plant-available iron did not affect the ability of the siderophore to further remove iron from fibers. Iron released after DTPA treatment is attributed to dissolution of iron from the crystal structure of chrysotile. Overall, our results show that siderophore can access and remove iron from chrysotile's structure.

4.2. Organic acid exposure had no effect on siderophore-mediated iron release

The short-chain organic acids that plants and fungi typically release to acquire elements in a nutrient-depleted environment play a critical role in cycling of nutrients including iron. Our results show that oxalic, malonic, and citric acid at 100 μM concentrations did not remove iron from chrysotile, suggesting that these organic acids have a limited role in removing iron from asbestos in environment. This result is in contrast to the finding of previous studies that examined the dissolution of chrysotile in the presence of organic acids [44, 51]. The

discrepancy is likely attributable to a difference in concentration of oxalic acid and pH of the solution. Both of the previous studies [44, 51] used oxalic acid at concentrations three orders of magnitude higher than what was used in our study. Furthermore, the pH of the solution in their studies was lower than the neutral pH used in our study. Solubility of iron increases with a decrease in pH. Furthermore, at pH 1, which was used in the previous study [51], the structure of chrysotile is not stable and deteriorates quickly, thereby releasing iron or any other impurity [52]. The pH of asbestos serpentine soil is typically above pH 7 due to the dissolution of the brucite-like outer layer [53]. At high pH, the dissolution of elements from chrysotile fibers is expected to be low and slower in the presence of organic acids. Thus, the contribution of organic acid exudates toward iron removal could be limited in the natural environment, although organic acids at high concentration can be effective in breaking down and detoxifying chrysotile fibers [54].

Although organic acids have a limited role in directly dissolving iron from asbestos minerals, they can increase the dissolution of other elements including Mg or remove other impurities to make iron-containing sites more accessible for siderophore-mediated dissolution [44]. Our results showed that pre-exposure to the three organic acids did not remove any more iron by DFO than the iron released when both organic acid and DFO were spiked simultaneously. As chrysotile was purified before use in this experiment, any possible coating that may hinder siderophore-mediated iron dissolution was already removed. Thus, future studies should use chrysotile fibers isolated from the environmental matrix and examine the effect of organic acids on siderophore-mediated iron removal.

The particular nature of the organic-acid exudates appears to be important in determining their effect on iron acquisition by fungi or microbes. This statement is based on there being no difference in iron release when DFO was used with or without oxalic or malonic acid while citric acid appears to enhance the siderophore-mediated iron dissolution. Organic acids cannot compete with DFO to release iron because the stability constant of DFO is orders of magnitude ($>10^{20}$) higher than the stability constant of organic acids. However, organic acids can dissolve magnesium from the brucite-like outer layer [44], thereby exposing any iron present in the outer layer. Among the three organic acids used, citric acid has a higher stability constant for magnesium. It is possible that citric acid dissolves more magnesium from the outer octahedral layer, exposing more iron for siderophore-mediate dissolution.

4.3. Siderophore treatment can reduce the toxicity of asbestos fibers

Removal of iron from chrysotile fibers by siderophores lowered the amount of reactive oxygen species generated by macrophages during exposure to fibers. This result indicates that a reduction in iron content will decrease the likelihood of inflammation in lungs when asbestos exposure occurs. This result confirms the finding from previous studies that correlated a decrease in toxicity of chrysotile fibers with decrease in iron content [40]. It should be noted that toxicity of fibers is due both to their physical properties—aspect ratio, rigidity—and chemical properties—iron content. Thus, although siderophores can lower the toxicity of fibers, they may not eliminate the risk completely. Nevertheless, removal of iron

by siderophores can form the basis of low-cost bioremediation strategies that can lower the risk from chronic exposure to toxic fibers in contaminated sites.

5. Conclusions

We examined the removal of iron from chrysotile fibers in the presence of one fungal and one bacterial siderophore with or without three commonly found organic-acid exudates and measured the fiber toxicity toward peritoneal macrophages before and after the treatment. Our results show that bioremediation of asbestos fibers is feasible. More specifically, we conclude:

- At natural concentration levels, siderophore can remove iron from chrysotile fibers. The fungal and bacterial siderophores were equally effective.
- Removal of plant-available iron did not lower siderophore-mediated iron removal, which indicates that siderophore can also remove iron from crystal structure.
- Organic acid exudates at environmentally relevant concentration did not remove iron from chrysotile fibers.
- Reactive oxygen species generated by macrophages upon exposure to asbestos fibers decreased when fibers were pre-treated with a siderophore.
- The fungal siderophore was more effective than the bacterial siderophore in reducing reactive oxygen species and thus, the toxicity of chrysotile.

Acknowledgments

This work was funded in part by: NIH-R03 CA180548 and 1P42ES023720-01. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIEHS, NIH.

References

1. Schreier, H. Asbestos in the natural environment. Elsevier; Amsterdam; New York: 1989.
2. Manning CB, Vallyathan V, Mossman BT. Diseases caused by asbestos: mechanisms of injury and disease development. International immunopharmacology. 2002; 2:191–200. [PubMed: 11811924]
3. Nielsen LS, Baelum J, Rasmussen J, Dahl S, Olsen KE, Albin M, Hansen NC, Sherson D. Occupational Asbestos Exposure and Lung Cancer-A Systematic Review of the Literature. Archives of Environmental & Occupational Health. 2014; 69:191–206. [PubMed: 24410115]
4. Shukla A, Gulumian M, Hei TK, Kamp D, Rahman Q, Mossman BT. Multiple roles of oxidants in the pathogenesis of asbestos-induced diseases. Free radical biology & medicine. 2003; 34:1117–1129. [PubMed: 12706492]
5. Lee RJ, Strohmeier BR, Bunker KL, Van Orden DR. Naturally occurring asbestos—A recurring public policy challenge. J. Hazard. Mater. 2008; 153:1–21. [PubMed: 18180100]
6. Frank AL, Joshi TK. The Global Spread of Asbestos. Ann. Glob. Health. 2014; 80:257–262. [PubMed: 25459326]
7. Bhattacharya S, John P, Ledwani L. Bacterial Weathering of Asbestos. Silicon. 2015:1–13.
8. Favero-Longo SE, Turci F, Fubini B, Castelli D, Piervittori R. Lichen deterioration of asbestos and asbestiform minerals of serpentinite rocks in Western Alps. International Biodeterioration & Biodegradation. 2013; 84:342–350.

9. Daghino S, Martino E, Perotto S. Fungal weathering and implications in the solubilization of metals from soil and from asbestos fiber. Current research, technology and education topics in applied microbiology and microbial biotechnology. 2010; 1:329–338.
10. Favero-Longo SE, Castelli D, Fubini B, Piervittori R. Lichens on asbestos-cement roofs: Bioweathering and biocovering effects. J. Hazard. Mater. 2009; 162:1300–1308. [PubMed: 18692312]
11. Daghino S, Turci F, Tomatis M, Girlanda M, Fubini B, Perotto S. Weathering of chrysotile asbestos by the serpentine rock-inhabiting fungus Verticillium leptobactrum. Fems Microbiology Ecology. 2009; 69:132–141. [PubMed: 19453742]
12. Daghino S, Martino E, Vurro E, Tomatis M, Girlanda M, Fubini B, Perotto S. Bioweathering of chrysotile by fungi isolated in ophiolitic sites. Fems Microbiology Letters. 2008; 285:242–249. [PubMed: 18616596]
13. Daghino S, Turci F, Tomatis M, Favier A, Perotto S, Douki T, Fubini B. Soil Fungi Reduce the Iron Content and the DNA Damaging Effects of Asbestos Fibers. Environ. Sci. Technol. 2006; 40:5793–5798. [PubMed: 17007142]
14. Emmanouil K, Kalliopi A, Dimitrios K, Evangelos G. Asbestos pollution in an inactive mine: Determination of asbestos fibers in the deposit tailings and water. J. Hazard. Mater. 2009; 167:1080–1088. [PubMed: 19304382]
15. Turci F, Favero-Longo SE, Gazzano C, Tomatis M, Gentile-Garofalo L, Bergamini M. Assessment of asbestos exposure during a simulated agricultural activity in the proximity of the former asbestos mine of Balangero, Italy. J. Hazard. Mater. 2016; 308:321–327. [PubMed: 26852207]
16. Tomatis M, Turci F, Ceschino R, Riganti C, Gazzano E, Martra G, Ghigo D, Fubini B. High aspect ratio materials: role of surface chemistry vs. length in the historical "long and short amosite asbestos fibers". Inhalation Toxicol. 2010; 22:984–998.
17. Miserocchi G, Sancini G, Mantegazza F, Chiappino G. Translocation pathways for inhaled asbestos fibers. Environmental Health. 2008; 7
18. Aust A, Lund L. The role of iron in asbestos-catalyzed damage to lipids and DNA. Biol. Oxid. Syst. 1990; 2:597–605.
19. Aust EA, Lund LG, Chao C-C, Park S-H, Fang R. Role of iron in the cellular effects of asbestos. Inhalation Toxicol. 2000; 12:75–80.
20. Fubini B, Mollo L. Role of iron in the reactivity of mineral fibers. Toxicol. Lett. 1995; 82–83:951–960.
21. Zhang Y, Choksi S, Chen K, Pobezinskaya Y, Linnoila I, Liu Z-G. ROS play a critical role in the differentiation of alternatively activated macrophages and the occurrence of tumor-associated macrophages. Cell Res. 2013; 23:898–914. [PubMed: 23752925]
22. Pascolo L, Gianoncelli A, Schneider G, Salome M, Schneider M, Calligaro C, Kiskinova M, Melato M, Rizzardi C. The interaction of asbestos and iron in lung tissue revealed by synchrotron-based scanning X-ray microscopy. Sci. Rep. 2013; 3:1123. [PubMed: 23350030]
23. Turci F, Tomatis M, Lesci IG, Roveri N, Fubini B. The Iron-Related Molecular Toxicity Mechanism of Synthetic Asbestos Nanofibres: A Model Study for High-Aspect-Ratio Nanoparticles. Chemistry-a European Journal. 2011; 17:350–358.
24. Hardy JA, Aust AE. Iron in Asbestos Chemistry and Carcinogenicity. Chemical Reviews. 1995; 95:97–118.
25. Pollastri S, D'Acapito F, Trapananti A, Colantoni I, Andreozzi GB, Gualtieri AF. The chemical environment of iron in mineral fibres. A combined X-ray absorption and Mössbauer spectroscopic study. J. Hazard. Mater. 2015; 298:282–293. [PubMed: 26073382]
26. Gazzano E, Turci F, Foresti E, Putzu MG, Aldieri E, Silvagno F, Lesci IG, Tomatis M, Riganti C, Romano C, Fubini B, Roveri N, Ghigo D. Iron-loaded synthetic chrysotile: A new model solid for studying the role of iron in asbestos toxicity. Chemical Research in Toxicology. 2007; 20:380–387. [PubMed: 17315889]
27. Jiang L, Akatsuka S, Nagai H, Chew SH, Ohara H, Okazaki Y, Yamashita Y, Yoshikawa Y, Yasui H, Ikuta K, Sasaki K, Kohgo Y, Hirano S, Shinohara Y, Kohyama N, Takahashi T, Toyokuni S. Iron overload signature in chrysotile-induced malignant mesothelioma. J. Pathol. 2012; 228:366–377. [PubMed: 22864872]

28. Foresti E, Fornero E, Lesci IG, Rinaudo C, Zuccheri T, Roveri N. Asbestos health hazard: A spectroscopic study of synthetic geoinspired Fe-doped chrysotile. J. Hazard. Mater. 2009; 167:1070–1079. [PubMed: 19264404]
29. Holmstrom SJM, Lundstrom US, Finlay RD, Van Hees PAW. Siderophores in forest soil solution. Biogeochemistry. 2004; 71:247–258.
30. Gadd GM, Bahri-Esfahani J, Li Q, Rhee YJ, Wei Z, Fomina M, Liang X. Oxalate production by fungi: significance in geomycology, biodeterioration and bioremediation. Fungal Biology Reviews. 2014; 28:36–55.
31. Hoffland E, Kuyper TW, Wallander H, Plassard C, Gorbushina AA, Haselwandter K, Holmström S, Landeweert R, Lundström US, Rosling A. The role of fungi in weathering. Frontiers in Ecology and the Environment. 2004; 2:258–264.
32. Martino E, Cerminara S, Prandi L, Fubini B, Perotto S. Physical and biochemical interactions of soil fungi with asbestos fibers. Environ. Toxicol. Chem. 2004; 23:938–944. [PubMed: 15095889]
33. Szaniszlo P, Powell P, Reid C, Cline G. Production of hydroxamate siderophore iron chelators by ectomycorrhizal fungi. Mycologia. 1981:1158–1174.
34. Favero-Longo SE, Turci F, Tomatis M, Castelli D, Bonfante P, Hochella MF, Piervittori R, Fubini B. Chrysotile asbestos is progressively converted into a non-fibrous amorphous material by the chelating action of lichen metabolites. Journal of Environmental Monitoring. 2005; 7:764–766. [PubMed: 16049575]
35. Martino E, Prandi L, Fenoglio I, Bonfante P, Perotto S, Fubini B. Soil fungal hyphae bind and attack asbestos fibers. Angew Chem Int Edit. 2003; 42:219–222.
36. Dehner CA, Awaya JD, Maurice PA, DuBois JL. Roles of Siderophores, Oxalate, and Ascorbate in Mobilization of Iron from Hematite by the Aerobic Bacterium Pseudomonas mendocina. Applied and Environmental Microbiology. 2010; 76:2041–2048. [PubMed: 20118367]
37. Carrasco N, Kretzschmar R, Xu J, Kraemer SM. Adsorption of hydroxamate siderophores and EDTA on goethite in the presence of the surfactant sodium dodecyl sulfate. Geochemical Transactions. 2009; 10
38. Hersman L, Lloyd T, Sposito G. Siderophore-Promoted Dissolution of Hematite. Geochimica Et Cosmochimica Acta. 1995; 59:3327–3330.
39. Kraemer SM. Iron oxide dissolution and solubility in the presence of siderophores. Aquatic Sciences. 2004; 66:3–18.
40. Chao CC, Aust AE. Effect of Long-Term Removal of Iron from Asbestos by Desferrioxamine-B on Subsequent Mobilization by Other Chelators and Induction of DNA Single-Strand Breaks. Arch Biochem Biophys. 1994; 308:64–69. [PubMed: 8311475]
41. Salamatipour A, Mohanty SK, Pietrofesa RA, Vann DR, Christofidou-Solomidou M, Willenbring JK. Asbestos Fiber Preparation Methods Affect Fiber Toxicity. Environmental Science & Technology Letters. 2016; 3:270–274. [PubMed: 27540559]
42. Lin FC, Clemency CV. THE DISSOLUTION KINETICS OF BRUCITE, ANTIGORITE, TALC, AND PHLOGOPITE AT ROOM-TEMPERATURE AND PRESSURE. American Mineralogist. 1981; 66:801–806.
43. Benson, RF. Method for leaching magnesium from magnesium hydroxide-containing composition. Carey Canada Inc.; 1982.
44. Holmes EP, Lavkulich LM. The effects of naturally occurring acids on the surface properties of chrysotile asbestos. Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering. 2014; 49:1445–1452.
45. Lindsay WL, Norvell WA. Development of a DTPA Soil Test for Zinc, Iron, Manganese, and Copper1. Soil Science Society of America Journal. 1978; 42:421–428.
46. Pietrofesa R, Velalopoulou A, Albelda S, Christofidou-Solomidou M. Asbestos Induces Oxidative Stress and Activation of Nrf2 Signaling in Murine Macrophages: Chemopreventive Role of the Synthetic Lignan Secoisolariciresinol Diglucoside (LGM2605). Int. J. Mol. Sci. 2016; 17:322. [PubMed: 26938529]
47. MacKenzie KJD, McGavin DG. Thermal and Mössbauer studies of iron-containing hydrous silicates. Part 8. Chrysotile. Thermochimica Acta. 1994; 244:205–221.

48. Reichard PU, Kretzschmar R, Kraemer SM. Dissolution mechanisms of goethite in the presence of siderophores and organic acids. Geochimica Et Cosmochimica Acta. 2007; 71:5635–5650.

49. Stewart AG, Hudson-Edwards KA, Dubbin WE. Mechanisms of goethite dissolution in the presence of desferrioxamine B and Suwannee River fulvic acid at pH 6.5. Geochimica Et Cosmochimica Acta. 2013; 115:1–14.

50. Zhong L, Yang J, Liu L, Li X. Desferrioxamine-B promoted dissolution of an Oxisol and the effect of low-molecular-weight organic acids. Biology and Fertility of Soils. 2013; 49:1077–1083.

51. Rozalen M, Javier Huertas F. Comparative effect of chrysotile leaching in nitric, sulfuric and oxalic acids at room temperature. Chemical Geology. 2013; 352:134–142.

52. Rozalen M, Ramos ME, Fiore S, Gervilla F, Huertas FJ. Effect of oxalate and pH on chrysotile dissolution at 25 degrees C: An experimental study. American Mineralogist. 2014; 99:589–600.

53. Hongo T. Dissolution of the chrysotile structure in nitric-acid solutions at different pH. Clay Miner. 2016; 51:715–722.

54. Turci F, Tomatis M, Mantegna S, Cravotto G, Fubini B. The combination of oxalic acid with power ultrasound fully degrades chrysotile asbestos fibres. Journal of Environmental Monitoring. 2007; 9:1064–1066. [PubMed: 17909639]

Highlights

- Fungal and bacterial siderophore removed iron from chrysotile fibers.
- Organic-acid exudates were ineffective in removing iron.
- Siderophore could remove exchangeable and structural iron.
- Siderophore-mediated iron removal lowered fiber toxicity.
- Results suggest that bioremediation of asbestos fibers is feasible.

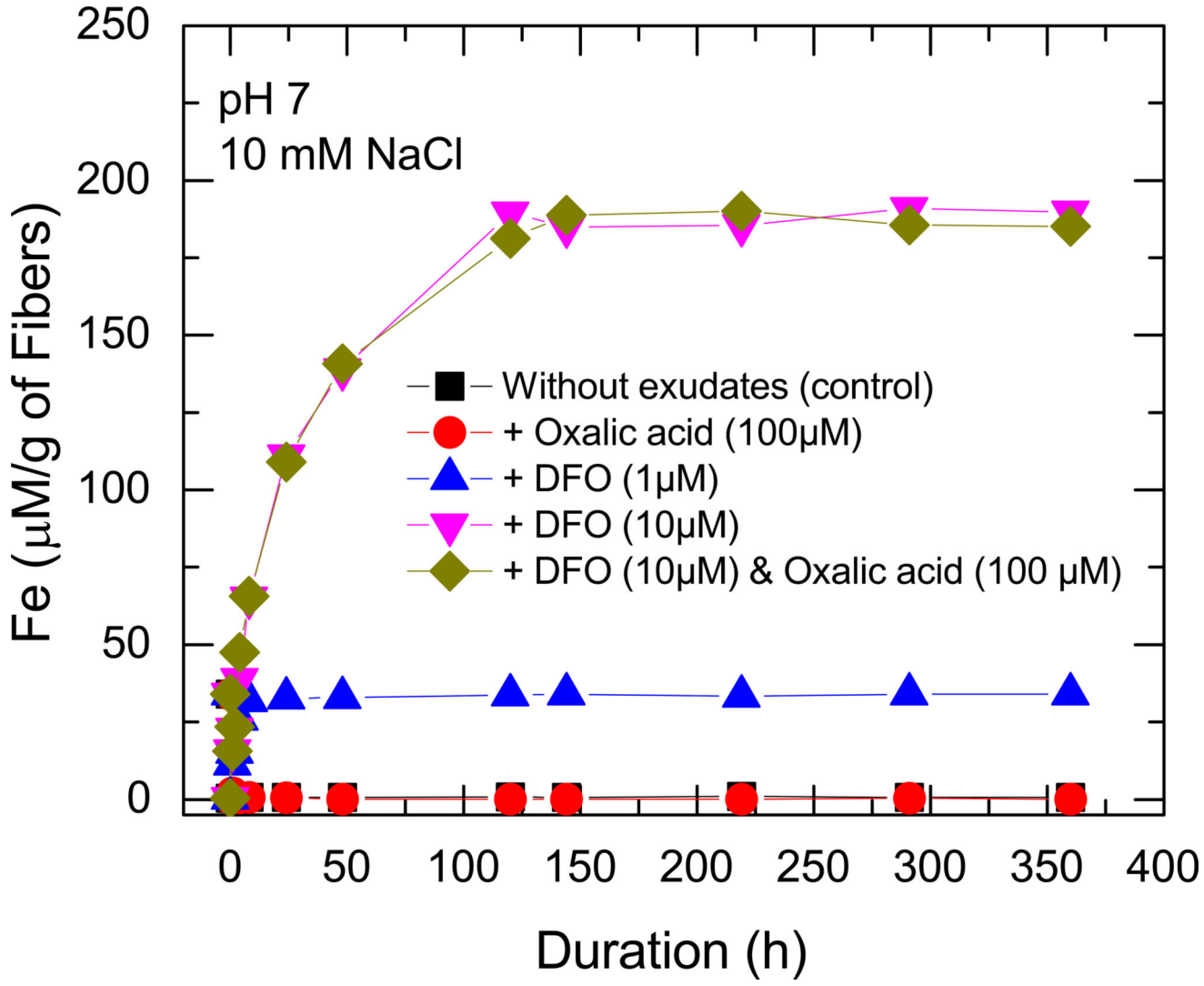


Figure 1.
Dissolution of iron from chrysotile fibers by DFO, with or without oxalic acid, at environmentally relevant concentrations

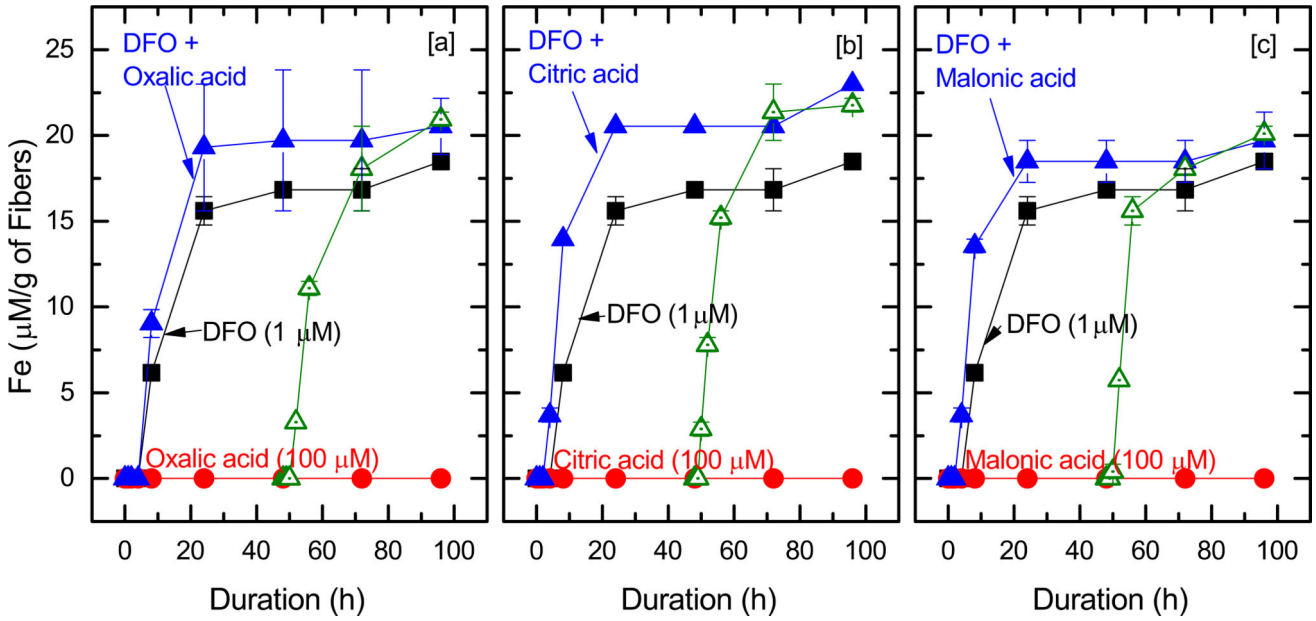


Figure 2.

Effect of organic acid exudates on siderophore-mediated iron release from chrysotile fibers. Pre-exposure to oxalic (a), citric (b), and malonic (c) acid released similar amounts of iron by siderophore as when fibers were exposed to siderophore and respective organic acid simultaneously. The open triangles represent the concentration of iron released by DFO from fibers that were pre-exposed to specific organic acid for 48h. Error bar indicates one standard deviation over mean from triplicate batch.

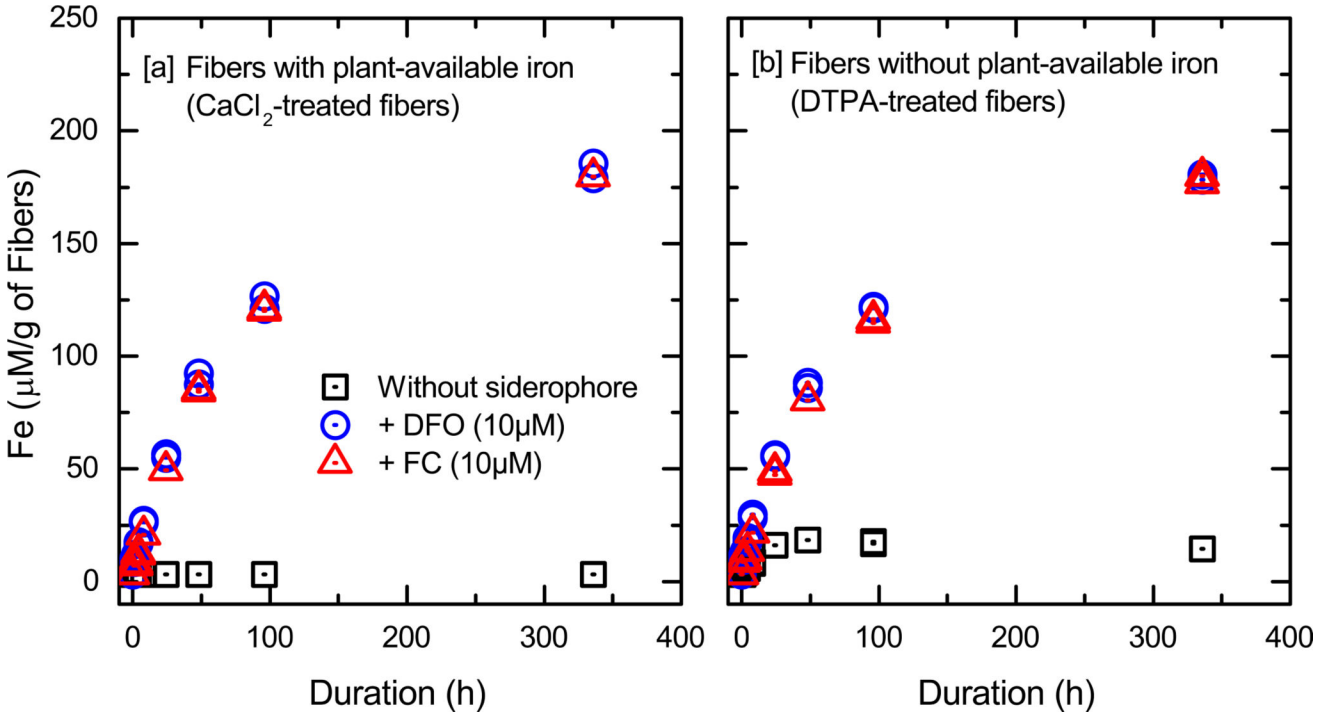


Figure 3.

Removal of iron by the bacterial (DFO) and fungal (FC) siderophore from fiber (a) with plant-available iron ($CaCl_2$-treated) or (b) without plant-available iron (DTPA-treated). Each treatment was carried out in duplicate batches.

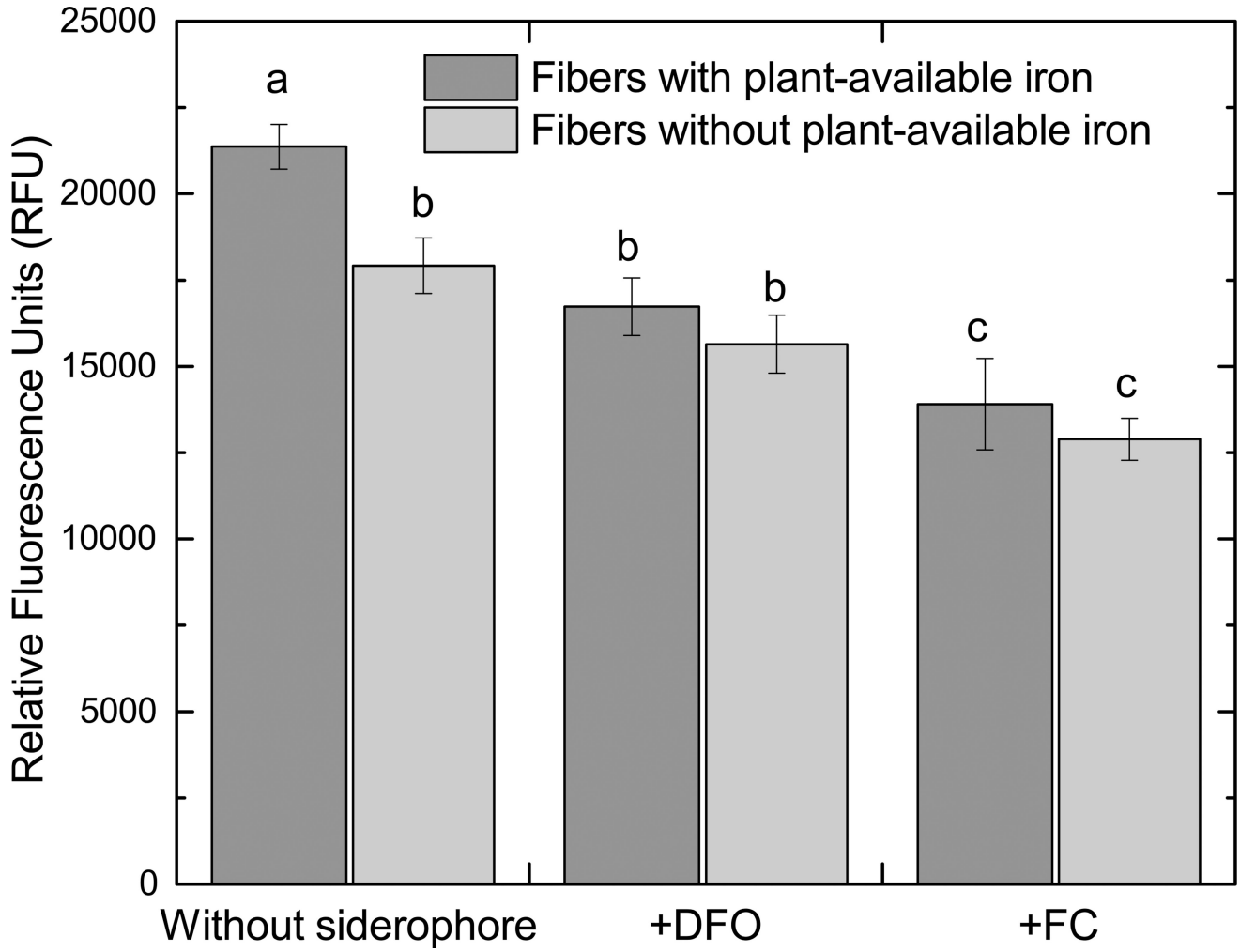


Figure 4.

Amount of ROS generated by macrophages as measured by relative fluorescent units exposed to two types of fibers, with or without plant-available iron, following the treatment without the bacterial (DFO) or fungal (FC) siderophore. Groups without significant different ROS level were indicated by a, b, and c.