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Fungal Degradation of Military Assets

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Three case histories of biodeterioration due to fungi are reviewed. Fungal accelerated deterioration has recently been documented in aircraft fuel storage tanks, painted interior surfaces of helicopters, and wire highlines stored in humid environments on wooden spools. In addition to the obvious esthetic problems requiring expensive, labor intensive amelioration, growth of fungi can cause microbiologically influenced corrosion (MIC).

microbiologically influenced corrosion, cellulosic biodeterioration, wire deterioration, polyvinyl deterioration, electrical deterioration, fungus and humid environments
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1435 Inhibition of Microbiologically Influenced Corrosion, P.M. Natishan, J. Jones-Meehan, B.J. Little, R. Ray, NRL; G.I. Loeb, N. Gray, American University; M. Beard, CDNSWC

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1600 Material Considerations for the Navy Shipboard Waste Destruction System, D.A. Shifler, C. Wong, CDNSWC
Fungal Degradation of Military Assets

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ABSTRACT

Three case histories of biodeterioration due to fungi are reviewed. Fungal accelerated deterioration has recently been documented in aircraft fuel storage tanks, painted interior surfaces of helicopters, and wire highlines stored in humid environments on wooden spools. In addition to the obvious esthetic problems requiring expensive, labor intensive amelioration, growth of fungi can cause microbiologically influenced corrosion (MIC).

INTRODUCTION

Biodeterioration due to fungi has been documented for the following: cellulosic materials (paper, composition board, and wood); communication wire; cable splices; telephone cable; cable sheaths; photographic film; polyvinyl chloride films; sonar diaphragm coatings; map coatings; paints; metals; crude oil; fuel oil; jet fuel; kerosene; greases; waxes; lubricants; adhesives; asphalt; hydraulic fluids; rain repellents; textiles (cotton and wool); vinyl jackets; leather shoes; feathers and down; natural and synthetic rubber; optical instruments; mechanical, electronic, and electric equipment (radar, radio, flight instruments, wire strain gauges, helicopter rotors); hammocks; tape; thermal insulation; brick masonry; medicines; museum valuables; concrete, and processed tobacco. Fungi are nonphotosynthetic organisms having a vegetative structure known as a mycelium that is the outgrowth of a single microscopic reproductive cell or spore. Mycelia are capable of almost indefinite growth in the presence of adequate moisture and nutrients so that fungi often reach macroscopic dimensions. Yeasts are fungi that multiply by forming buds instead of mycelia. Fungi are ubiquitous in atmospheric and aquatic environments where they assimilate organic material and produce organic acids including oxalic, lactic, acetic, and citric. Fungi produce spores, non-vegetative dormant stages, that can survive long periods of unfavorable growth conditions, e.g., drought and starvation. When conditions for growth are favorable, spores germinate. Despite the extensive data base that has been developed on biodeterioration and biocides, fungi remain a modern problem for shipment, storage, and use of military assets. The following case studies document problems in wire rope storage, jet fuel storage, and helicopter operation due to the presence and activities of fungi.

Wire Rope

One-inch carbon steel wire ropes or highlines used for transporting equipment and people between U. S. Navy ships are prepared according to a federal specification with six
individual strands around an independent wire core coated with a thick maintenance grease and wrapped onto wooden spools (Fig. 1). U. S. Navy standards further require that spools be wrapped in plastic prior to storage. Wire rope may be stored for weeks to months before being transported to U. S. Navy vessels. Any visible sign of corrosion disqualifies the rope for its intended purpose. Between May 1992 and February 1993, corrosion inspection statistics were compiled for 117 spools and compared with storage conditions. Five of fifty-eight highlines stored indoors showed some sign of corrosion whereas forty-two of the fifty-nine spools stored outdoors were unsuitable for use because of localized corrosion. Time in storage was not a factor. Further statistics were collected for highlines that had been removed from plastic packaging and were currently in use. Four of thirty-four highlines installed on winches were rusty when inspected. Fungal growth was observed on interiors of some wooden spools stored outdoors. Corrosion was most severe on wraps of wire in direct contact with the wooden spool flanges. There were numerous reports of a musty odor associated with spools when plastic packaging was removed.

Sections of wood from spool interiors and pieces of brown shipping paper from spool rim edges (Fig. 2) were removed. Wood and paper samples with fungal fruiting bodies, spores, or mycelia were placed in Petri dishes with potato dextrose agar (PDA) and incubated in a dark room at 37°C. After a 1-week incubation, fungal species were identified as Aspergillus niger and Penicillium sp. The pH of the condensate within Petri dishes and in areas adjacent to growing fungi were monitored using a microelectrode with a tip diameter of 2.4 mm vs. saturated calomel reference electrode. Condensate in Petri dishes where either isolate was grown dropped from a pH of 7.0 to 4.9 in three weeks. Coated wire rope was exposed to pH 5.0 citric acid solution. Localized corrosion (Fig. 3a, b) was observed in each location the acid solution touched the rope.

Wire rope received directly from the manufacturer was exposed on wooden tongue depressors in enamel trays containing PDA. Some trays were inoculated with fungi, covered with a clear plastic, and placed in a dark, 30°C environment. Other trays were inoculated but remained uncovered. Controls were uninoculated and covered. Wire rope in contact with wooden tongue depressors inoculated with either isolate developed localized corrosion in areas in direct contact with either the wood or the agar within four days (Fig. 4a, b). Fungi were attached directly to the wire and were associated with corrosion products in all inoculated trays (Fig. 5a, b, c). In the open, inoculated tray no additional corrosion was observed. No corrosion was observed in the abiotic, covered control. In the covered inoculated trays, corrosion developed in areas where condensate dripped onto the wire from the clear plastic in addition to areas in direct contact with wood and agar. Localized corrosion was observed on wire surfaces where the grease coating was thin or missing due to handling. In these cases, fungal cells were not associated with pitting and corrosion products. The maintenance coating is an amber-colored wax with organic corrosion inhibitors. The product, which has negligible solubility in water, was evaluated as a source of nutrients for the fungi by melting the grease in a Petri dish, allowing it to solidify, inoculating it directly with the isolates, and comparing to an abiotic control. Fungal isolates did not grow on the maintenance coating. Fungal growth could not be demonstrated on corroded rope sections not in direct contact with wood or agar.

Both Aspergillus niger and Penicillium sp. produce copious amounts of citric acid as a result of hydrocarbon degradation.2 Localized corrosion of the wire rope in direct contact
with fungal-contaminated wood may be due to either organic acid production or to initiation of corrosion nucleation sites. Citric acid solution at pH 5.0 did penetrate the protective grease and cause corrosion. Wire rope in direct contact with inoculated wood and agar was colonized by fungal mycelia and fungi were associated with corrosion products. In addition to organic acid production, fungi produce copious amounts of carbon dioxide. Carbon dioxide reacts with water to form carbonic acid. In our laboratory studies, condensates in covered, inoculated trays were in the range of pH 4.0–5.0. The acidic condensate penetrated the protective maintenance coating. Areas where the maintenance coating had been mechanically removed were particularly vulnerable to attack by acidic condensates.

Laboratory data and field statistics strongly implicate the practice of storing carbon steel highlines in humid conditions wrapped in plastic with MIC. The replacement of plastic wrapping with paper packaging and redesign of wooden spool flanges to include slats one-half inch apart to promote ventilation are currently being evaluated as corrosion control measures.

Microfungal Degradation of Polyurethane Paint and Corrosion of an Aluminum Alloy in Military Helicopters

Military helicopters operating in humid environments mildew or mold on interior surfaces (Fig. 6). Cleaning accounts for a significant amount of the labor expended in field maintenance activities. In extreme cases, fungal growth has been known to form thick masses, especially in areas of the craft not regularly cleaned in the field, such as the bilge, behind sound insulation blankets, within tight spaces in the overhead, and behind fixed equipment such as electronics racks. Growth in such areas may not be discovered until aircraft are sent for depot-level maintenance after 5 to 7 years. Occasionally, aircraft placed in storage for a short time are so badly contaminated with noxious growth that they are unusable and must be returned for costly, depot-level recleaning.

Five H-53 aircraft at various stages of depot-level maintenance, including salvage storage, were surveyed and whole portions of contaminated surfaces were sampled for microscopic examination in the laboratory. Fungal contamination was observed in all aircraft examined. To isolate and identify fungi, culture plates containing potato-dextrose agar (PDA) were inoculated by pressing the agar surface directly against contaminated surfaces of the aircraft. Plates were incubated in the laboratory at room temperature until colonies could be picked for isolation, identification and maintenance. The following nine genera of fungi were identified: Pestalotia, Trichoderma, Epicoccum, Phoma, Epicoccum, Aureobasidium, Stemphylium, Penicillium, and Hormodendrum.

Paint exposure tests were carried out using 1-inch square coupons of 2024 T-6 aluminum alloy coated with chromate primer and type 36321 polyester polyurethane paint according to Federal Specifications TT-P-2756A, MIL-P-85582 and TT-L-20A. A parallel set of coupons was prepared for comparison using lacquer coating. Coatings were scratched vertically down the center to expose bare aluminum to simulate mechanical damage that might be expected in the field. Coupon surfaces were swabbed thoroughly with 75% ethanol and dipped (with the scratch vertical) halfway into a solution of household bleach (~5.25% sodium hypochlorite). One third of the coupons was used without further treatment, while
one third of the coupons was swabbed on one side with hydraulic oil, and the remaining third was swabbed on one side with a lanolin-based preservative to simulate operating and storage conditions, respectively. Sets of three coupons (one each of alcohol, hydraulic oil, and lanolin treatments) were placed on PDA in Petri dishes with treated sides up, and agar surrounding each coupon was inoculated with fungal spores from an isolate to initiate growth of a thick fungal colony around each coupon that would either overgrow the coupon or provide a source of inoculum to the coupon surface. To further encourage growth, inocula were smeared on the center of each coupon. The following five fungal isolates were used as inocula: *Trichoderma, Aureobasidium, Stemphylium, Penicillium*, and *Hormodendrum*. The resulting experimental matrix was: 5 isolates × 2 bleaching treatments (bleached or not) × 3 surface treatments (alcohol only, oil, or lanolin). Incubation was carried out in the dark at 26°C.

Coupons were photographed to document the extent of growth over their surfaces at 4, 8, 23, and 32 days. At 32 days, coupons were examined visually with a binocular microscope and qualitatively scored for surface contamination. Cleaning efficacy was tested using a 75% ethanol-dipped swab to clean a spot on each coupon. Both swabbed and unswabbed areas were examined using environmental scanning electron microscopy (ESEM). ESEM micrographs of coupon surfaces before exposure to fungi demonstrate that the polyurethane coating was noticeably smoother (Fig. 7a) than the sharp, porous surface of the lacquer (Fig. 7b). After 30-day exposure to growing fungi, polyurethane-coated coupons exhibited no corrosion or blistering; however, blisters were observed close to the experimental scratch on lacquer-coated coupons.

Qualitative estimates of fungal growth on the surfaces of painted coupons after 30 days were made. Lacquer-coated coupons were more susceptible to fungal colonization than polyurethane-coated coupons. Of the 5 isolates tested, *Hormodendrum* was the most aggressive in its growth on both coatings; almost half of each coupon was covered after only 4 days exposure. Many fungi grow on solid surfaces given a modicum of organic material as a carbon source. During normal operation, interiors of military helicopters usually become coated with a thin film of hydraulic oil, and during storage, a lanolin-based coating is applied to interior surfaces as a preservative. Both materials are potential carbon sources, and, along with normal dirt accumulation, increase fungal growth, and exacerbate deleterious effects on surface coatings.

Fungi on polyurethane surfaces appeared well established, with mycelia and conidia (Fig. 8a), and germinating spores (Fig. 8b), but there was no obvious degradation at attachment points. Figure 9 illustrates incomplete removal of fungal structures by simulated field cleaning procedures. Hyphae were present in the experimental scratches on many coupons, and occurred in association with both occasional corrosion products in unbleached portions and thick corrosion products on the bleached portions.

Fungi isolated from these aircraft are ubiquitous, so contamination of the airframes could have occurred virtually anywhere during their duty cycle. At least one of the isolates, *Aureobasidium*, has been reported to cause superficial discoloration on latex paint. *Hormodendrum* (reported as *Cladosporium resinae*) causes corrosion of 2024-T6 aluminum alloy in the presence of water, salts, and diesel oil, a situation that might be expected in
the bilge areas of aircraft operating in a maritime environment. Aluminum alloy exposed in scratches or other mechanical damage to painted surfaces may be particularly vulnerable to corrosion exacerbated by fungal growth. In this study, although there was some spatial relationship between fungi and corrosion products in the unbleached portion of the experimental scratches, the data do not indicate a causal relationship. Corrosion in the bleached portion of the experimental scratches was due to oxidation by the bleach solution. In addition to exposing aluminum, a broken paint surface may be susceptible to additional failure at the paint-substratum interface, as illustrated by blisters observed in lacquer paint near the experimental scratch. The data cannot support a conclusion of a causal relationship between blisters and fungi, although the spatial relationship suggests an interaction.

The lower colonization rates for polyurethane compared to lacquer suggest this coating was less susceptible to initiation of fungal growth. In this short experiment, no surface degradation of polyurethane coatings was observed microscopically where fungal hyphae were attached. Simulated field cleaning/disinfectant procedures using 75% ethanol physically removed most fungal structures from paint surfaces; the viability of the few remaining fungal structures was not assessed. Operationally, short term exposure of polyurethane paints to fungi (on the order of weeks) appears to result in no physical damage to the coating surface, and alcohol cleaning appears to be effective. Prolonged or multiple exposures to fungal contamination may have a cumulative effect on coating performance. Performance also may be a function of substratum, as indicated by the observation that polyurethane paint disbonded from a glass fiber composite structure under a thick fungal colony while the surrounding surface remained intact.

Fungal influenced corrosion may occur in two ways. Fungi may directly attack unprotected aluminum alloys. At least one species of the genus *Cladosporium* in liquid culture can accelerate corrosion of unprotected 2024 T-6 alloy, the type used in military helicopters, by organic acids production during metabolic breakdown of hydrocarbons. Fungi may indirectly cause corrosion when bleach (~5.25% sodium hypochlorite) is used to remove fungal stains untouched by the authorized cleaning/disinfectant agent, 100% isopropyl alcohol. Sodium hypochlorite solution readily corrodes aluminum alloy upon contact in scratches or other holidays in the protective coating.

**Jet Fuel Storage**

MIC of metals is a problem in the extraction, production, distribution, and storage of hydrocarbon fuels. MIC has been identified for coated and uncoated carbon steel and aluminum. Ships with seawater displacement fuel tanks, in which seawater replaces fuel as it is consumed, are particularly susceptible. In addition, bacterial and fungal contamination in jet fuel causes fuel pump, gauge, filter, and coalescer malfunction due to clogging and blockages. Fungal contamination of fuel oils causes a deterioration in the quality of the product and, when contaminated fuel is used, can cause plugging in fuel lines and filters. Mechanisms proposed for MIC in fuel water systems are as follows: production of acids derived from the degradation of hydrocarbon chains; establishment of concentration cells; formation of tubercles by the symbiotic association with bacteria; and direct removal of metallic ions from the surface by extracellular enzymatic activity.
Extensive localized corrosion of the bottoms of carbon steel jet fuel storage tanks with subsequent leakage of fuel oil was recently investigated. *Hormoconis* (formerly *C. resinae*), *Aspergillus, Penicillium* and *Fusarium* were identified in the fuel/water mixtures. Corrosion attack was located near the tank bottom at the fuel/water interface where an active microbiological population was associated with free water. Fungi were observed growing over the entire surface of oil droplets and within suspended water droplets (Fig. 10). In the absence of water, no fungi were detected. Water soluble biocides are routinely added to fuel to prevent microbial growth. Problems occur when the biocides are exhausted or the amount of water has been underestimated. Attempts to form emulsions of fuel/water combinations accelerate fungal growth by increasing the surface fuel/water interfacial area and, therefore, the availability of oil, oxygen, and nutrients.

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REFERENCES


Figure 1 – Wooden spool on which wire rope was stored; pieces of paper are attached to rim edges.

Figure 2 – Inside surface of wooden spool; white deposits are the result of fungal growth.
Figure 3 – (a) Strand of wire with protective grease intact before exposure to acidic solution, 30x. (b) Localized corrosion after exposure to citric acid solution at pH 5.0, 40x.

Figure 4 – (a) Wire rope placed on wooden tongue depressors in a tray containing PDA inoculated with Aspergillus niger. (b) Wire rope that had been in contact with wood and PDA inoculated with Aspergillus niger. Rope rolled aside to show localized corrosion after contact with agar or wood.
Figure 5 – (a) Light microscope photograph showing fungal mycelia attached directly to wire and associated with localized corrosion, 40x. (b) ESEM micrograph showing mycelia in association with corrosion products, 500x. (c) ESEM micrograph showing mycelia in association with corrosion products, 1000x.
Figure 6 — Fungal growth on interior of H-53 helicopter.

Figure 7 — (a) Polyurethane paint surface before exposure (500×) and (b) lacquer paint surface before exposure (500×).
Figure 8 — (a) Mycelial mass on polyurethane coated coupon after 30 days (250×) and (b) germinating mycelia on polyurethane coated coupon after 30 days (250×).

Figure 9 — Polyurethane coated coupon surface after alcohol cleaning (1000×).
Figure 10 — Fungal mycelia in association with water droplets in jet fuel.