Heliyon 5 (2019) e02210

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.heliyon.com

Evidence of pyrite dissolution by *Telephora terrestris* Ehrh in the Libiola mine (Sestri Levante, Liguria, Italy)

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ARTICLE INFO

Keywords:
Environmental science

ABSTRACT

Evidence of pyrite dissolution by *Telephora terrestris* Ehrh were observed for the first time in the abandoned sulphide Libiola mine in May 2017 (Sestri Levante, Liguria, Italy). This fungus is an ectomycorrhizal species able to colonize this extreme environment and bioaccumulate metals such as copper and silver in its fruiting bodies, and it is known to establish symbiosis with maritime pines present in the area, thus favouring their recolonization of the site. This paper presents evidence of *T. terrestris* promoted dissolution of sulphide minerals. This species can remove from soil not only metals possibly toxic to the pine trees, but it can also contribute to the ions bio-accumulation through the bioweathering of sulphide mineral grains (especially pyrite).

1. Introduction

Pyrite alteration is a complex process occurring in a wide range of geological settings and it involves chemical, electrochemical, and biochemical reactions (i.e. galvanic dissolutions, oxidation by O_2 e Fe^{3+}; [1]), resulting in acid generation and toxic element mobilization. This process is often mediated by the activity of iron-oxidizing bacteria and archea, such as *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* [2, 3, 4, 5], which are able to continuously oxidise Fe^{2+} perpetuating pyrite oxidation [3]. Recently, Zotti et al. [6], Cecchi et al. [7, 8] and Cecchi et al. [9] showed that native microfungal strains developed the capability to actively trigger sulphide weathering in order to increase the bioavailability of essential ions and micronutrients, and to modify the pH of the microenvironment. It is well known that not only microfungi but also ectomycorrhizal (ECM) fungi can dissolve minerals and bioaccumulate metals by employing several mechanisms, including protonation (acidolysis), chelation (complexolysis), and metal accumulation [10, 11, 12].

This paper reports the first evidence of mineral-ECM interactions on partially altered pyrite mineralization sampled in May 2017 within a waste-rock dump of the Libiola mine. The Libiola mine (Sestri Levante, Eastern Liguria, Italy) is an abandoned Fe–Cu sulphide mine occurring within the Jurassic ophiolites of the Northern Apennine (Vara Supergroup; [13]), which consist of an ultramafic/gabbroic basement overlain by a volcano-sedimentary sequence. Non-mineralised rocks (mainly basalts and serpentinites), non-valuable minerals (low-grade chalcopyrite and pyrite), and tailings derived from mechanical grinding, milling, and handpicking were dumped in five main open-air waste-rock dumps [14].

Previous research evaluated fungal and plant colonization and the biodiversity in the Libiola mine [15, 16, 17]. These studies showed that the high concentration of trace metals, the paucity of nutrients, and the soil acidity, caused by pyrite oxidation, induced an extremely poor flora, vegetation and a peculiar fungal community. In this site, a few individuals of maritime pine (*Pinus pinaster* Aiton) along with a few herbaceous species grew close to the border of the dump, together with two ectomycorrhizal macrofungal species (*Telephora terrestris* Ehrh. and *Scleroderma polyrhizum* (J.F. Gmel.) Pers.). Both fungal species also established ECM symbiosis with pine. ECM fungi are known to form symbiotic relations with *P. pinaster* [18]. Maritime pine is an undemanding hardly species able to grow in infertile, sandy, and slightly acid soils [19]. Moreover, the success of *P. pinaster* as invader of eroded disturbed soils such as those occurring at the Libiola mine, may be attributed in part to its compatibility with many different ECM fungi [19], such as *Telephora terrestris* and *Scleroderma polyrhizum*. In fact, mycorrhyza-root association improves plant health helping the plant to resist stress caused by heavy metals as well as drought, salinity, and pathogens [20]. Moreover, other investigations [16, 21] demonstrated that these ECM macrofungi are able to accumulate high levels of metals, in particular Cu $>$ 1000 mg/kg in *Telephora terrestris* and Ag$>$50000

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https://doi.org/10.1016/j.heliyon.2019.e02210
Received 18 January 2019; Received in revised form 20 March 2019; Accepted 30 July 2019

μg/kg in *Scleroderma polyrhizum*. These fungi are able to actively sequester most of the potential toxic elements occurring within dump sediments. Khan et al. [22] showed that ECM fungal mycelium could be a physical barrier or shield against heavy metals, protecting plant roots, actively bioaccumulating metals, or precipitation of metals by exudates.

2. Materials and methods

The studied samples represent the first occurrence of altered pyrite mineralization clasts crossed and twisted with mycelium and rhizomorphs of *Telephora terrestris*. The analysed samples were collected at the base of a waste-rock dump, from Libiola mine, containing both non-valuable pyrite-bearing minerals and non-mineralized host rocks (mainly basalts and serpentinites). They were collected close to the *T. terrestris* fruiting bodies by sampling the first 25 cm from the surface with a shovel. The soil colour was determined *in situ* according to the Munsell® Colour Soil Charts [23]. Mineralogical, micromorphological and microtextural analyses were carried outby means of Polarized-Light Optical Microscopy (PLOM) and Environmental Scanning Electron Microscopy with attached X-ray Energy Dispersive System (ESEM-EDS). The PLOM analyses were carried out using a BX-41 Olympus Microscope equipped with 2x, 4x, 10x, 20x, 40x, 50x objective magnifications and 10x and 12,5x eyepieces. High-resolution images were acquired using OLYMPUS COLOR VIEW II-SET cameras and processed using OLYMPUS C-VIEW II – BUND- CellB software. The ESEM-EDS investigations were performed SEM Vega3 – TESCAN type LMU system equipped with an EDS EDAX APOLLO XSDD and a DPP3 analyzer. EDS spectra were collected at 20 kV accelerating voltage and 1.2 nA beam current at the specimen level for 60 live second counting time with a spot size of 370 nm and a working distance of 15 mm. Concentrations of major and minor elements were calculated on an anhydrous basis with ZAF (Z, atomic number; A, absorption; F, fluorescence) corrections and using natural silicates and oxides for calibration. ESEM-EDS was also used in low-vacuum mode for micromorphological analyses to characterize the fungal colonization on mineral surfaces.

3. Results

The studied samples can be classified as sandy-gravely sediment, characterized by heterogeneous materials comprised of basalt and serpentinite clasts and pyrite with different degree of alteration. The silty and clayely fractions are mostly composed of Fe-oxide and oxy-hydroxides, which confer to the sediment a reddish-yellow colour (7.5YR 6/8-; [23]). The unaltered pyrite clasts are composed of idiomorphic pyrite crystals with minor quartz and chalcopyrite without evidence of fungal colonization (Fig. 1A). On the other hand, most of the mineralized clasts exhibited significant fungal colonization with extensive pyrite alteration (Fig. 1B-E). *T. terrestris* rhizomorphs and hyphae progressively coated and enveloped the pyrite crystals starting from grain boundaries and grain microfractures (Fig. 1B). Where the fungal colonization is less developed (Fig. 1B, C), evidence of bioweathering, such as etching and dissolution channels, is present on the crystals surface in correspondence with *T. terrestris* hyphae. Where the fungal colonization is more developed (Fig. 1D, E) pyrite mineralizations clasts are strongly altered and partially (Fig. 1D) to completely (Fig. 1E) replaced by secondary authigenic minerals. The EDS spectra on these strongly altered clasts (e.g. Fig. 1F) showed that S, Fe, and Ca are the only chemical elements present in detectable amounts, thus suggesting that iron-oxides/oxyhydroxides and subordinately calcium/iron sulphates are the main authigenic secondary minerals.

4. Discussion

Mycorrhizal fungi are recognized for their importance for plant foraging of soil resources [20]: however, how mycorrhizal traits mediate soil-plant relationships is still an open question [24]. In the Libiola mine, ECM fungi enhance maritime pine nutrition, stress tolerance and soil structure and, consequently, promote the recovery of the vital functions

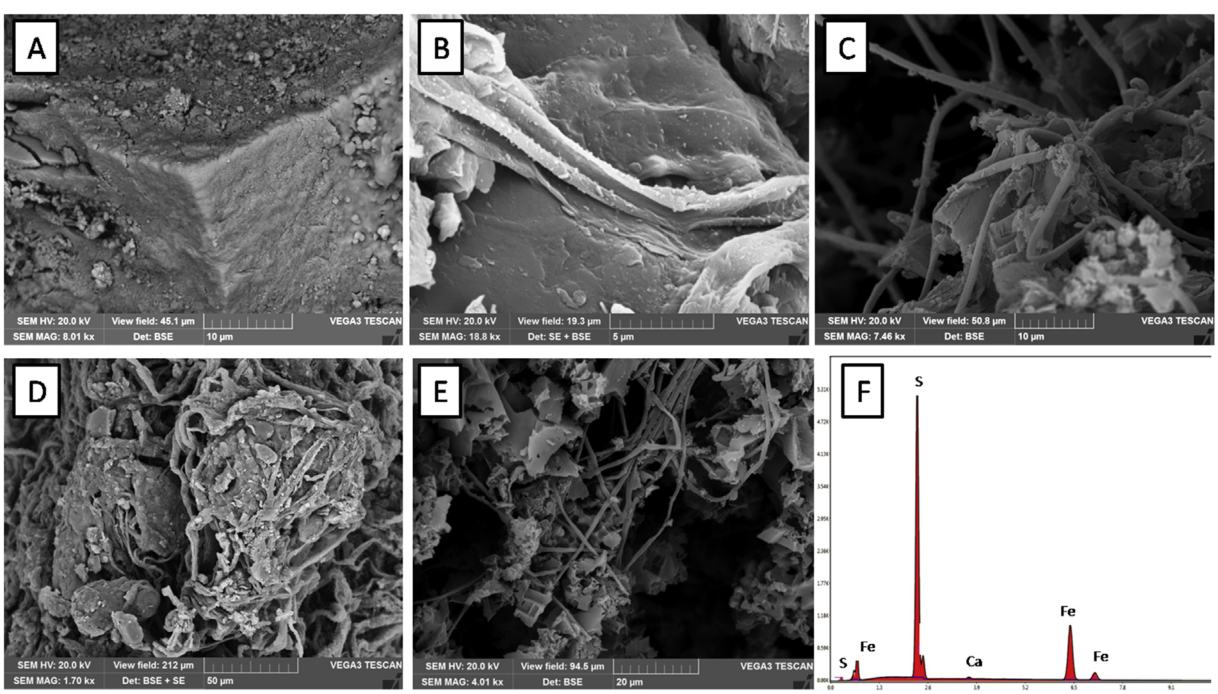

Fig. 1. A) ESEM image of unaltered pyrite crystal without evidence of mycological biofilm and hyphae. B) Detail of a pyrite crystal surface with evidences of bioweathering represented by pitting, etching and dissolution channel placed in correspondence of *T. terrestris* hyphae. C) ESEM image showing altered pyrite crystal completely enveloped by *T. terrestris* hyphae. D) Detail of *T. terrestris* hyphae enveloping pyrite crystal. E) ESEM image evidencing the high degree of colonization by *T. terrestris* mycelium with interstitial idiomorphic pyrite crystals, lamellar calcium-sulphate (presumably gypsum) and microcrystalline aggregates of Fe-oxyhydroxides. F) EDS spectra of the area reported in Fig. E.

Fig. 2. Fruiting bodies of *T. terrestris* at the Libiola mine.

in the degraded soil of the waste-rock dump. In particular, *T. terrestris* (Fig. 2) is a common symbiotic ECM fungus with beneficial effects for trees growing under stressful conditions, such as those prevailing in the mine areas [20]. Moreover, this fungal species is characterised by rhizomorph formation, which increases water and phosphate uptake through a long-distance exploration mechanism [20]. *Telephora terrestris* is also known to solubilise zinc phosphate [11] and it confirms that this fungus can dissolve salts and probably also pyrite crystals and sulphide mineralizations [16, 17, 21]. Van Tichelen et al. [25] also showed that *T. terrestris* plays a central role in the P nutrition of the host plant in P-limited and Cu-contaminated soils. Several studies demonstrated that macrofungi may bioaccumulate heavy metals in their fruiting bodies, owing to enzymes or organic acids chelating metals ions and actively transporting them into the cells [11, 26, 27]. Moreover, Jongmans et al. [28] found tunnels inside mineral grains formed by hyphae of ECM fungi, which were the first indications of the role played by macrofungi in mineral dissolution, thus implying that fungi were able to dissolve mineral grains.

Our ESEM analyses reveal the direct and active role of *T. terrestris* in the bioweathering of pyrite, where hyphae play a central role in natural corroded samples. The bioweathering scheme of corrosion observed here differs significantly from the abiotic pyrite alteration documented from the same area [14, 29]. The abiotic pyrite weathering commonly develops from the crystal edges and surfaces, following specific crystallographic planes or weakness, and involves the progressive replacement of pyrite crystals by secondary minerals through pseudomorphic or sub-pseudomorphic mechanisms [30]. Pyrite bioalteration observed in our study occurs randomly on the crystal surfaces and initially consists of the formation of scattered etch pits due to organic acids and other excreted metabolites [8]; at this stage, crystal surfaces appear pitted and pocked in correspondence to hyphae adhesion. Where the interaction is more developed, hyphae completely envelop crystals and are intimately intermixed with pyrite relics and newly forming authigenic minerals.

Trace metals contained in pyrite (such as Cu, Ni, Co, Zn) are mostly incorporated in new forming Fe-oxides and oxyhydroxides [14], but a significant metals accumulation, particularly copper, has been demonstrated for *T. terrestris* spontaneously growing in Libiola mine [17].

Our results further confirm the active role of *T. terrestris* in pyrite alteration and support the results of previous studies [16, 17, 21] which also showed its role in roots protection of *P. pinaster* in the waste-rock dump from the Libiola mine. This fungus not only favours plant re-colonization in a strongly contaminated environment, but also assists plants (*P. pinaster)* in the acquisition of mineral nutrients triggering weathering and increasing their bioavailability. Smits et al. [31] showed for the first time grain-scale effects of ECM fungi, in symbiosis with a host plant, on mineral weathering under sterile conditions. Moreover, most of the experimental research has been carried out in controlled conditions in a laboratory [31, 32, 33]. As far as we know, our results are the first evidence of direct mineral-macrofungi interaction in contaminated sites under natural environmental conditions. In fact, *in situ* evidence of these phenomena are well documented in uncontaminated podzols in temperate and boreal zones and in acid brown forest soils [28, 34].

5. Conclusion

The hypothesis that ECM fungi favour the bioaccumulation and transport to the tree of ions through the bioweathering of soil mineral grains leads to define a new role of ECM fungi in plant nutrition and biogeochemical cycles of essential elements [12]. *T. terrestris,* collected in the Libiola mine, can play this central role favouring *P. pinaster* colonization of the mine dumps and accumulating toxic metals by corroding sulphide minerals (mainly pyrite crystals). However, further investigations on minerals or on biotic components of the system will be the next steps to confirm these promising results.

Declarations

Author contribution statement

M. Zotti: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

G. Cecchi: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

S. Di Piazza: Performed the experiments; Analyzed and interpreted the data.

P. Marescotti: Analyzed and interpreted the data.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

We thank Laura Negretti (DISTAV - Dipartimento di Scienze della Terra dell'Ambiente e della Vita, University of Genoa) for assistance with scanning electron microscopy.

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