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Effects of concrete properties and nutrients on fungal colonization and fouling

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ABSTRACT

This study describes the fouling of concrete surfaces by diverse fungal genera under controlled laboratory conditions. A circulating flow-through chamber was designed for testing the effects of different concrete compositions and exogenously added nutrients on fungal colonization and fouling. Fungal strains belonging to the genera *Alternaria*, *Cladosporium*, *Epicoccum*, *Fusarium*, *Mucor*, *Penicillium*, *Pestalotiopsis*, and *Trichoderma* were cultured directly from visibly fouled concrete structures and used individually and in a mix to inoculate mortar tiles varying in cement composition, supplementary cementitious material additions, water-to-cement ratio, and surface roughness. A strong positive relationship was observed between tile water-to-cement ratio and the amount of biofouling. In addition, cement containing photocatalytic titanium dioxide and exposed to artificial sunlight strongly inhibited fungal colonization and fouling. Mortar tiles coated with form-release oil and incubated with sterile rainwater were also capable of supporting fungal colonization. Our results indicate that the fouling of concrete surfaces by fungi can be influenced by variations in concrete composition variations and available nutrients.

1. Introduction

The fouling of concrete and stone surfaces by microorganisms can have deleterious effects on structural integrity and aesthetic appeal (Gaylarde and Morton, 1999; Dubosc et al., 2001). The deteriogenic biofilms inhabiting these surfaces can expedite the destruction of historic structures resulting in the loss of national symbols of cultural heritage (Warscheid and Braams, 2000). Similarly, biologically mediated corrosion of infrastructure, including buildings, highways, and sewers represents a significant economic loss in the form of maintenance and repair costs (Gu et al., 1998). Exposure to biofilms colonizing buildings is also of concern for public health (Gaylarde and Morton, 1999; Shirakawa et al., 2003). For these reasons, it is important to understand the composition and diversity of these microbial communities, and to begin developing strategies to mitigate colonization.

While diverse groups of bacteria and fungi are capable of fouling concrete surfaces, the discoloration of structures is thought to be largely due to fungi (Gaylarde and Morton, 1999). During growth, some fungi produce pigments (e.g. melanins), which are postulated to cause the dark staining patterns characteristic of concrete biofouling (Sterflinger, 2000; Warscheid and Braams, 2000). Numerous fungi have been implicated in biofouling, including *Cladosporium* spp. on the interior mortars of buildings (Shirakawa et al., 2003), and the genera *Alternaria*, *Aspergillus*, *Epicoccum*, *Mucor*, *Penicillium*, and *Trichoderma* on other concrete and stone surfaces (Gaylarde and Morton, 1997; Mitchell and Gu, 2000). Fungi are often considered to be secondary colonizers that succeed autotrophic primary communities (Gaylarde and Morton, 1999; Warscheid and Braams, 2000). However, heterotrophic fungi may also be capable of primary colonization if suitable exogenous carbon sources are available in dust, rainwater runoff, or vehicular air pollution (Warscheid et al., 1991; Warscheid and Braams, 2000). Some fungi may also be able to utilize carbon derived from surface coatings, including paint (Shirakawa et al., 2002; Gaylarde and Gaylarde, 2005). In addition to the visible discoloration, fungi can also have deteriogenic effects on concrete and stone surfaces (Gu et al., 1998; Gaylarde and Morton, 1999). A number of genera including *Alternaria*, *Fusarium*, *Penicillium*, and *Trichoderma* are known to produce organic acids such as acetic, glucuronic and oxalic acid as a byproduct of metabolism (Sterflinger, 2000). The excretion of these biogenic acids can damage concrete through the formation of insoluble calcium complexes, which precipitate from the structure, resulting in weight loss and increased permeability and porosity (Gu et al., 1998). The fungi *Fusarium* sp. and *Penicillium frequentans* are reported to deteriorate concrete and stone, respectively, through this process. In addition to organic acid production, fungi such as *Fusarium* sp. can damage concrete and stone by physical means, particularly through the penetration of hyphae into the concrete surface (Gaylarde and Morton, 1997; Gu et al., 1998).

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Concrete has a number of properties that can be utilized as measures of the overall quality (Mehta and Monteiro, 2006). These characteristics, including compressive strength, permeability, moisture content, porosity, and surface roughness, as well as cement composition, addition of supplementary cementitious materials (SCMs), and water-to-cement ratio (*w*/cm), are interrelated and postulated to influence a surface's susceptibility to biofouling (Guillitte and Dreesen, 1995; Dubosc et al., 2001; Shirakawa et al., 2003; Pinheiro and Silva, 2004; Mehta and Monteiro, 2006). For example, concrete with a lower *w*/cm has been reported to be less susceptible to fouling by algae (Dubosc et al., 2001). In addition, greater surface roughness and capillary porosity have been observed to increase concrete's bioreceptivity to filamentous algae as well as *Cladosporium sphaerospermum* (Guillitte and Dreesen, 1995; Pinheiro and Silva, 2004). Cements or coatings containing photocatalytic substances such as titanium dioxide (TiO_2) can drive the oxidation of organic compounds on the surfaces of outdoor structures exposed to sunlight (Blob and Elfenthal, 2007; Cassar et al., 2007). This 'self-cleaning' capability may help to prevent or severely retard concrete surface biofouling, although the mitigation of fungal growth on photocatalytic concrete surfaces has not been previously examined.

Laboratory-controlled biofouling tests can be performed to evaluate concrete surface colonization, biological or chemical degradation, and material bioreceptivity (Guillitte and Dreesen, 1995; Gu et al., 1998; Dubosc et al., 2001; Shirakawa et al., 2003). There have been a number of variations that simulate the *in vitro* biofouling of concrete surfaces. To understand the colonization and corrosion of concrete sewers, Gu et al. (1998) constructed a miniaturized concrete pipe system from which a concrete specimen was hung and sprayed with culture medium inoculated with *Thiobacillus intermedius* or a *Fusarium* sp. The bioreceptivity of mortar to colonization by *C. sphaerospermum* has been tested by drying media onto a mortar cube, inoculating with the fungal isolate, and suspending the specimen in a chamber at 100% relative humidity (Shirakawa et al., 2003). Concrete and other building materials with varied *w*/cm, surface roughnesses, and porosities have been tested for their susceptibility to algal and cyanobacterial colonization, using incubation systems that sprinkle and recycle the biological inoculum onto test specimens (Guillitte and Dreesen, 1995; Dubosc et al., 2001).

In this study, we examine the ability of diverse fungal genera to colonize and discolor concrete surfaces under controlled laboratory conditions testing a variety of concrete and nutrient parameters. Specifically, fungal isolates were tested for their ability to foul an array of concrete compositions, which varied in cement composition (including the examination of photocatalytic cement), water-to-cement ratio, addition of SCMs, and surface finishes. Additionally, fungal isolates were tested for their ability to foul concrete under settings that designed to more closely resemble *in situ* field conditions, where nutrients were provided through sources such as rainwater, form-release oil, or vehicular pollutants. To our knowledge, this is one of the first studies to examine both the effects of a wide array of concrete characteristics and nutrient amendments on biofouling caused by different fungal populations.

2. Materials and methods

2.1. Site description and culturing

Four outdoor concrete sites located throughout the state of Georgia, USA (Atlanta, 33°48′N, 84°22′W; Gainesville, 34°15′N, 83°57′W; LaGrange, 33°03′N, 84°57′W; Savannah, 32°13′N, 81°37′W) were sampled in this study. All four of the study sites had visibly fouled surface characteristics, i.e., black crusts on the concrete appearing as either vertical striations or covering the entire surface (Giannantonio et al., 2008). Sampling was performed as previously described (Giannantonio et al., 2008). Agar strips (5 cm × 1 cm) composed of potato dextrose agar were aseptically pressed against the concrete surfaces. Strips were then placed on solid media and grown at room temperature for 7 days. Isolates were repeatedly sub-cultured to fresh media until pure strains were obtained.

2.2. Fungal identification

Cultures were identified morphologically by microscopy and molecularly by analysis of the internal transcribed spacer (ITS) region rRNA genes as previously described in Giannantonio et al. (2008). DNA was extracted from fungal biomass using an UltraClean™ Soil DNA Isolation Kit (Mo Bio Laboratories, Inc.) according to manufacturer's instructions. The ITS1, 5.8S, and ITS2 regions of fungal rRNA genes were amplified using primers ITS1 and ITS4 (White et al., 1990) and PCR conditions previously described (Michaelsen et al., 2006). Purified amplicons were sequenced at the Georgia Institute of Technology Genome Center using a BigDye Terminator v3.1 Cycle sequencing kit on an automated capillary sequencer (model 3100 Gene Analyzer, Applied Biosystems). Sequences were identified using Basic Local Alignment Search Tool (BLAST) against the GenBank database provided by the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/).

2.3. Mortar tile compositions

In order to determine the effects that different concrete properties had on susceptibility to biofouling, mortar tiles (6 × 6 × 0.4 cm) of varying compositions were constructed. Mortar (i.e., mixes with only sand fine aggregate), instead of concrete (i.e., mixes with fine and coarse aggregate), was used as it was more conducive for the casting of small tiles used in this study. Cement paste was mixed with a sand fine aggregate, cast into tiles against flat, galvanized steel using a plastic framework, and cured in limewater at 25 °C for 28 days, during which time varying surface finishes were applied as necessary (i.e., for brushed surface finish was applied before curing, for polished 120 grit after 1 day of curing, and for 600 grit after 2 days of curing). Following curing, tiles were carbonated in a chamber (Nuaire US Autoflow NU-4850) at 20% CO_2, 40 °C, and 55% relative humidity for 30 days to reduce their surface pH to that of field conditions (i.e., pH less than 9). The pH was confirmed by spraying a phenolphthalein indicator solution on the mortar tile surfaces. Tiles were sterilized by autoclaving prior to fungal inoculations.

A list of the mortar tile mixes, which varied in cement composition, water-to-cement ratio, addition of SCMs, and surface finishes, is shown in Table 1. A standard mortar mix was created using an American Society for Testing and Materials (ASTM) Type I/II General Use (GU) cement (ASTM, 2007b) provided by Holcim, a water-to-cement ratio of 0.5, no SCM additions, and a brushed surface finish (mortar mix A; Table 1). The composition and properties of this mortar mix are similar to mixes commonly used in Georgia Department of Transportation (GDOT) construction projects (GDOT, 2001). In addition to the standard cement chosen for analysis, we also tested a commercially available GU Type I/II cement interground with 5% limestone provided by Holcim (mortar mix B; Table 1). Also examined were a Type I/II cement provided by Essroc Co. (mortar mix C; Table 1), and Essroc Type I/II doped with a small percentage of nano-anatase TiO_2 (mortar mix D; Table 1) designed to impart photocatalytic properties to UV-exposed surfaces. All cements were predominately composed of CaO (60–64%) and SiO_2 (19–20%), with smaller amounts of Al_2O_3 (5–6%), Fe_2O_3 (2–4%), and carbon and other impurities (1–4%), which was

Table 1
Compositions of mortar mixes used in fouling tests.

Parameter examined	Mix variation	Mix ID
Standard mix:	Holcim GU I/II cement 0.5 water-to-cement ratio no SCM addition brushed surface	A
Cement composition:	Holcim GU + limestone	B
	Essroc I/II	C
	Essroc I/II + TiO_2	D
Water-to-cement ratio:	0.3	E
	0.4	F
	0.6	G
SCM addition:	fly ash (10%)	H
	fly ash (18%)	I
	fly ash (25%)	J
	slag (10%)	K
	slag (25%)	L
	slag (50%)	M
	silica fume (5%)	N
	silica fume (10%)	O
	silica fume (15%)	P
	metakaolin (8%)	Q
Surface finish:	polished (120 grit)	R
	polished (600 grit)	S

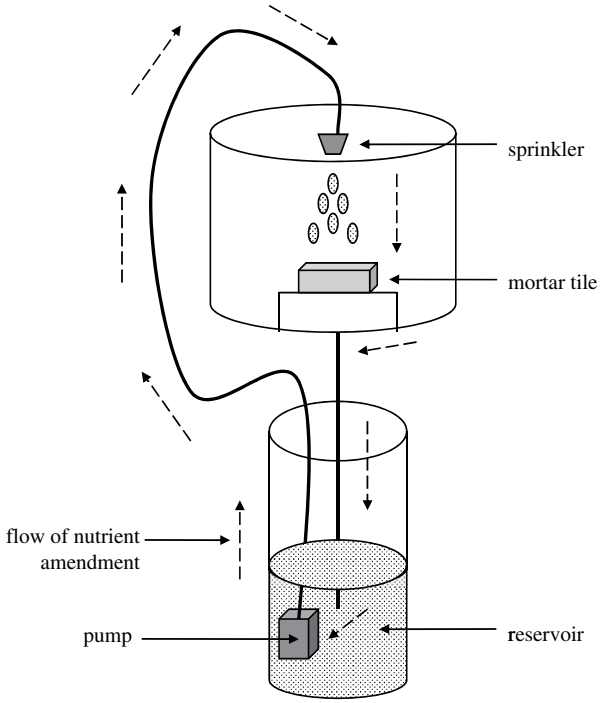

Fig. 1. Schematic diagram of the test chamber used in this study. Nutrient amendments were introduced over inoculated tiles as shown and runoff was drained into a reservoir, where it was recirculated through the sprinkler system (per directions of arrows).

determined by oxide analysis and loss on ignition (Kurth, 2008) as per ASTM standards (ASTM, 2007a,c).

Various water-to-cement ratios were tested for their effect on biofouling. In addition to the standard mortar mix with a *w*/cm of 0.50, mixes with ratios of 0.30, 0.40, and 0.60 were chosen for examination (mortar mixes E–G; Table 1). The effect of surface roughness on biofouling was examined by testing three surface finishes, including a standard brush finish (mortar mix A; Table 1), a 120-grit paper polished finish (mortar mix R; Table 1), and a 600-grit paper polished finish (mortar mix S; Table 1). Surface roughness was quantified using a Leica SP-1 Confocal Microscope to generate a roughness number as previously described (Kurtis et al., 2003; Chinga et al., 2007).

A number of SCMs typically used in Georgia concrete construction projects (GDOT, 2001) were also examined. These included Class C fly ash (Holcim), blast furnace slag (Holcim), silica fume (W.R. Grace Force 10000D), and metakaolin (Thiele) added to mortar mixes at different replacement percentages by mass (mortar mixes H–Q; Table 1). Oxide analysis and loss on ignition were performed to determine the composition and carbon content, respectively, of each SCM used in this study (Kurth, 2008). Fly ash was composed mainly of SiO_2 (35%), CaO (25%), and Al_2O_3 (18%), with small amounts of Fe_2O_3 (7%) and MgO (6%). Blast furnace slag contained mostly SiO_2 (38%), CaO (36%), MgO (18%), and Al_2O_3 (8%). Silica fume was almost entirely composed of SiO_2 (97%), and metakaolin was predominately made up of SiO_2 (52%) and Al_2O_3 (44%). All SCMs had relatively small amounts of carbon and other impurities (0–1%).

2.4. Biofouling incubations

In order to investigate the biofouling of concrete surfaces under controlled laboratory conditions, incubation chambers were designed as previously described (Giannantonio et al., 2008). Chambers distributed nutrient amendments over inoculated mortar tiles using a sprinkler; runoff was collected in a reservoir and then recirculated (Fig. 1). Prior to experiments, incubation chambers were sterilized by flushing with 10% (v/v) bleach solution followed by sterile water, each for 24 h. Sterile mortar tiles were placed into incubation chambers and inoculated with 100 μl aliquots of each fungal isolate suspended in 0.85% saline at a density of 10^6–10^7 cells (or spores) per ml. Incubations were run at 25 °C at 95–100% relative humidity as 300 ml of liquid nutrient amendment was sprinkled on tiles over 6-h on/off time intervals.

Incubations examining variations in fungal strains, mortar mixes, and nutrient amendments were performed. First, isolates cultured from sample sites were individually tested for their ability to foul tiles cast from the standard mortar mix (mortar mix A; Table 1). Next, incubations using mixed fungal communities were performed to test the biofouling susceptibility of the array of mortar mixes used in this study. These tiles were inoculated with equal cell densities (i.e., 10^6 cells per ml each) of different fungal cultured isolates organized by sample site origin (i.e., Atlanta, Gainesville, LaGrange, Savannah). In addition, the type strain *Trichoderma viride* 52438 obtained from the American Type Culture Collection was inoculated onto multiple mortar mixes in order to compare mixed community and single strain incubations. These incubations were run for one week under dark conditions using a 20% potato dextrose broth (PDB) nutrient amendment to provide sufficient time for any potential colonization to occur.

Incubations testing nutrient amendments that more closely simulated field conditions were also performed. Each tile composition was inoculated as described above with suspensions of all isolates cultured from the field. Nutrient amendments of sterile deionized water and sterilized rainwater (collected in the city of Atlanta) were tested. Also, sterile rainwater incubations were performed with tiles coated with a DuoGuard form-release agent composed of fuel oil (diesel-based) and petroleum oil-base stock (W.R. Meadows, Inc.) immediately prior to inoculation. An additional nutrient amendment tested consisted of sterilized rainwater doped with particulates derived from vehicular exhaust. Particulates were leached from a five-year-old diesel truck air filter submerged into sterilized rainwater shaking (24 h, 200 rpm). All field-simulating nutrient amendment incubations were run in dark conditions for one month to provide sufficient time for any potential colonization to occur.

The ability of cultured isolates to foul mortars containing photocatalytic cement was also examined. A mix of suspensions of each cell densities (as described above) from all field-cultured isolates was inoculated onto mortar tiles composed of Essroc Type I/II cement either containing (mortar mix D; Table 1) or not containing (mortar mix C; Table 1) photocatalytic TiO_2. During the one week incubations, tiles were exposed to 6-h cycles of artificial sunlight at approximately 10 W/m^2 near-UV irradiation generated by Ultra-vitalux Daylight lamps (Osram), which overlapped for 3 h with sprinkler cycles of 20% PDB, so that incubations experienced equal time under light/dark and rain/non-rain conditions.

2.5. Quantification and analysis of biofouling

A number of analyses were performed to quantify the biofouling coverage observed on mortar tiles and correlate coverage to the variables tested. To quantify the fouling observed, a flatbed scanner with 1200 dpi resolution was used to image tiles 48 h following inoculation. Images obtained were visually partitioned into 4 × 4 grids. Each image fragment was analyzed by confirming the presence of growth. Specifically, three independent observations identified any growth apparent in each image fragment. These data were compiled and used to calculate the coverage of biofouling for each tile as a percentage of surface area. Further details on this procedure can be found in Kurth (2008). To determine any relationships between the amount of biofouling observed and the incubation variables tested, multiple linear regression using the econometric software Limdep 7.0 was performed. Measurements were fit to linear models, and R^2values for closeness of fit were obtained. In the case of categorical variables (e.g. cement composition), *P*-values were obtained using a standard *T*-test to determine significant differences in biofouling. Selected mortar tiles from biofouling experiments were imaged on an FEI Quanta 200 environmental scanning electron microscope (ESEM) at the Tennessee Technological University (Cookeville, TN). Samples were examined in an 85% relative humidity atmosphere at stage temperature 5 °C and 733.3 Pa. Samples containing abundant growth were gold-sputtered and viewed in high vacuum mode to maintain adherence for high-resolution imaging.

3. Results

3.1. Biofouling by individual fungal isolates

The eight fungal isolates cultured from concrete sample sites (Table 2) were all able to successfully colonize the surfaces of standard mortar mix A tiles when provided with a nutrient-rich source (i.e., 20% PDB) (Fig. 2). Many these isolates (e.g. *Alternaria*, *Cladosporium*, *Epicoccum*, *Fusarium*) have been previously implicated in the fouling of concrete and stone surfaces (Gaylarde and Morton, 1997; Mitchell and Gu, 2000; Shirakawa et al., 2003). Tiles inoculated with *Alternaria* sp. (Fig. 2A), *Cladosporium cladosporioides* (Fig. 2B), and *Epicoccum nigrum* (Fig. 2C) isolates exhibited similar discoloration to that recently observed in the field (Giannantonio et al., 2008). Standard tiles inoculated with *Fusarium* sp., *Mucor* sp., *Penicillium oxalicum*, and *Trichoderma asperellum* isolates exhibited robust fouling that ranged in color from green to dark grey evident within the one week incubation (Fig. 2D–G). The tile inoculated with the *Pestalotiopsis maculans* isolate exhibited light beige fouling that nearly covered the entire tile surface (Fig. 2H). The standard mortar tile which served as a negative control exhibited no discoloration (Fig. 2I).

Table 2
List of isolates cultured from sample sites.

Isolate	% Similarity	Site acquired
Alternaria sp.	100	Atlanta
Cladosporium cladosporioides	99	Atlanta, LaGrange
Epicoccum nigrum	100	Gainesville
Fusarium sp.	99	Savannah
Mucor sp.	99	LaGrange, Savannah
Penicillium oxalicum	100	Gainesville, Savannah
Pestalotiopsis maculans	100	Atlanta
Trichoderma asperellum	100	Atlanta, Gainesville, LaGrange

3.2. Effects of mortar tile variations on biofouling

The array of mortar mix tiles inoculated with a mixture of fungal isolates derived from each of the four sites (Atlanta, Gainesville, LaGrange, and Savannah; Table 2), or the *T. viride* type strain exhibited a variety of fouling patterns when provided with 20% PDB (Fig. 3). Fouling by mixed fungal communities generally appeared as dark patches of tan, grey, and black discoloration, which, as expected, was visually different than tiles inoculated with individual isolates (Figs. 2 and 3A–E). ESEM images of selected tiles inoculated with mixed fungal communities or *T. viride* revealed fungal structures such as hyphae present on the surfaces (Fig. 3F–J).

The amount of fouling observed on mortar tiles by mixed fungal communities and *T. viride* incubations were compared to the various cement compositions used in this study. Analysis revealed no significant differences when comparing fouling on the standard cement in mortar mix A to the other cements in mixes B–D when incubated in the dark (data not shown). The addition of SCMs at different replacement percentages by weight was also tested for its effect on biofouling. Similar to primary cement variations, the addition of fly ash, slag, silica fume, or metakaolin did not significantly affect biofouling in the mixed fungal community or *T. viride* incubations (data not shown). Similarly, when the effect of surface roughness on fungal biofouling was examined through the use of three different surface finishes, mixed fungal community and *T. viride* incubations did not reveal a statistically significant relationship (data not shown).

When the amount of fouling observed was compared to different water-to-cement ratio variations, however, a strong positive trend ($R^2 = 0.98$) was revealed when a mixed fungal community and *T. viride* incubations were incorporated into a linear regression model (Fig. 4). The strongest positive trends were exhibited in incubations with fungi cultured from Atlanta ($R^2 = 0.96$), Gainesville ($R^2 = 0.97$), and *T. viride* ($R^2 = 0.95$), though positive trends were also present in incubations with fungi cultured from LaGrange ($R^2 = 0.65$) and Savannah ($R^2 = 0.79$) (Fig. 4).

3.3. Effects of nutrient amendments simulating field conditions on biofouling

Several nutrient amendments that were designed to represent natural field conditions were tested for their ability to induce biofouling. All mortar tile types were inoculated with a mixed fungal community composed of all eight isolates cultured from the four test sites (Table 2). Incubations using sterile deionized water, sterilized rainwater, and sterilized rainwater exposed to vehicular exhaust did not successfully induce fouling by the mixed fungal community. However, an incubation of tiles coated with form-release oil and provided with sterilized rainwater was able to produce a small amount of biofouling on one mortar mix D tile (Fig. 5). This fouling consisted of dark rings of discoloration on the tile surface (Fig. 5A), which upon closer examination was found to consist of black, conidia-like structures (Fig. 5B). ESEM imaging revealed a meshwork of hyphae-like filaments along the tile's surface (Fig. 5C). Though attempts to re-culture the small amount of fouling to solid media for conclusive identification were

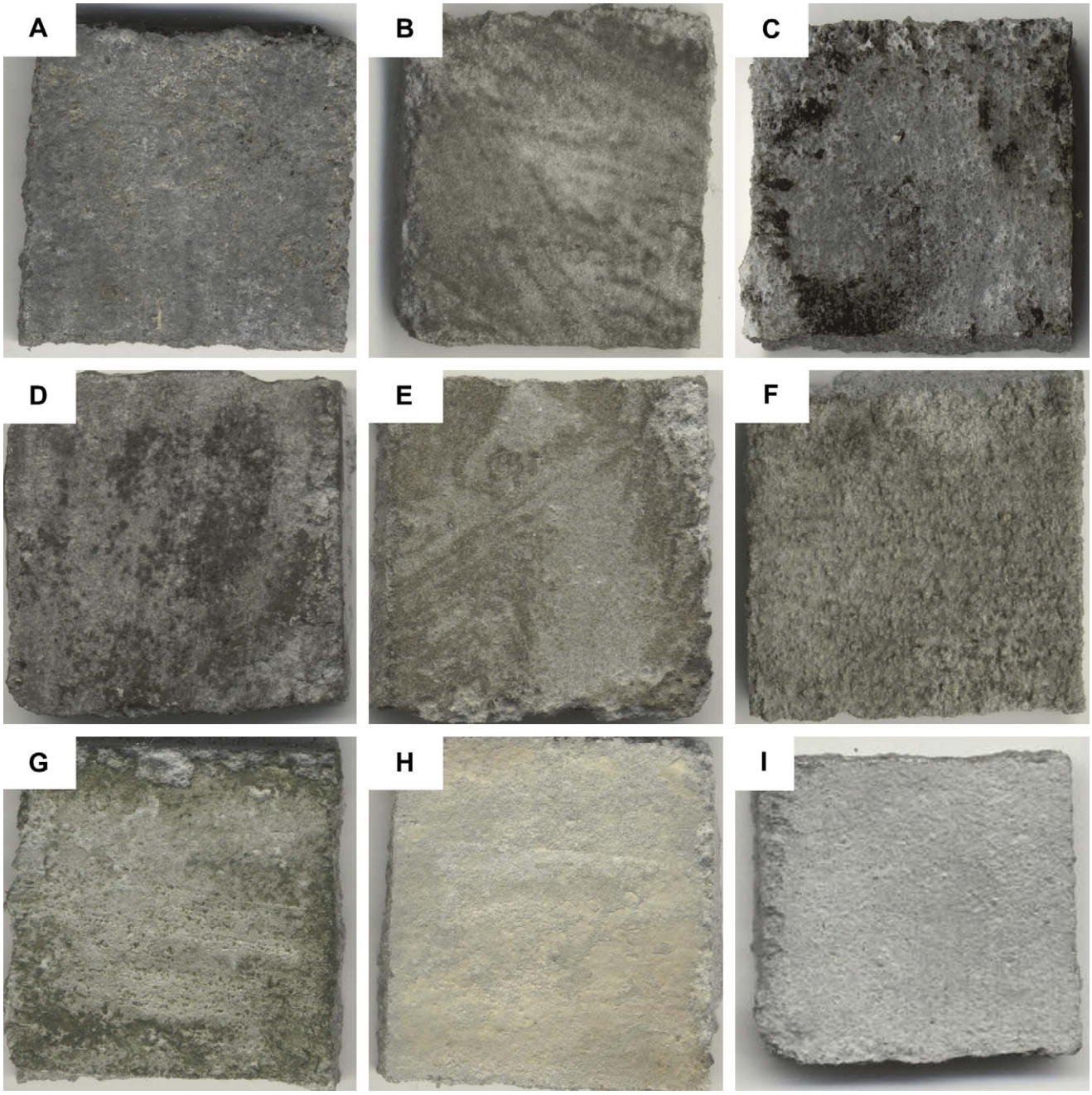

Fig. 2. Biofouling of standard mortar tiles by individual fungal isolates: (A) *Alternaria* sp.; (B) *Cladosporium cladosporioides*; (C) *Epicoccum nigrum*; (D) *Fusarium* sp.; (E) *Mucor* sp.; (F) *Penicillium oxalicum*; (G) *Trichoderma asperellum*; (H) *Pestalotiopsis maculans*; (I) negative, uninoculated abiotic control.

unsuccessful, the black, crust-like fouling resembled that observed in *E. nigrum* incubations.

3.4. Effects of photocatalytic cement on biofouling

To test the antimicrobial activity of photocatalytically active cement, a mixed community consisting of all eight fungal isolates (Table 2) was inoculated onto tiles composed of cement either containing TiO_2 (mortar mix D; Table 1) or controls without TiO_2 (mortar mix C; Table 1). These incubations used 20% PDB and included cycles of artificial sunlight exposure. Incubations using non-photocatalytic tiles exposed to the light source exhibited a moderate amount of black, tan, and red fouling (Fig. 6A). However, tiles containing photocatalytic TiO_2 exhibited only a small amount of a single type of tan-colored fouling (Fig. 6B and C). The reduction in biofouling observed was found to be statistically significant ($P = 0.05$). ESEM imaging revealed hyphae-like structures attached to the surface of the tile (Fig. 6D). This tan-colored fouling was re-cultured onto solid media and identified as the *T. asperellum* isolate, which was cultured from three of the four study sites (Table 2).

4. Discussion

The biofouling of concrete and stone surfaces can damage both the appearance and functionality of structures such as bridges, walls, and monuments. The production of pigments such as melanins by fouling fungal communities can cause severe discoloration of the structures they inhabit (Gaylarde and Morton, 1999;

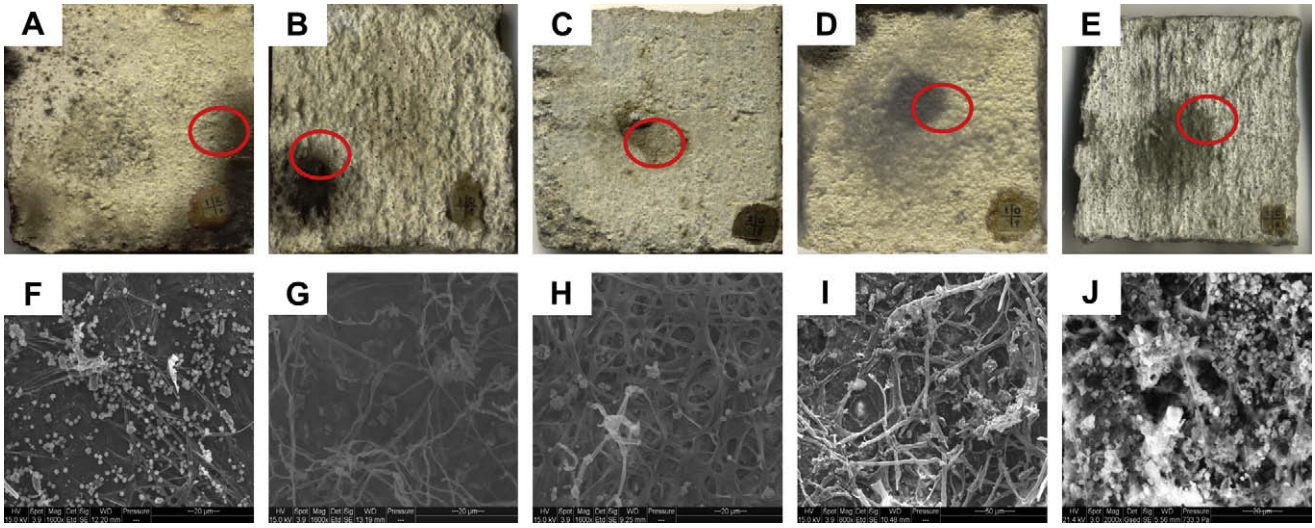

Fig. 3. Examples of biofouling by site-specific mixed fungal community and *T. viride* type strain incubations: (A) mortar mix D tile fouled by Atlanta-derived mixed fungal community; (B) mortar mix G tile fouled by Gainesville-derived mixed fungal community; (C) mortar mix P tile fouled by LaGrange-derived mixed fungal community; (D) mortar mix B tile fouled by Savannah-derived mixed fungal community; (E) mortar mix A tile fouled by *T. viride*; and (F–J) corresponding ESEM images. Images were taken from the edges of the observed growth patterns.

Warscheid and Braams, 2000). Additionally, fungi can damage these structures through the production of biogenic acids and surface penetration of fungal hyphae (De La Torre et al., 1993; Gu et al., 1998). A greater understanding of the biological, physical and chemical factors that promote and affect the colonization and fouling by naturally occurring fungal communities is needed in order to formulate a strategy for mitigation.

This study, to our knowledge, is one of the first to examine the ability of fungal isolates cultured from the field to foul an array of mortar surfaces varying in composition under controlled-laboratory settings, testing both nutrient-rich conditions and amendments designed to simulate *in situ* field conditions. Diverse genera of fungi were able to foul mortar tiles when provided with a nutrient-rich source, i.e., 20% PDB. The appearance of fouling varied between individual isolate incubations and incubations of mixed fungal communities. Site-derived mixed fungal community incubations often exhibited dark patches of fouling that were different from any individual isolate, suggesting that the visual appearance of biofouling is influenced by the composition of the endogenous fungal community. The fouling observed also suggests, perhaps not surprisingly, that fungal isolates can exhibit different morphologies (and pigmentation patterns) under varying incubation conditions. This was best exemplified by the *T. asperellum* isolate, which exhibited green fouling on tiles incubated in the dark, and tan fouling on tiles when exposed to artificial sunlight.

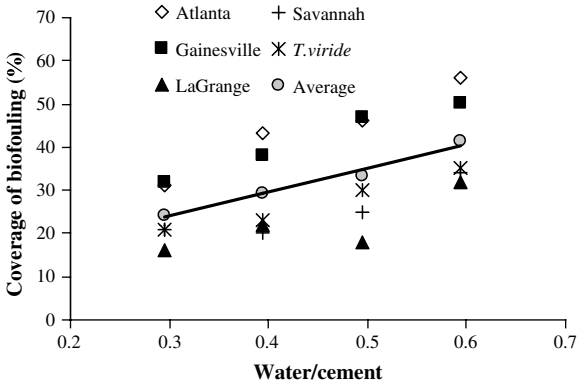

Fig. 4. Effect of water-to-cement ratio on biofouling of mortar tiles. A strong positive trend ($R^2 = 0.98$) was observed for the average of all incubations. Atlanta ($R^2 = 0.96$), Gainesville ($R^2 = 0.97$), and *T. viride* ($R^2 = 0.95$) incubations exhibited stronger trends than LaGrange ($R^2 = 0.65$) and Savannah ($R^2 = 0.79$) incubations.

Several mortar mix composition variables were examined for their effects on biofouling susceptibility. Of these, water-to-cement ratio variation exhibited the strongest relationship. The positive relationship observed suggests that a higher *w*/cm makes concrete more susceptible to biofouling. This may be expected, as increasing the amount of water used in cement hydration can result in greater porosity, which can decrease a structure's overall strength as well as increase permeability (Mehta and Monteiro, 2006), resulting in greater surface area and possible moisture and nutrient availability for colonizing communities. Indeed, these results agreed with previous work that linked susceptibility of biofouling by algae to greater concrete *w*/cm (Dubosc et al., 2001). The numerous effects that *w*/cm has on concrete properties as well as its strong relationship to biofouling in this study suggest that it may be a good predictor of a structure's bioreceptivity. Furthermore, water-to-cement ratio is a parameter which can by limited in design specifications, allowing for a potential means for limiting biofilm growth in practice.

While a greater surface roughness has been reported to be more conducive to biofouling by filamentous algae (Guillitte and Dreesen, 1995) as well as the fungus *C. sphaerospermum* (Pinheiro and Silva, 2004), in our study variations in surface roughness did not show a strong relationship to biofouling susceptibility. While these results suggest that surface roughness may not have a strong influence on concrete bioreceptivity, only three different surface finishes were examined in this study, two of which generated similar, smooth surfaces. Surfaces with a greater range of roughness may therefore need to be examined in order to distinguish a relationship. Differences in cement composition and SCM additions did not seem to affect the biofouling susceptibility of mortar mixes used in this study. This in part may be due to the relative similarity of the chemical composition of cement and SCM reaction products (e.g. both produce hydrous calcium silicates and hydrous calcium aluminates (Mehta and Monteiro, 2006)). In addition, though the different cements and SCMs tested contained various proportions of metals as determined by oxide analysis, none studied had particularly large or different amounts of carbon impurities as

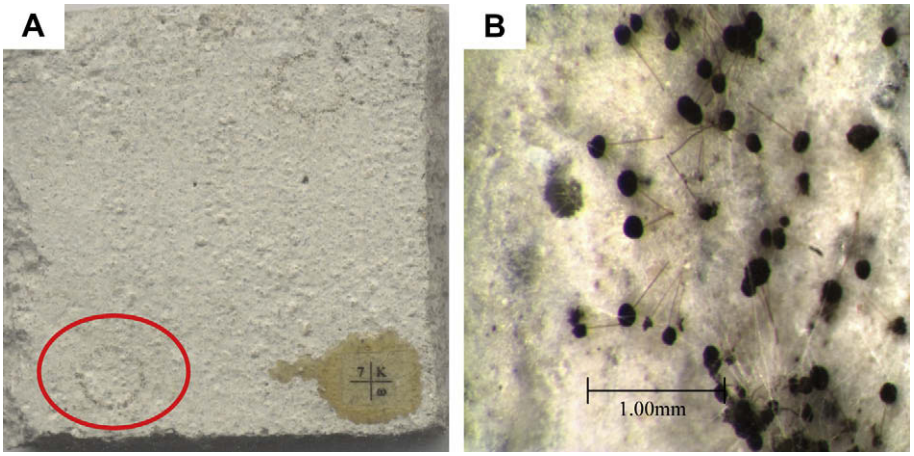

Fig. 5. Biofouling of a mortar mix D tile coated with form-release oil, inoculated with all eight fungal isolates (Table 2), and incubated with sterilized rainwater: (A) image of tile exhibiting dark "rings" of fouling, which are circled; (B) stereomicroscope image of the dark fouling; (C) ESEM image of the tile surface.

determined by loss on ignition (Kurth, 2008). Specifically, the range of SCM compositions used in this study was quite narrow, and those used were of relatively high quality, having less than 2% carbon by mass, compared to the 6% loss on ignition allowed by ASTM (ASTM, 2008). The effects on biofouling by carbon impurities in our cements and SCMs were likely muted by the high carbon content of the 20% PDB amendment used to maximize fungal colonization and fouling on test tiles. Furthermore, SCMs may slow the rate of carbonation in cement-based materials, which could potentially delay biofilm growth; this effect was not considered, as all mortar tiles were carbonated prior to testing. Thus, while the amount of biofouling was not correlated to cement composition or SCM additions in this study, it is possible that these variables could have an effect *in situ*.

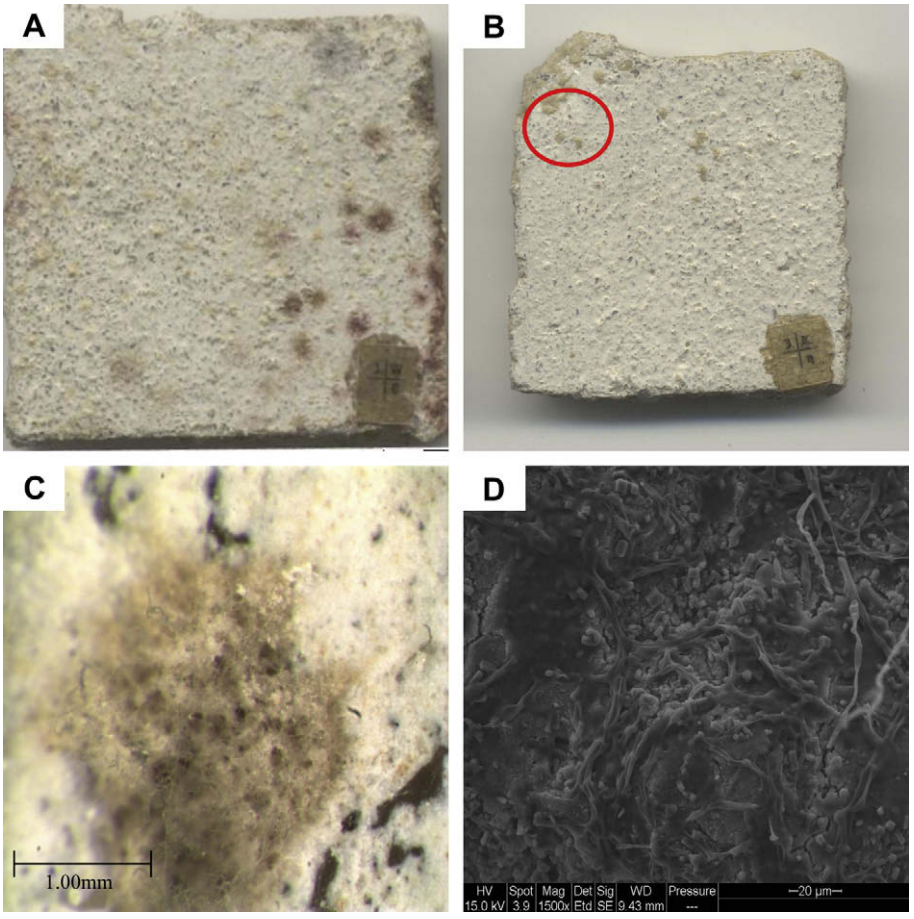

Fig. 6. Fouling of tiles inoculated with all eight fungal isolates (Table 2), incubated with 20% PDB, and exposed to artificial sunlight: (A) multi-colored fouling of a mortar mix C tile composed of non-photocatalytic cement; (B) fouling of a mortar mix D tile composed of cement containing photocatalytic TiO_2, circle indicates location of growth; (C) stereomicroscope image of tan-colored fouling on the photocatalytic tile; (D) ESEM image of fouling on the photocatalytic tile.

Photocatalytic cement under artificial sunlight exposure exhibited strong resistance to biofouling compared to the base cement of the same composition without TiO_2. The data suggest that the photocatalytic cement was capable of inhibiting biofouling when activated by artificial sunlight. These results agree with previous studies that report the photocatalytic disinfection by TiO_2 of liquid suspension of fungi (Lonnen et al., 2005; Sichel et al., 2007); though to the best of our knowledge, this is the first report of such antifungal properties tested on photocatalytic cement surfaces. However, it should be noted that the *T. asperellum* isolate was capable of surviving and being re-cultured from photocatalytic tile surfaces. This suggests that the use of TiO_2-containing cements, though effective, may not completely inhibit fungal biofouling of concrete structures.

Biofouling tests utilizing nutrient amendments designed to simulate conditions *in situ* were performed to determine if fungal biofouling could occur when nutrient sources were only available through the concrete intrinsically, rainwater, vehicular exhaust/air pollution, or carbon sources remaining on concrete from construction practices (i.e., use of form-release oil). Laboratory testing of nutrient limited conditions did not result in the robust biofouling observed when using a 20% PDB nutrient amendment. This in part may be due to the one-month duration of these incubations, as it likely takes years for an appreciable amount of biofouling to manifest *in situ*, even after surface carbonation of concrete has occurred. It is also possible that many fungal isolates require autotrophic microorganisms to be present on or initially colonize concrete surfaces if a suitable or sufficient nutrient source is not readily available. However, at least one culturable isolate, likely the *E. nigrum*, was able to establish itself on a mortar tile when given sterilized rainwater and form-release oil as a nutrient amendment, suggesting that over time, fungal isolates may indeed be able to initially colonize and foul concrete surfaces in the field if provided an exogenous nutrient source.

In summary, this study examines an array of materials and environmental factors that may influence the fungal biofouling of concrete structures. Laboratory-controlled incubations of mortar tiles inoculated with fungal isolates cultured from fouled concrete surfaces revealed that biofouling may be related to concrete *w*/cm. In addition, the use of photocatalytic cement may help to significantly mitigate and deter biofouling caused by fungi. The results from this study also suggest that it may be possible for fungal isolates to initially colonize and foul concrete structures using rainwater and form-release oil as a sole nutrient source. While more temporal studies and field examinations must be performed in order to better understand colonization, succession, energy-flow, and inhibitory influences on concrete surfaces, this study provides a foundation for identifying and testing the parameters that may have a significant effect on fouling communities.

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