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Fungal degradation of calcium-, lead- and silicon-bearing minerals

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Abstract

The aim of this study was to examine nutritional influence on the ability of selected filamentous fungi to mediate biogenic weathering of the minerals, apatite, galena and obsidian in order to provide further understanding of the roles of fungi as biogeochemical agents, particularly in relation to the cycling of metals and associated elements found in minerals. The impact of three organic acid producing fungi (*Aspergillus niger*, *Serpula himantioides* and *Trametes versicolor*) on apatite, galena and obsidian was examined in the absence and presence of a carbon and energy source (glucose). Manifestation of fungal weathering included corrosion of mineral surfaces, modification of the mineral substrate through transformation into secondary minerals (i.e. crystal formation) and hyphal penetration of the mineral substrate. Physicochemical interactions of fungal metabolites, e.g. H^+ and organic acids, with the minerals are thought to be the primary driving forces responsible. All experimental fungi were capable of mineral surface colonization in the absence and presence of glucose but corrosion of the mineral surface and secondary mineral formation were affected by glucose availability. Only *S. himantioides* and *T. versicolor* were able to corrode apatite in the absence of glucose but none of the fungi were capable of doing so with the other minerals. In addition, crystal formation with galena was entirely dependent on the availability of glucose. Penetration of the mineral substrates by fungal hyphae occurred but this did not follow any particular pattern. Although the presence of glucose in the media appeared to influence positively the mineral penetrating abilities of the fungi, the results obtained also showed that some geochemical change(s) might occur under nutrient-limited conditions. It was, however, unclear whether the hyphae actively penetrated the minerals or were growing into pre-existing pores or cracks.

Introduction

Fungal weathering of rocks and minerals is closely linked with growth characteristics. Hyphal extension, the characteristic mode of growth, enables fungi to explore and penetrate their substrates in search of nutrients and new environments (Gooday 1995; Jacobs *et al.* 2002). Hyphae may follow fissures and cavities found in rocks and cause physical damage but are also capable of excreting inorganic and organic metabolites, such as H^+, CO_2, siderophores and carboxylic acids, which contribute to biochemical weathering (Burford *et al.* 2003). Fungal metabolites may interact with rocks and minerals in various ways. Firstly, dissolution may be manifest by etching of the rock surfaces (Callot *et al.* 1987; Lee & Parsons 1999; Adamo & Violante 2000). Secondly, chelation of metal cations by organic ligands, e.g. citrate and oxalate, weaken metal–oxygen bonds in minerals and can increase the solubility of metals such as Al (Banfield *et al.* 1999; Harley & Gilkes 2000). Thirdly, neogenesis of minerals may occur. This includes the formation of secondary precipitates by the actions of organic acids, e.g. oxalic acid secretion can lead to the precipitation of insoluble

metal oxalates (Gadd 1999). Overall, the resulting change(s) in the distribution and speciation of various elements as a result of rock breakdown may ultimately affect their respective elemental cycles in the biosphere and their availability for microbial and plant uptake (Harley & Gilkes 2000; Wallander 2000; Blum *et al.* 2002; Guidry & Mackenzie 2003).

Apatite is a common primary source of phosphorus in the soil (Rogers *et al.* 1998; Taunton *et al.* 2000). In many environments, phosphorus is growth-limiting largely because of its involvement in crucial roles such as energy production and storage via ATP (Ehrlich 1981; Taunton *et al.* 2000). Its regulatory capabilities affect processes such as climate, environmental and ecological change, an example being the control of conversion rates of atmospheric carbon dioxide to organic matter (Föllmi 1996). On addition to the soil as mineral phosphates, the bioavailability of phosphorus is often reduced (Lapeyrie *et al.* 1991; Whitelaw *et al.* 1999; Whitelaw 2000). The weathering of apatite becomes significant in this regard as it leads to the release of constituent elements, calcium and phosphorus, the latter in the form of phosphates (Wallander 2000; Blum *et al.* 2002). Lead is ubiquitous in the soils and rocks of the earth and can substitute for calcium in some minerals, e.g. (hydroxy) apatite (Burau 1982; Ma *et al.* 1993; Ruby *et al.* 1994). In nature, lead deposits usually occur in the form of the sulphide galena, a common mineral ore (Greninger *et al.* 1974; Blaskett & Boxal 1990; Davies 1995). Anthropogenic sources also contribute to its occurrence in the environment: biotransformations of Pb are therefore of relevance to processes such as the bioremediation of contaminated soil and groundwater (Sayer *et al.* 1999). Obsidian is a glassy, silicon-containing volcanic rock. Silicon is one of the most abundant constituents of the lithosphere (Pokrovski & Schott 1998) and, in volcanic regions, the weathering of volcanic glass controls the build-up of soil as well as the composition of the waters that drain them (Kurtz *et al.* 2002; Gordon & Brady 2002). Such weathering also plays important roles in nutrient availability in terrestrial and marine ecosystems in addition to representing a major sink for atmospheric CO_2 (Harris *et al.* 1998; Kurtz *et al.* 2002; Taylor *et al.* 2000).

Fungal interactions with mineral surfaces leading to weathering are important not only because they promote mineral neogenesis, transformation and dissolution (Gadd 1993; Sayer *et al.* 1995; Sayer & Gadd 1997; White *et al.* 1997; Ehrlich 1998; Gharieb *et al.* 1998), but also because they impact architecture through the biodeterioration of stone and concrete used as construction materials for monuments and historic buildings (Diercks *et al.* 1991; Bock & Sand 1993; Ascaso *et al.* 1998; Gu *et al.* 1998; Banfield *et al.* 1999). The main purpose of this work was to investigate the degradation of apatite, galena and obsidian by oxalic acid-producing strains of fungi under conditions of nutrient scarcity and availability. The parameters used (individually and collectively) to assess weathering by the fungi were corrosion (i.e. etching) of mineral surfaces, crystal precipitation (through oxalic acid production) and/or hyphal penetration into minerals.

Materials and methods

Organisms

The following fungi – *Aspergillus niger* (ATCC No. 201373), *Serpula himantioides* (fr:fr) P.karst (from Dr N. White, University of Abertay Dundee, UK) and *Trametes versicolor* (from Prof. C. Evans, University of Westminster, UK) were used. These were maintained on an experimental medium (tap water agar) which consisted of 15 g l^{-1} agar (Lab M, No.1; International Diagnostics Group, UK) and tap water, with and without 20 g l^{-1} glucose, and grown at 25 °C in the dark for 10 days prior to the start of the experiment.

Mineral preparation

Natural samples of the following rock minerals were used – apatite [$Ca_5(PO_4)_3F$; Wilberforce, Ontario, Canada], galena (PbS; Derbyshire, UK) and obsidian (SiO_2; Lake County, Oregon, USA). They were crushed using a ball mill and then sieved to obtain a final size of about 2.5 mm in diameter. The resulting rock pieces were sterilized according to the methods of Gu *et al.* (1998). Briefly, they were rinsed (a) in running tap water for 2 h and (b) with deionized water for at least 8 h with intermittent agitation on an orbital shaker. The samples were then immersed in 70% ethanol

for at least 24 h after which the solution was decanted and the samples left to dry in a sterile flow hood. Once dry, they were oven-sterilized at 80 °C for at least 24 h.

Media preparation and experimental set-up

Media was prepared (with and without the addition of 20 g l^{-1} glucose) consisting of agar (Lab M, No.1, 15 g l^{-1}) and tap water and poured into 90 mm diameter Petri dishes. Before solidification of the agar, four rock pieces were placed in the agar with exposed surfaces approximately 15 mm from the centre of each 90 mm Petri dish. Dialysis membranes cut to ~80 mm diameter discs were sterilized by autoclaving for 15 min at 121 °C and placed on the surfaces of half the plates of each agar type to prevent hyphal penetration while permitting diffusion of fungal metabolites. Using 7 mm diameter corers, all the Petri dishes (except the controls) were inoculated with cores obtained from the margin of 10-day-old grown mycelia of the fungi mentioned above and placed in the centre of the plates. The control plates were prepared in the same manner except without the inoculation of fungi. All plates were incubated in the dark at 25 °C over an eight week period. Each treatment had three replicates.

Rock surface and crystal analyses

Rock pieces were removed from agar in plates with and without membranes for each fungus, air dried and sputter coated with 10 nm gold–palladium. They were viewed under a Philips XL 30 environmental scanning electron microscope (ESEM) for any changes in the rock surface and compared with images obtained from the control. Crystals precipitated on the rock surface were observed with the ESEM and those produced around the rock in the agar were observed with a light microscope. Energy dispersive X-ray analysis (EDX) linked to the ESEM was used to determine the constituent elements in the crystals.

In order to obtain crystals for X-ray powder diffraction (XRPD), the crystal-containing agar observed underneath and in close proximity to the rock pieces in each media type was collected with a spatula and put into a crystallizing dish. Sterile distilled water was added and the dish was put in a microwave oven at full capacity (950 W) for 2–3 min until the agar dissolved completely, leaving behind the crystals in the resulting solution. The solution was pipetted out of the dish and the process was repeated twice. Crystals which settled at the bottom of the dish were then transferred into an Eppendorf tube and placed in a desiccator until fully dry crystals were obtained. XRPD analysis was carried out with a Philips PW1050/Hiltonbrooks DG2 X-Ray diffractometer.

Organic acid determination

Organic acid determination in the agar was carried out using a HPLC system comprising a Waters 600 controller, a Waters 486 turnable absorbance detector, a Waters 600 pump and a Waters 717 autosampler controlled by Millipore (Waters) millennium chromatography software. Two 9 mm diameter cores of agar were made from each agar plate and put in different plastic tubes to which 2.5 ml of ddH_2O and 1 M HCl were added to obtain a water fraction (containing water-soluble organic acids) and a HCl fraction (containing water-insoluble precipitates e.g. oxalate), respectively. The tubes were then placed in a water bath at 75 °C for 30 min until the agar dissolved. The resulting samples were mixed with a cation exchange resin (Bio-Rad AG 50W-X4) in order to remove free metal species and filtered through Whatman 0.45 μm filters. The eluent used was 4 mM sulphuric acid that was pre-filtered using a Whatman 0.45 μm cellulose nitrate membrane filter. The column was an Aminex® HPX-87H ion exclusion column. About 20 μl duplicates of the samples and standards were run for 30 min and detection was carried out at 210 nm. Organic acids were identified and quantified by comparison to retention times of standards.

Staining of rock samples

Using 9 mm diameter corers, agar cores were made from all plates incubated without membranes and put into 20 ml glass vials. Each agar core contained a rock piece. To each vial, an aqueous solution of glutaraldehyde (2.5% v/v) was added gently until the rock sample was completely immersed to fix the hyphae. The vials were then placed in a desiccator and a pressure of ~50 kPa was applied for 10 min, then left to incubate at room temperature for a further 1.5 h, at the end of

which the solution was removed. Afterwards, the samples were stained in 0.45 μm Whatman cellulose nitrate membrane filtered fluorescent brightner (0.2% w/v Fluostain1, i.e. Calcofluor White; Sigma-Aldrich Co.). The vials were again placed in a desiccator to which a pressure of $\sim$50 kPa was applied for another 10 min. After this, they were left to incubate at room temperature for 2.5 h, at the end of which the solution was removed. The samples were then dehydrated with a graded series of ethanol/water once for 20 min in 50%, 60%, 70%, 80%, 90% ethanol solutions and twice for 20 min each in 100% ethanol.

Fluorescence microscopy

Each sample was affixed on its side unto a microscope glass slide (25 × 47 mm) using a mixture of EPO-THIN^{R} resin and hardener (Buehler, UK). The glass slide with each sample was put into a Buehler Precision slide holder and ground down to between one and two millimetres with the aid of a P800 grit BUEHLER-MET^{R} silicon carbide abrasive disc. Observations were made with an Olympus BH2 fluorescence microscope equipped with a mercury lamp and using a green–blue filter. The emission filter used was an Olympus O515. Photographs were taken using a Kodak Ekta 160T colour slide film with an Olympus C-35AD-4 camera attached to the microscope.

Results

Corrosion of rock surfaces

The corrosion abilities of the fungi varied with rock type and medium. The use of dialysis membranes to prevent direct contact between fungal hyphae and rock surface excluded the mechanical role of hyphae in corrosion. The results presented in this section therefore only show the consequence of the interactions between metabolites secreted by the fungi and the minerals. In general, the addition of glucose to tap water agar (TWA) lead to an increase in the etching abilities of the fungi tested. All three fungi were able to corrode apatite in glucose-amended TWA (Figure 1) while only *S. himantioides* and *T. versicolor* showed etching abilities in TWA (Figure 2). Figure 3 shows that

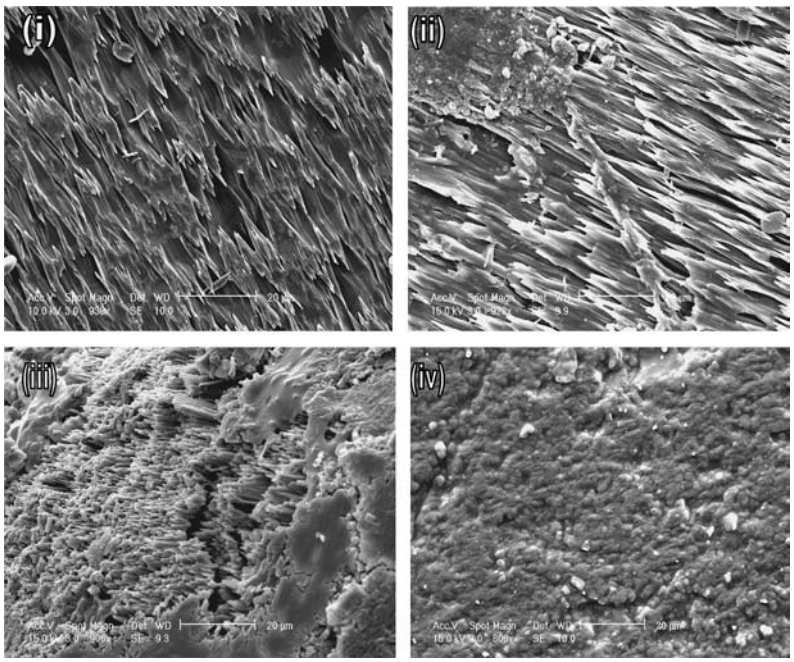

Figure 1. Typical SEM micrographs of apatite showing corrosion by (i) *A. niger*, (ii) *S. himantiodes* and (iii) *T. versicolor* grown on 20 g l^{-1} glucose-amended TWA while (iv) shows an uninoculated and uncolonized apatite rock surface. These fungi were grown on agar containing exposed apatite surfaces and were incubated in the dark at 25 °C over an eight week period. All SEM images shown are representative of at least five replicate determinations.

these two fungi were also able to corrode the surface of galena in glucose-amended TWA. None of the three fungi did so in TWA. With obsidian, only *A. niger* and *S. himantioides* produced weathered grains in the presence of glucose (Figure 3). No etching was observed in TWA. The changes which occurred were obvious when compared to control samples maintained in uninoculated sterile medium throughout the duration of the experiment. These results are summarized in Table 1.

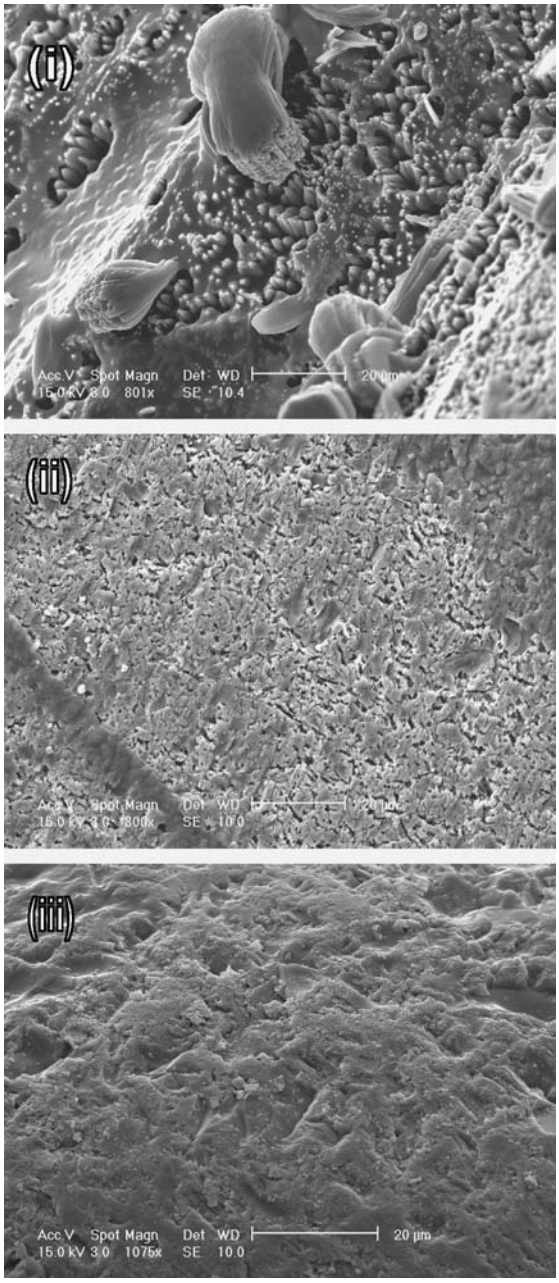

Figure 2. Typical SEM micrographs of apatite showing corrosion by (i) *S. himantioides* grown on TWA. Image (ii) suggests evidence of marginally increased weathering by *T. versicolor* grown on TWA while (iii) shows the surface of an uninoculated and uncolonized apatite rock surface.

Crystal formation on minerals

Crystal formation by the fungi in the presence and absence of glucose is summarized in Table 2. Different morphologies were observed alongside crystal precipitation by the fungi and these are presented in Figures 4 and 5. Crystals were formed with apatite in TWA by *S. himantioides* and *T. versicolor* only (Figure 4) while all three fungi produced crystals with apatite (Figure 4) and galena (Figure 5) both on the rock surface and in the agar in glucose-amended TWA. No crystal formation was observed on galena during fungal growth on TWA. With obsidian, crystals were precipitated by *S. himantioides* and *T. versicolor* on their hyphae both in the presence and absence of glucose (Figure 6). *A. niger* did not precipitate any crystals with obsidian whether in the presence or absence of glucose.

EDX and XRPD

The profiles in Figure 7 show the EDX spectra for apatite, galena and obsidian. The profiles confirmed the presence of calcium in apatite, lead in galena and silicon in obsidian, among other elements. EDX analysis of the crystals produced by the fungi with the different minerals showed them to contain calcium (apatite), lead (galena) and calcium (obsidian). XRPD profiles shown in Figure 8 confirmed the crystals produced with apatite and galena were calcium oxalate and lead oxalate, respectively.

Organic acid production by the fungi

The fungi produced a range of acids under different growth conditions with the minerals as shown in Table 3. The most common acids produced by all three fungi were malic, oxalic and fumaric acids. Malic acid was secreted by the fungi on different rock types both in the absence and presence of glucose. With apatite, the amount of oxalic acid produced by the fungi in the presence of

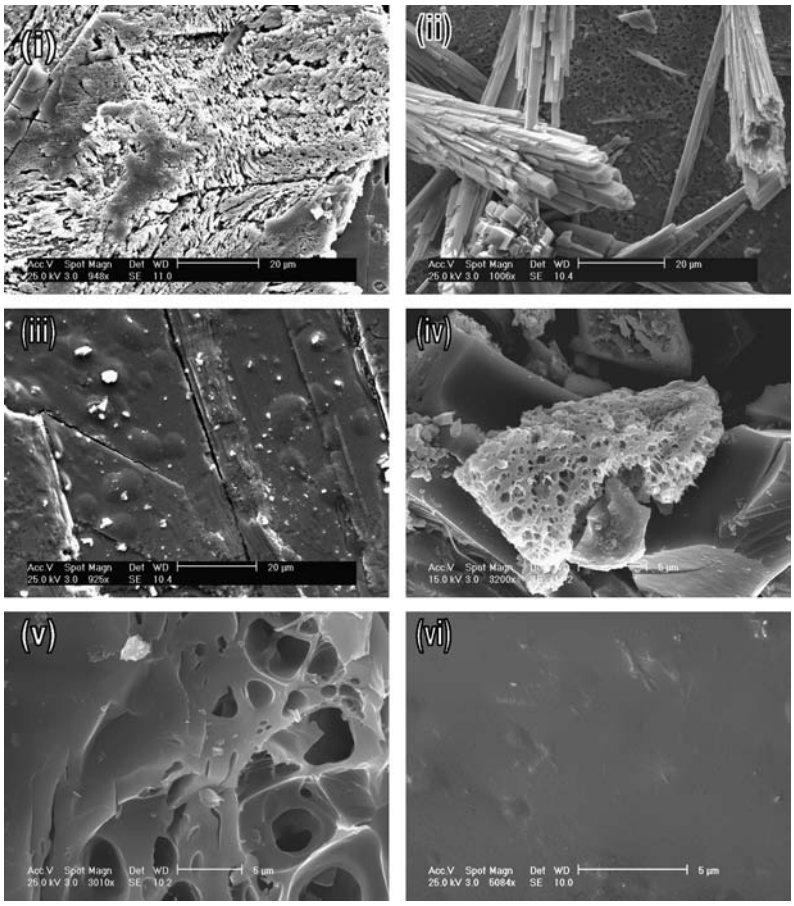

Figure 3. Typical SEM micrographs showing corrosion of galena by (i) *S. himantioides*, (ii) *T. versicolor* (i.e. underneath the crystals) grown on 20 g l^{-1} glucose-amended TWA. Image (iii) is the control for galena (uninoculated, therefore uncolonized galena surface). Images (iv) and (v) show tunnel structures of weathered grains of obsidian by *A. niger* and *S. himantioides* in 20 g l^{-1} glucose-amended TWA respectively while image (vi) is the uninoculated control for obsidian.

glucose was greater than that produced when glucose was absent. In the presence of glucose, the amount produced by the fungi was in the order *A. niger* > *S. himantioides* > *T. versicolor*: in the absence of glucose, the amount produced by *S. himantioides* was greater than that of either *A. niger* or *T. versicolor*.

The amount of oxalic acid secreted with galena in the absence of glucose by each fungus was greater than that produced in the presence

Table 1. Corrosion of various mineral surfaces by the fungi in the presence and absence of glucose.

Rock mineral	Agar type	
	Glucose-amended TWA	TWA
Apatite	*A. niger, S. himantioides* *T. versicolor*	*S. himantioides,* *T. versicolor*
Galena	*S. himantioides,* *T. versicolor*	–
Obsidian	*A. niger, S. himantioides*	–

Table 2. Crystal formation by the fungi with the various minerals in the presence and absence of glucose.

Rock mineral	Agar type	
	Glucose-amended TWA	TWA
Apatite	*A. niger, S. himantioides* *T. versicolor*	*S. himantioides,* *T. versicolor*
Galena	*A. niger, S. himantioides* *T. versicolor*	–
Obsidian	*S. himantioides,* *T. versicolor*	*S. himantioides,* *T. versicolor*

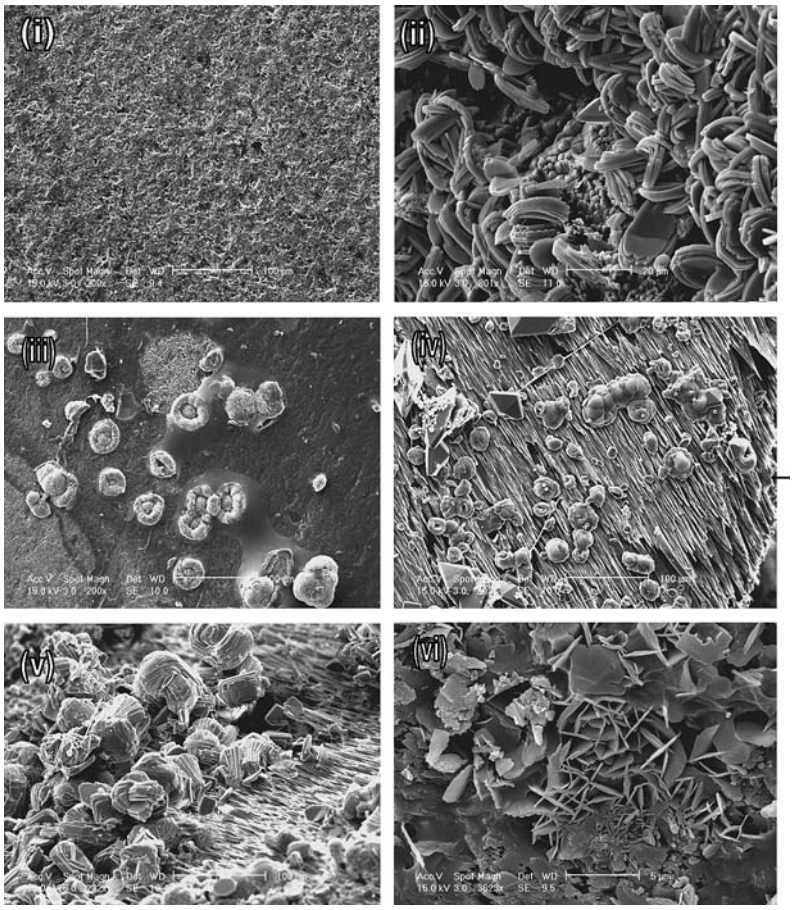

Figure 4. Typical SEM micrographs showing (i) no crystal production on apatite by *A. niger* grown on TWA, crystal production on apatite by (ii) *S. himantioides* and (iii) *T. versicolor* grown on TWA. Images (iv), (v) and (vi) show crystal production on apatite in glucose-amended TWA by *A. niger*, *S. himantioides* and *T. versicolor* respectively.

of glucose, the only exception being *S. himantioides*. The presence of glucose had no influence on oxalic acid secretion in media with obsidian and amounts produced in media without glucose were greater than that produced in glucose-amended media for all three fungi.

With fumaric acid, amounts produced by the fungi with apatite were greater in the absence of glucose, with *S. himantioides* being the only exception as it produced no fumaric acid in the absence of glucose. No fumaric acid was produced with galena by *A. niger* and *S. himantioides* in the presence of glucose although the amount produced by *T. versicolor* in the presence of glucose was greater than that produced in its absence. With obsidian, *S. himantioides* and *T. versicolor* produced greater amounts of fumaric acid in the presence of glucose than in its absence. Fumaric acid was not produced by *A. niger*.

Observation of hyphae within minerals

The fluorescence microscopy technique aided the visualization of fungal hyphae in the various minerals under the differing glucose conditions. A summary of the results obtained is shown in Table 4. Figure 9 shows fungal hyphae in some of the minerals. No particular pattern of hyphal penetration by the fungi was observed. However, hyphae of all three fungi were seen in all minerals especially in the presence of glucose. Only hyphae of *A. niger* and *T. versicolor* were observed in galena in the absence of glucose while only hyphae of *S. himantioides* and *T. versicolor* were seen in obsidian in the absence of glucose.

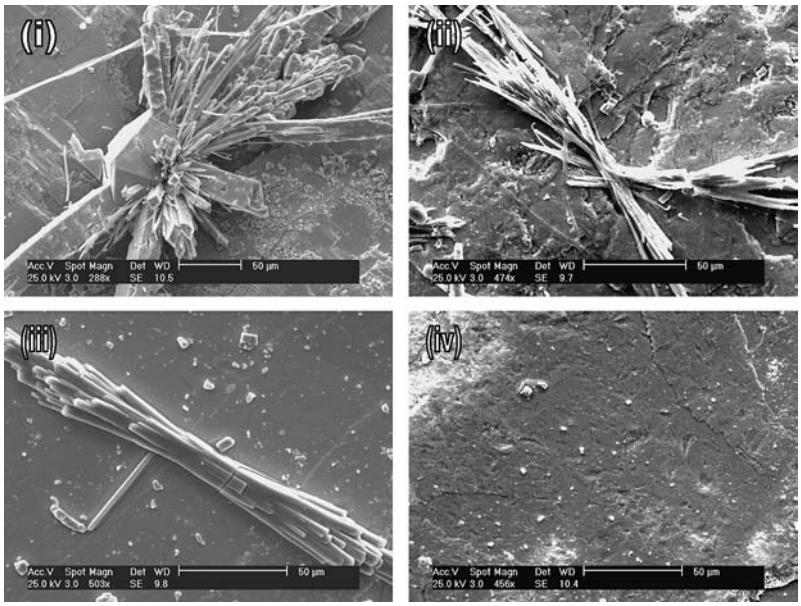

Figure 5. Typical SEM micrographs of galena showing crystal formation by (i) *A. niger*, (ii) *S. himantioides* and (iii) *T. versicolor* grown on 20 g l^{-1} glucose-amended TWA while image (iv) shows the control i.e. uninoculated galena surface.

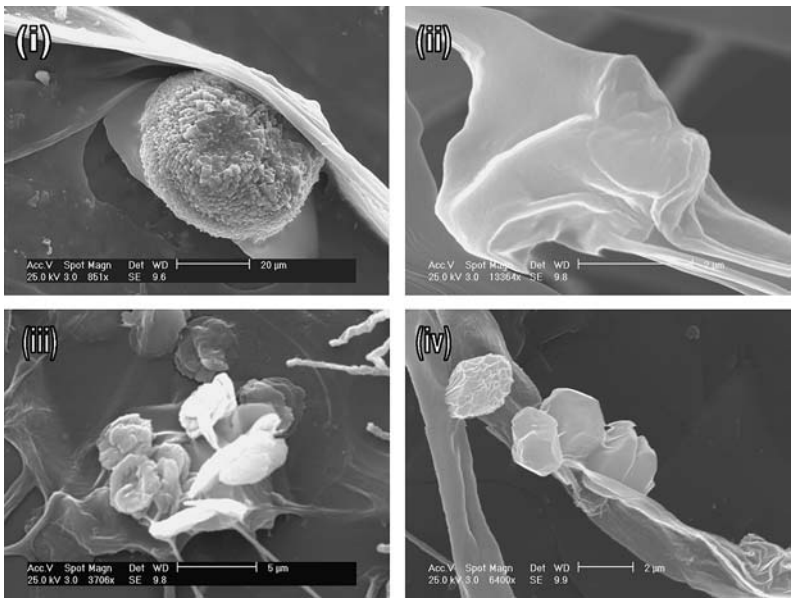

Figure 6. Typical SEM micrographs showing crystal precipitates in hyphae of *S. himantioides* grown on agar containing exposed obsidian surfaces in the (i) absence and (ii) presence of 20 g l^{-1} glucose in TWA and *T. versicolor* also grown on agar containing exposed obsidian surfaces in the (iii) absence and (iv) presence of 20 g l^{-1} glucose in TWA.

Discussion

This work has demonstrated the influence of glucose on the corrosion of apatite, galena and obsidian, and penetration into the interior of the mineral substrate as well as precipitate (i.e. crystal) formation by fungi. Of all three minerals, apatite appeared to be the most susceptible to weathering

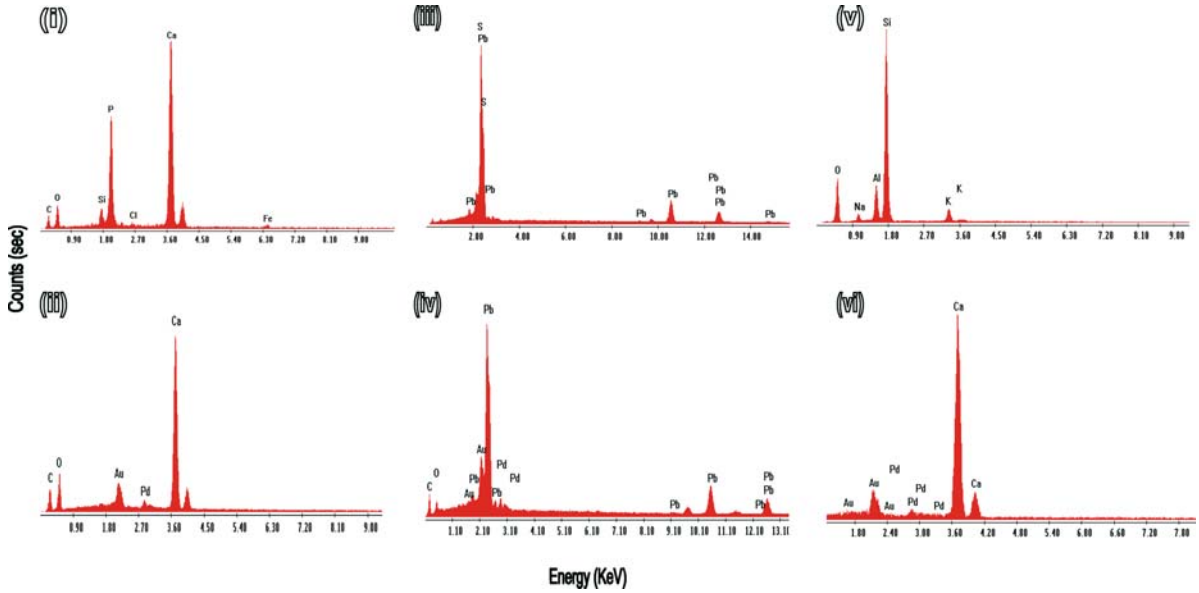

Figure 7. Typical EDXA profiles of (i) apatite, (ii) crystals produced by *A. niger*, *S. himantioides* and *T. versicolor* on apatite in agar with and without glucose; (iii) galena, (iv) crystals produced on galena by *A. niger*, *S. himantioides* and *T. versicolor* grown on glucose-amended TWA; (v) obsidian, and (vi) the crystal-like precipitates produced in the hyphae of *S. himantioides* and *T. versicolor* grown in the presence and absence of glucose with obsidian. The Au and Pd peaks in (ii, iv, and vi) are as a result of coating the minerals with 10 nm gold and palladium prior to viewing under the SEM. The profiles shown are representative of at least five replicate determinations.

with the tested parameters especially in glucose-amended TWA. The presence of glucose in the media tended to confer etching abilities on the fungi although this was not found in all cases. For example, all three fungi were able to etch the surface of apatite under conditions of glucose availability but only two – *S. himantioides* and *T. versicolor* – were able to do so in the absence of glucose. In addition, these two fungi were also able to etch galena in glucose-amended agar while none of the fungi were capable of doing so in the absence of glucose. Weathered grains of obsidian were observed with *A. niger* and *S. himantioides* grown on glucose-amended media. Increased acidity due to the presence of glucose in the media is thought to be responsible for the etching of the mineral surfaces. Acidity could arise as a result of organic acids being released during growth, the exudation of protons via the proton translocating ATPase, absorption of nutrients in exchange for protons and carbonic acid formation as a result of respiratory CO_2 production (Burgstaller & Schinner 1993). A range of organic acids – especially fumaric, malic and oxalic acids – were produced by the three fungi in different media with and without the addition of glucose. It was expected that amounts of acids produced in the presence of glucose would be greater than when absent. This was not found in all cases. The production of these acids was generally in the order malic > oxalic > fumaric although levels of oxalic acid were higher than malic acid in some cases. Despite the greater quantities of malic acid produced, precipitate formation is not known to occur with this acid. While amounts of malic acid produced tended to be greater than the amounts of oxalic acid produced with the only exceptions being *S. himantioides* in TWA with apatite and *A. niger* and *S. himantioides* in glucose-amended TWA with apatite, the production of insoluble crystals characterized as oxalates confirmed interaction with oxalic acid (Lee & Parsons 1999). Corrosion of minerals without concomitant precipitation of insoluble oxalates may imply interaction with other organic acids (in this case, malic and fumaric acids), as well as other acidification routes.

In this work, the formation of calcium and lead oxalates by the fungi on the surface of

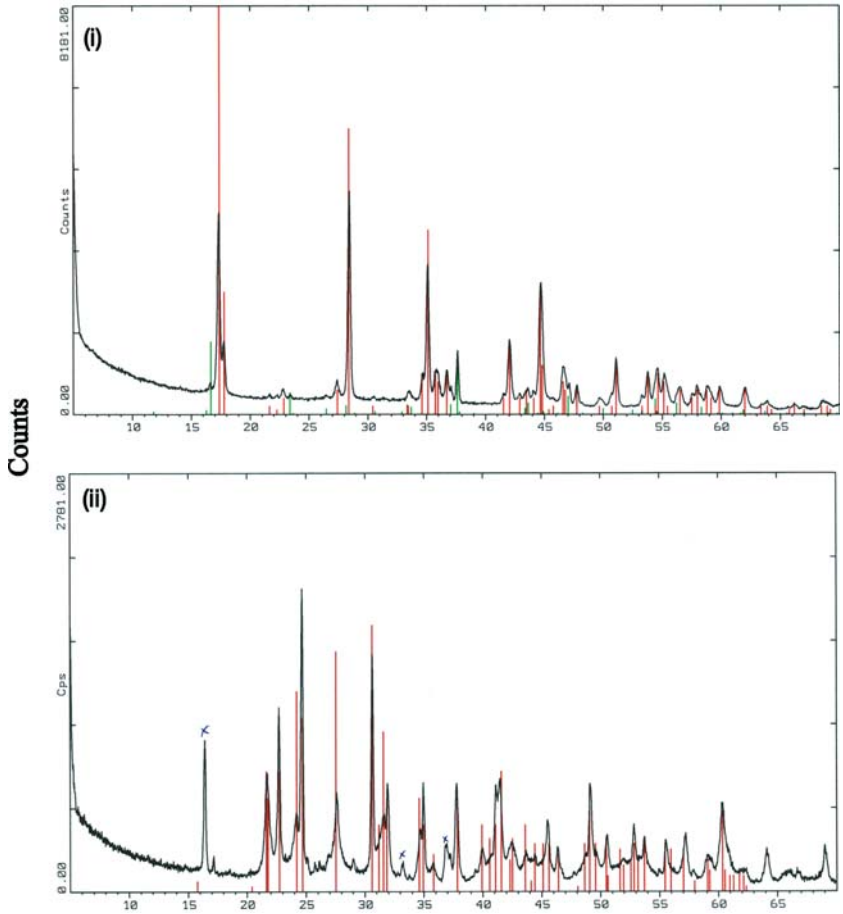

Figure 8. Typical XRPD profiles of precipitated crystals confirming (i) calcium oxalate produced from apatite and (ii) lead oxalate obtained from galena by *A. niger*, *S. himantioides* and *T. versicolor*.

apatite and galena under differing conditions occurred. The formation of oxalates is important because it represents a first step in biomineralization, i.e. the biogenic formation of new minerals (Verrecchia *et al*. 1993). Corrosion of the minerals by fungi and associated crystal formation in each instance, coupled with penetration of hyphae were only linked in glucose-amended TWA and apatite, suggesting a dual role for oxalic acid in the weathering of minerals – etching and oxalate formation. Fungal production of oxalate on apatite was partly affected by glucose availability in the medium and no crystals were produced by *A. niger* in the absence of glucose. With galena, no crystals were produced in the absence of glucose. In glucose-supplemented medium, crystals of different morphologies were observed on the rock surface and none were observed in the agar. For obsidian, the presence or absence of glucose in the medium had no effect on the abilities of *S. himantioides* and *T. versicolor* to produce crystals on the rock surface and in the agar. However, druse-like crystals were precipitated on their hyphae (Arnott 1995). Using X-ray microanalysis, these crystals were revealed to be chiefly calcium-containing. The formation of these calcium containing crystal precipitates on the hyphae of *T. versicolor* and *S. himantiodes* grown on obsidian is significant since calcium comprises less than 1% of the elemental composition (result not shown).

The fungi used for this work were capable of penetrating apatite, galena and obsidian

Table 3. Amounts of organic acids (in mM) secreted by *A. niger*, *S. himantioides* and *T. versicolor* with apatite, galena and obsidian in the absence and presence of glucose.

Rock type and nutrient state	Fungi	Acetic	Citric	Fumaric	Gluconic	Malic	Oxalic	Succinic (mM)
Apatite – glucose	*A. niger*	–	–	0.0007	–	0.67	0.01	–
	S. himantioides	–	–	–	–	0.23	0.33	–
	T. versicolor	–	–	0.004	–	0.52	0.01	–
Apatite + glucose	*A. niger*	–	–	–	–	0.64	2.21	–
	S. himantioides	–	–	0.002	–	0.59	1.91	–
	T. versicolor	–	–	0.0006	–	0.53	0.45	–
Galena – glucose	*A. niger*	–	–	0.0002	–	0.56	0.080	–
	S. himantioides	–	–	0.0005	–	0.59	0.130	–
	T. versicolor	–	–	0.0003	0.21	0.57	0.070	–
Galena + glucose	*A. niger*	–	0.19	–	0.39	0.29	0.010	–
	S. himantioides	0.01	0.57	–	–	0.64	0.540	–
	T. versicolor	–	0.64	0.001	–	0.46	0.003	–
Obsidian – glucose	*A. niger*	–	–	0.0060	–	0.77	0.0600	–
	S. himantiodes	–	–	0.0002	–	0.36	0.0004	0.02
	T. versicolor	–	–	0.0003	–	0.57	0.0100	–
Obsidian + glucose	*A. niger*	–	–	–	0.70	1.06	0.0100	–
	S. himantiodes	–	0.03	0.0035	0.15	0.30	–	–
	T. versicolor	–	–	0.0010	0.91	0.79	0.0040	–

The figures shown are mean values of three replicates and were calculated by comparing the values derived with the retention times of the different organic acid standards of known concentrations.

although under different conditions. With hyphal penetration into the minerals, the addition of glucose in the media appeared to influence positively the penetrating abilities of the fungi as the hyphae of all three fungi were observed within the different minerals in glucose-amended media. With galena and obsidian, only hyphae of *A. niger* and *T. versicolor*; and *S. himantioides* and *T. versicolor* were observed, respectively. What is unclear about the observations of hyphae in the minerals is whether they actively penetrated the minerals themselves or were just merely growing into pre-existing pores or cracks formed through rock mineral interactions with prior abiotic weathering processes (such as freeze-thaw cycles, etc.) or by the exudation of organic acids from hyphal tips of fungi (Jongmans *et al*. 1997). In summary, what has been shown through this work is that fungal-driven geochemical change(s) might occur under nutrient-limited conditions. Such changes can affect soil fertility through release of nutrients such as calcium and phosphates from minerals for, e.g. plant use, or immobilization of potentially toxic species (e.g. lead) in soil.

Table 4. Hyphae of fungi observed in the different minerals in the presence and absence of glucose.

Rock mineral	Agar type	
	Glucose-amended TWA	TWA
Apatite	*A. niger, S. himantioides* *T. versicolor*	*A. niger,* *S. himantioides* *T. versicolor*
Galena	*A. niger, S. himantioides* *T. versicolor*	*A. niger,* *T. versicolor*
Obsidian	*A. niger, S. himantioides* *T. versicolor*	*S. himantioides,* *T. versicolor*

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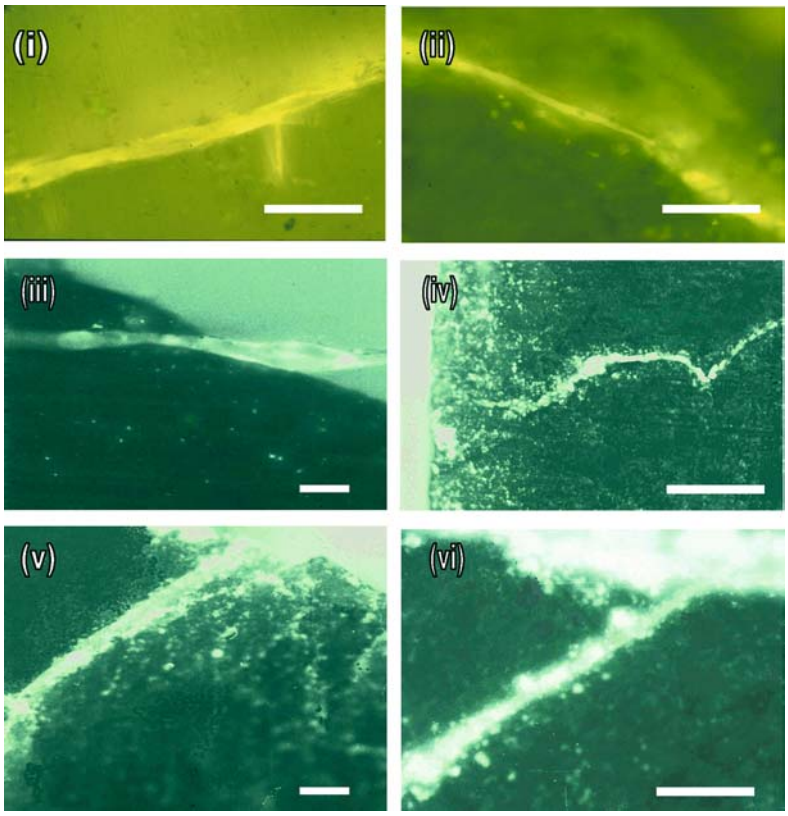

Figure 9. Hyphae of (i) *A. niger* in apatite in the presence of glucose, (ii) *S. himantioides* in apatite in the absence of glucose, (iii) *S. himantioides* in galena in the presence of glucose, (iv) *T. versicolor* in galena in the absence of glucose, (v) *T. versicolor* in obsidian in the presence of glucose and (vi) *S. himantioides* in obsidian in the absence of glucose. (Bar = 10 μm).

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