Rock Varnish: A Nature’s Shield

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Graphical Abstract
Abstract

Bare rock surfaces in dry to semi-arid places of the world usually have a black-brown, metallic sheen covering known as "rock/desert varnish." Rock varnishes are naturally occurring surface textures rich in Mn and Fe that are deposited on rocks. The varnish surface looks to be an ideal environment for microbial development. The function of bacteria in varnish formation, on the other hand, is currently being debated. Therefore, researchers throughout the world have long been interested in the biogeochemical fingerprints of life in severe settings, and the identification of organic entities is an important aspect of the quest for early life in extreme circumstances. High-altitude Ladakh, one of the world's harshest dry deserts, was chosen as the study's sample location due to its severe climatic circumstances. The current study illustrates significant microbial fingerprinting using organic biomarkers and isotopic analyses in conjunction with electron microscopy, revealing the presence of organic metabolites such as fatty acids, alkyl benzenes, oxime, amide, and fatty acids on the varnish layer as a result of mineral-microbial interactions. We propose for the first time that how change in surface wettability characteristics from hydrophilic (in host rock) to hydrophobic (in varnish) allowed microbial life to thrive in extreme environments. The current work provides evidence for a long-standing question about microbial affinity for the varnish layer and illustrates the significance of hydrophobicity in the varnish layer as a crucial component for understanding biogeochemical processes related to varnish growth.

Keywords

Rock Varnish; Surface Wettability; Extreme Environment; Ladakh; Mineral-microbe interaction; organic molecules; isotopic studies
1. Introduction

Rock varnish is a thin, dark, lustrous coating frequently seen on exposed rock surfaces worldwide in desert environments (Dorn 2009; Otter et al. 2020). It hosts range of microorganisms (Dorn and Oberlander 1981; Nagy et al. 1991) as well as clay minerals amalgamated with iron and manganese oxides and hydroxides (Perry and Adams 1978; Chaddha et al. 2021). Depending on the environmental factors, the varnish layer can range in thickness from 10 μm to several 100μm and increases at a rate of the 1-15μm over thousands of years (Perry and Adams 1978; Dorn et al. 1992; Liu and Broecker 2000). The abiotic hypothesis, which holds that minute changes in pH can concentrate manganese through geochemical processes (Dorn and Meek 1995), and biotic hypothesis, which holds that bacteria and other microorganisms are responsible for manganese concentration (DiGregorio 2005) are the two main hypotheses that have been used to explain the origin of rock varnishes. However, it is still unclear/controversial how exactly rock varnish is formed. Intriguingly, current studies favours the importance of biotic interventions in rock varnish development, and the augmentation of manganese has always been a crucial factor in determining the process of rock varnish production (Engel and Sharp 1958; Dorn et al. 2012).

It is observed that Mn and Fe both act as a UV shields for any micro species existing below and inside the layers of varnish, shielding them from the intense UV irradiation and extreme temperatures (DiGregorio 2002). The varnish layer’s protective properties also increase curiosity in the possibility of materials resembling varnish that have been detected in Martian rocks and boulders, indicating that this layer will play a significant part in our knowledge of life on other planets (Guinness et al. 1997; Allen et al. 2001).

Additionally, it has been demonstrated that bacteria and fungus in rock varnish have been detected on and within desert varnish coats from dry environments, pointing towards the possibility that these species are actively involved in varnish creation (Taylor-George et al.
1983; Kuhlman et al. 2006; Krinsley et al. 2009; Lang-Yona et al. 2018; Lingappa et al. 2021). Bacterial species often prefer hydrophobic solid surfaces to grow on, and there is a positive association between bacterial adhesions and hydrophobicity (Forson et al. 2020). We propose that the rock varnish’s hydrophobic properties allow microbiome to flourish within and beneath these surfaces because the hydrophobicity of cell’s surface is a crucial factor in how well it adheres to or detaches from a given surface (Krasowska and Sigler 2014). More specifically, the hydrophobins facilitate the attachment of hyphae to attach on hydrophobic surfaces and promote fungal growth on the substrate (Wösten et al. 1994; Temple et al. 1997). In addition to the varnish layer’s hydrophobic properties, the presence of birnessite (MnO$_2$) nanoparticles in it (Chaddha et al. 2022) shields bacteria from harmful UV radiations due to the MnO$_2$ nanoparticles’ well known UV shielding properties (Ghosh et al. 2016) and creates an ideal habitat for the development of the microbiome. In this study, we present the previously unknown hydrophobic feature of the varnish layer may have protected and nourished microbiomes for years after the varnish layer is formed in extreme conditions and may have its important role in understanding the overall biogeochemical process involved in varnish formation.

2. Materials and Methods

A number of sophisticated techniques were applied to characterize this unique material such as FESEM with EDS to understand surface morphology and chemical composition; IRMS and GC-MS/MS for the source identification and biomarker present in the varnish layer in terms of organic metabolites present in the varnish layer; Contact angle study to understand the surface wettability properties. The details of each technique are given below.
2.1 FE-SEM-EDS: The surface morphology and elemental composition of the varnish layer and its associated host rock samples were examined using a JEOL FESEM 7610F electron microscope. To eliminate cross-contamination, all mounting and other processes were carried out in a sterile environment. Specimens were taken at various magnifications using a secondary electron detector at a 15 KV acceleration voltage to examine their morphological features. EDS spectra were taken from an EDAX Octane + detector using TEAM software. Element scanning and point mapping were done at 15 KV.

2.2 Contact angle study: Surface wettability studies were performed on the visibly smooth surface of the varnish layer and host rock using the Kruss Drop Shape Analysis System, model no. DSA10MK2 (Kruss GmbH, Germany). Distilled water was used as a wicking liquid and contact angle with respect to the varnish and the host rock surfaces were determined for comparison.

2.3 IRMS: Carbon and nitrogen isotopic investigations were carried out for the varnish layer and associated host rock using an Isotope Ratio Mass Spectrometer (IRMS; MAT 253, Thermo Finnegan). Signals corresponding to masses 44, 45, and 46 for both the sample and a reference gas were detected, and the isotopic composition was computed. Running standards IAEA, CH3, and CH6 were used to monitor analytical precision. A routine accuracy of ±0.1 ‰ has been obtained for δ13C values. The carbon isotopic data is reported against VPDB. Similarly, Nitrogen isotope measurements were performed using IAEA N1 and NO3 running standards. Total organic carbon (TOC) and total nitrogen (TN) were determined by using peak area obtained from the sum of the integrated m/z 44, 45, and 46 signals and m/z 28 and 29, respectively.
2.4 GC-MS/MS: Trace GC Ultra TSQ 8000 Evo Mass spectrometer (Thermo, USA) fitted with a TG 5MS capillary column (30 m X 0.25mm, 0.25 μm film thickness of stationary phase, 5% phenyl and 95% dimethyl polysiloxane was used to analyse the sample extract of varnish and host rock samples by GC-MS/MS. As a part of silylation, powdered materials were derivatized with BSTFA (N,O-Bis(trimethylsilyl)trifluoroacetamide) and 1% TMCS (trimethylchlorosilane). The GC instrument was operated in split injection mode (1:10 split ratio) with an injector port temperature of 80 °C. Helium (99.999%) was used as carrier gas with a flow rate of 1.0 mL min\(^{-1}\). The following temperatures were set in the GC oven: 60 °C (hold for 3 minutes), then the temperature was increased up to 200 °C at 8 °C min\(^{-1}\) rate (hold for 5 minutes), then the temperature was increased up to 230 °C at a 6 °C min\(^{-1}\) rate (hold for 5 minutes), and finally the temperature was increased up to 290 °C at a 10 °C min\(^{-1}\) rate (hold for 20 minutes), with a total run time duration of 60 minutes. The temperatures of the ion source and interface were set at 230 °C and 300°C, respectively. The mass spectrometer's electron energy was set to 70 eV, and assessed using a full scan mode of mass between 50 and 850 m/z. 1µl of the derivatized sample was injected into GC-MS/MS system for final analysis of the rock samples.

3. Results

3.1 Surface morphology and elemental analysis

Rock varnish formation is understood to be a surface phenomenon, which is supported by current study. Understanding the surface morphological variations between the varnish layer and the host rock is crucial. By employing the Field Emission Scanning Electron Microscopy-Energy Dispersive X-Ray Spectroscopy (FESEM-EDS), it was possible to perform Back Scattered Electron imaging (BSE) of the varnish layer in relation to the
host/parent rock (Fig. S1). The dark varnish layer and the lighter host rock, which has a distinct texture which is clearly contrasted in the BSEs. As shown by yellow arrows (Fig. 1 a-d), it is interesting to notice that Microcolonial fungus (MCF) was also found in the varnish samples, especially in rock surface pits, as supported by previous studies (Perry and Kolb 2004; Parchert et al. 2012). In the varnish layer, decomposing MCF with a sponge-like shape has also been discovered (Fig. 1 e-f). These species, as was previously said, mimic the ascomycetes fungi’s cell walls (Vasilyeva et al. 2010). Additionally, the coccoid shaped nostoc type cyanobacterium cells and the microbial mat (Fig. S3), which has unique pores in its sheath (indicated by yellow dotted lines and arrows) (Honegger et al. 2013) enable microbial interventions in varnish production. Additionally, the elemental analysis (EDAX) demonstrates that the varnish samples are enriched in iron and manganese oxides (see Spot S3, Fig.1b). The MCF also establishes the function of organic entity in varnish production through the enrichment of C, P, and S components on spots S1 and S2 and an increase in C content at cocci-shaped cells (Fig. S2). It’s interesting to observe that the varnish layer has a distinct smoothness with different microbial entities embedded in the Mn and Fe rich coating.
Fig. 1 (a-d) MCF (multi-colonial fungi) embedded in the surface of varnish coated rock, with multi-spot elemental analysis demonstrating the elemental contrast in the biotic species and the varnish layer; (e-f) Cross-section of decaying MCF observed embedded in the varnish layer with sponge-like morphology.
3.2 Surface wettability study

Fig. 2 The sessile drop method is vividly demonstrating the hydrophobic character of the varnish surface in comparison to the host rock surface.
It must be proved that the varnish, which is a sub-micron thick layer, is smooth and impermeable. In order to ascertain the wettability of the varnish layer's surface, contact angle analysis is carried out. Water is a universal fluid that interacts with rocks, soil, and other natural materials through moisture, vapours, and precipitation. Understanding the driving forces at the interface of the varnish layer and the surrounding environment requires knowledge of the rock varnish-fluid system. An ordinary, distinctly smooth (polished) linear section of varnish coated sample served as the test subject (Fig. S4). With a measured contact angle of 81.08°, the Contact Angle measurement (CA), which gauges the affinity between the rock varnish solid surface and the liquid (water in this case), reveals moderate hydrophobic surface properties. In comparison, the host rock’s contact angle with the water was only 13.78°, indicating hydrophilic characteristics (Fig. 2). A surface is sub-critically water-repellent if the contact angle (CA) is between 0° and 90°, a non-wettable hydrophobic surface if the CA is more than 90° (Woche et al. 2017).
3.3 Organic source identification analysis

Fig.3 The δ\textsuperscript{13}C values of rock varnish layer in rock varnish sampled at four different sites in Leh-Ladakh (~ 3000-3600 m a.s.l) (in black square, current study) and its comparison with the available isotopic data of varnish from Western North America, Israel, and the Sinai Peninsula from (Dorn and DeNiro, 1985)(Dorn and DeNiro 1985). Negative δ\textsuperscript{13}C values represent depletion. The values in the pink inverted triangle indicate values from different elevations at Kitt Peak, Arizona (3,4,6, and 9) and from various places in the central Mojave Desert, California (1,2,5,7, and 8).
Table 1 Total organic carbon (TOC), total nitrogen (TN), and stable isotopic carbon composition expressed as depletion relative to the VPDB standard ($\delta^{13}C$), with TOC/TN ratios for four rock varnish samples 1V, 2V, 3V, and 4V obtained from the respective samples RV1, RV2, RV3, and RV4.

<table>
<thead>
<tr>
<th>Rock Varnish</th>
<th>TOC (%) (Decarb.)</th>
<th>TN (%) (Decarb.)</th>
<th>$\delta^{13}C$ (Decarb.) (‰, VPDB)</th>
<th>TOC/TN</th>
</tr>
</thead>
<tbody>
<tr>
<td>1V</td>
<td>0.77</td>
<td>0.11</td>
<td>-22.4</td>
<td>7.0</td>
</tr>
<tr>
<td>2V</td>
<td>0.20</td>
<td>0.03</td>
<td>-21.9</td>
<td>6.7</td>
</tr>
<tr>
<td>3V</td>
<td>0.64</td>
<td>0.09</td>
<td>-20.7</td>
<td>7.5</td>
</tr>
<tr>
<td>4V</td>
<td>0.70</td>
<td>0.08</td>
<td>-23.6</td>
<td>8.2</td>
</tr>
</tbody>
</table>

To further comprehend the connection between the $\delta^{13}C$ values of organic components present in the varnish layer and their surrounding environment, elemental and stable carbon-nitrogen isotopic analysis were performed. There aren’t many isotopic studies of the varnish layer itself since the influence of the host rock beneath cannot be disregarded. In order to address this, we removed rock varnish sample’s micro-level varnish layer and used the fresh host underneath as a control for the measured values. Following analysis of the rock varnish samples, TOC (%), TN (%), TOC/TN, and $\delta^{13}C$ values of decarbonated samples were presented in Table 1. While the TN content of the rock varnish sample ranges from 0.03 to 0.1 percent, the TOC content ranges from 0.20 to 0.77 percent in mass. The stable isotopic carbon ratios in the varnish sample ranged from -20 to -23 per mil (‰). The results for the respective host rock samples 1H, 2H, 3H and 4H on which varnish was produced were -27.6
(‰), -31.6 (‰), -29.3 (‰), and -27.9 (‰), respectively. Each decarbonated parent host rock 1H, 2H, 3H, and, 4H had a TOC (%) of 0.05(%), 0.04(%), 0.06(%) and 0.07(%), respectively. The average TOC concentration in lacustrine sediments (Seiter et al. 2004) was around 0.5 percent; in rock varnish samples, it was typically higher and almost on par with the TOC concentrations in dry soils (Carr et al. 2013) at about 0.8 percent. A significant amount of organic material must have been present in or on the desert varnish coatings that were the subject of this enquiry based on carbon content. Here, it’s crucial to remember that while the presence of C and N in adequate abundance favours the claim of microbial interventions, the organic molecular analyses are necessary to back up the claim.

### 3.4 Organic biomarker study

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Mol. Wt.</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.64</td>
<td>234</td>
<td><img src="image" alt="trimethylsilyl 2-((trimethylsilyl)oxy)propanoate" /></td>
</tr>
<tr>
<td>10.3</td>
<td>180</td>
<td><img src="image" alt="5-(tert-butyl)-2-methylbenzenethiol" /></td>
</tr>
<tr>
<td>12.83</td>
<td>423</td>
<td><img src="image1.png" alt="Structure" /></td>
</tr>
<tr>
<td>15.55</td>
<td>198</td>
<td><img src="image2.png" alt="Structure" /></td>
</tr>
<tr>
<td>18.23</td>
<td>218</td>
<td><img src="image3.png" alt="Structure" /></td>
</tr>
<tr>
<td>19.70</td>
<td>232</td>
<td><img src="image4.png" alt="Structure" /></td>
</tr>
<tr>
<td>20.07</td>
<td>246</td>
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<tr>
<td>#</td>
<td>Molecular Weight</td>
<td>Molecular Structure</td>
</tr>
<tr>
<td>-----</td>
<td>------------------</td>
<td>---------------------------------------------------------</td>
</tr>
<tr>
<td>21.13</td>
<td>246</td>
<td><img src="image" alt="dodecan-2-ylibenzene" /></td>
</tr>
<tr>
<td>22.83</td>
<td>366</td>
<td><img src="image" alt="11-(pentan-3-yl)hexicosane" /></td>
</tr>
<tr>
<td>23.68</td>
<td>338</td>
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</tr>
<tr>
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<tr>
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<td>40.80</td>
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<tr>
<td>40.89</td>
<td>502</td>
<td><img src="image" alt="2,3-bis((trimethylsilyloxy)propyl stearate" /></td>
</tr>
<tr>
<td>45.30</td>
<td>424</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>-----</td>
<td></td>
</tr>
<tr>
<td><img src="image1.png" alt="Chemical Structure 1" /></td>
<td>((6-methoxy-2-phenylhexahydropyrano[3,2-d][1,3,2]dioxaborinine-7,8-diylbis(oxy))bis(trimethylsilane)</td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>49.83</th>
<th>646</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image2.png" alt="Chemical Structure 2" /></td>
<td>tris(2,4-di-tert-butylphenyl) phosphite</td>
</tr>
</tbody>
</table>
Fig. 4 Relative abundance of different type of organic compounds detected in the varnish samples 1V, 2V, 3V, and 4V, respectively. Note that fatty acids, alkyl benzene and phosphite are relatively more abundant compared to other compounds.

The organic biosignatures that are present in the varnish layer have been identified using GC-MS-MS analysis of rock varnish samples taken from diverse locations. While scraping the microscopic thin varnish layer off the parent rock, one cannot ignore the parent’s rock impact. All the varnish samples contain organic compounds similar to those in Table 2, with a TMS (trimethyl silane) group connected to the molecule seen in the spectrometer as a result of trimethylsilylation carried out on the sample before injecting it into the GC-MS/MS. n-alkanes, branched alkyl benzenes, organic acids including lactic acid, and fatty acids predominated in all four varnish samples (1V, 2V, 3V, and 4V) (Fig. 4). The varnish layer also contained benzenethiols, pyrrole-ester derivatives, tetra-hydropyran derivatives, phosphite, long chain amide, and oxime, among other specific substances. Box plot
examination revealed the presence of organic compounds in the varnish layer with a variety of abundances, including fatty acids, alkyl benzenes, oxime, amide, and fatty acids (Fig. S7). It suggests that the rock varnish contains a significant amount of these compounds. Although the idea of microbial interactions in varnish production is supported by both the elemental and molecular composition of the organic matter.

4. Discussion

The presence of different forms of micro-biota on the varnish layer provides evidence that microorganisms may be involved in the varnish layer’s creation, as shown by FE-SEM analysis. Most often, fungus and bacteria have been found in the most severe environments where varnish is present. Because they are sensitive to extreme temperature changes, these organisms are extremophiles. The existence of MCF supports the global findings (Staley et al. 1982, 1983; Jones 1991) and further supports the notion that MCF exists in the frigid desert of Ladakh, India. Therefore, it is important to remember that varnish occurs in a natural setting and the bacteria and/or their by-products may have some impact on this process. Additionally, organics may be introduced to varnish surfaces as aerosols or as compounds bound to or bonded to dust particles.

According to the contact angle study, the rock varnish has a CA of = 81.08°, which is close to having hydrophobic surface character. This finding suggests that the varnish layer has less surface energy than the host rock surface, which has a CA of = 13.78°, indicating that it has a hydrophilic nature. This comparison of the varnish layer’s and the host rock’s surface characteristics shows how crucial surface chemistry is to varnish layer’s formation. It is possible to use the concept of water repellence in soil-water interaction (Doerr et al. 2000) in this context to understand the significance of the surface characteristics of the two distinct solid surfaces. Due to the fact that non-polar organic surfaces, including interstitial particulate
organic debris or organic substances adsorbed on particle surfaces, can produce the contrast
surface property behaviour on the varnish layer (Ma’Shum et al. 1988). The adsorption of
inorganic and organic materials as well as biogenic elements like microbial remains creates
biogeochemical interfaces (Chorover et al. 2007; Totsche et al. 2010). According to
petrographic study, they may also serve as a transition zone between the solid surface and the
pore space, containing different amounts of adsorbed particulate organic matter (Rennert et
al. 2012) with varying film thickness (Fig. S5). The atoms C, O, and H are the ones that are
largely involved in particle wettability. Non-polar functional groups in varnish layers may
consist of C-H bonds (for example, -CH\textsubscript{3}), while polar functional groups typically include an
O-H or a C-O bond (for example, -OH, -COOH). Liquid movement will generally be
encouraged in a matrix with a high proportion of hydrophilic surfaces and will be inhibited as
the proportion of hydrophobic surfaces rises. At both the micro and macro levels,
hydrophobic surface heterogeneity can lead to favoured flow paths (Dekker and Ritsema
1994; Bachmann et al. 2013). It is also believed that decreased wettability promotes OM
(organic matter) stability (Goebel et al. 2007). A wettable (hydrophilic) surface should
always result from the presence of just polar C species (Pronk et al. 2017). Krueger had
hypothesised that decreased wettability would be a function of the quantity of non-polar C
species (Krueger et al. 2016). The contact angle analysis indicates that there are interactions
between minerals, organic compounds, and microbes at the varnish layer's so-called
biogeochemical interfaces. The rock-varnish layer’s nearly hydrophobic nature creates a
niche for microbial life to flourish at the interface between the host rock and varnish layer in
addition to protecting against the environmental influences including UV radiation, abrasion
by particles, and chemical change by precipitation, etc.

The stable carbon isotope ratios in the four samples of desert varnish agreed with earlier
findings for other samples that were similar (Dorn and DeNiro 1985; Dorn et al. 1987). The
majority of the data from the $\delta^{13}$C technique were consistent with organic material produced by C3 photosynthetic plants and may be altered by desert varnish bacteria (Malherbe et al. 2017). The samples of desert varnish examined here might then contain some antiquated organic stuff. It should be noted, that the isotopic values of the corresponding host rock samples 1H, 2H, 3H, and 4H were significantly depleted in comparison to the varnish, so values reported here were largely obtained for the varnish coating and the influence of the underlying rock materials is not included. The varnish layer was studied for rocks from either Indus molasses (sedimentary clastic rock, 1H, 2H and 3H) or the Ladakh batholith (igneous, 4H). For the Indus molasses, the $\delta^{13}$C values ranged from -27.6 (‰) to -31.6 (‰), and very well agree with the average $\delta^{13}$C (‰) for the clastic rock-hosted organic carbon (Canfield et al. 2020). The Ladakh batholith’s isotopic value, -27.9 (‰), is also connected with the isotopic make up of granitic rocks (Zhao et al. 2001). The association between the $\delta^{13}$C values of varnish organic matter and the environment can be explained by the difference in isotopic values between the varnish layer and the host rock, which points to a distinct origin source. Micrometre-sized primarily shrubby plant debris in the air that accumulates on rock surfaces becomes varnish organic matter (Simoneit et al. 1981). On varnish, bacteria, micro-colonial fungus, and other microorganisms break down some of this plant matter (Krumbein and Jens 1981; Staley et al. 1982, 1983, 1992). The isotopic makeup of the plant detritus (Dorn and DeNiro 1985) that is locally accessible and the microbial organic components that are incorporated into varnish are comparable. In contrast to C4 and CAM plants in the surrounding vegetation (Deines 1980), the relative amount of C3 plants and heterotrophic bacteria is depicted by the $\delta^{13}$C levels of entering plant material (Boschker and Middelburg 2002). The $\delta^{13}$C values of varnish organic matter may be inferred to reflect the local environment in which it grows because C4 and CAM plants are more common in warm arid conditions than the C3 plants in cold habitats (Mooney et al. 1973; Troughton et al. 1977);
despite the fact that vegetation is limited in Ladakh, C3 plants in the form of shrub species are present (Raina et al. 2012; Husain et al. 2018). The varnish’s microbial mediation is supported by the varnish’s strong association between TOC and TN (Fig. S6), which shows that both substances are produced from similar organic sources. The TOC/TN ratio of <10 suggests the presence of microorganisms during the growth of varnish layer (Meyers 1997).

In soils and sediments, where microbial immobilisation of nitrogenous material is paired with carbon remineralisation, C/N ratios are declining (Sollins et al. 1984). The current study’s results are in agreement with the global data set on the $\delta^{13}C$ isotopic variability of rock varnishes (Fig. 3). The isotopic values of varnishes from the semi-arid regions around the world are complemented by the $\delta^{13}C$ levels of varnish samples from the current investigation. It also accords with the results from the altitudinal climatic-transect, which unmistakably shows that the varnish $\delta^{13}C$ values drop with elevation. Finally, it can be deduced that there is a strong correlation between the local environment and the organic matter $\delta^{13}C$ levels.

Considering that some autotrophic organisms that exists in habitats where varnish develops are capable of producing varnish and cyanobacteria despite their relatively scarce occurrence, the microbial role to the development of the varnish layer is somewhat confirmed (Dorn and Oberlander 1982). While some fungus may incorporate atmospheric CO$_2$ into their biomass, it seems that the micro colonial fungi that are usually spotted on varnish derive their carbon from airborne organic matter acquired from nearby soil and plants (Staley et al. 1982; Taylor-George et al. 1983)

Additionally, the GC-MS/MS examination of different varnish samples turned up a number of compounds, confirming the bacterial origin (Malherbe et al. 2017). Microorganisms often create organic acids as metal chelators and weathering agents, such as lactic acid and fatty acids (Yeager et al. 2019). Since microbial production of these acids is well recognised, their presence in varnish layer is a certain indicator of the influence of microbial activity on the
varnish layer (Naraian and Kumari 2017). It has already been documented that soil microorganisms rapidly and extensively break down lactic acid as a source of carbon and energy for the bacteria that live there (Macias-Benitez et al. 2020). Because of this, the isolation of lactic acid from soil and sediments confirms the claim that it is present on the varnish layer and may have been deposited there by nearby soil aerosols (Chen et al. 2005). Incomplete substrate oxidation causes organic acid build-up occurs in aerobic bacteria and fungi, which has been connected to dietary imbalances such as mineral ions (Papagianni 2011). Since both bacteria and fungi can produce large amounts of various organic acids under certain circumstances, organic acid build up is probably the best-known example of primary metabolic overflow. Since many bacterial taxa, including Acinetobacter, may utilize long chain alkanes as a carbon source and breakdown the hydrocarbons present in the varnish, the presence of n-alkanes (C-24, C-26) in the varnish layer also suggests the presence of bacterial populations (Park et al. 2017). Through a series of oxidation processes in which alkane is transformed into alcohol, aldehyde, and finally, fatty acid, the higher relative abundance of fatty acids in varnish samples can be linked with aerobic alkane breakdown by microbial species (Van Beilen et al. 2003). Short chain fatty acids of methyl ester (FAME) with C18 were found in the samples in the form of palmitate and stearate indicating that organic material was created through bacterial action (Malherbe et al. 2017). In fact, the saturated fatty acids (C-14 to C-18 for bacteria, C-16 to C-26 for algae, and C-28 to C-36 for plants) are typically found in the range of lipids of cell membranes (Jetter et al. 2006). Fatty acids in glyco and phospholipids are typically crucial bacterium makers. The lack of long-chain FAMEs in the rock varnish suggests that the surrounding flora’s leaf waxes did not significantly influence the GC-MS/MS results. Long chain alkyl benzenes (LCAB) are produced as a result of many biochemical and thermochemical reactions between organic molecules found in the environment and rock varnish (Razwan Sardar et al. 2011).
Since the synthesis of alkyl benzenes is caused by the breakdown of β-carotenoids at low temperatures, the existence of a larger abundance of LCAB in the varnish layer suggest a microbiological influence (Byers 1983). Further fatty acid cyclisation may lead to the formation of these substances; long chain fatty acids found in the varnish layer can also serve as a precursor to LCAB. As a result, fatty acids played a major role in the process of dehydration, cyclization, and decarboxylation during the synthesis of LCABs (Saiz-Jimenez 1995). Thus, the biological source of LCABs in the varnish may be fatty acids. Thiol fragments could suggest a microbiological origin, if they are present (Hand and Honek 2005). Given that pyrrole-carboxylate fragments are a well-known biological form of pyrrole present in the microbial environment, the presence of these fragments in the sample may be due to biosynthetic metabolites of various microbial strains (Walsh et al. 2006; Maharjan et al. 2012). While these fragments constitute structural subunits of many bioactive natural compounds, there presence in the sample cannot be associated with the presence of bacteria (Ghosh and Brindisi 2016). Given that oxime-ether are flexible precursors in organic synthesis, the discovery of oxime-ethers molecule fragments in the varnish layer is intriguing (Mirjafary et al. 2015). They serve as a good starting materials for the synthesis of nitrogen-containing compounds like amines, 1,2-aminoalcohols, α and β-amino acids, nitriles, lactams, and nitrogen heterocycles with three to eight members (Moody 2004). Given that the prebiotic synthesis of self-replicating genetic sequences is still a challenging but necessary job in the study of life origins, these chemicals are recognised as being essential synthetic antecedents of early life molecules. In order to comprehend how nucleotide oligomers (such as DNA and RNA) first arose on the early earth, it may be able to link the presence of an oxime ether molecule to the prebiotic synthesis of nucleotides, which serve as the building blocks of RNA and DNA, respectively. Since bacteria produce 13-Docosenamide in response to glucose and it may play a role in host-bacteria interactions, the presence of this chemical in
varnish layer is proof of microbial involvement (Tamilmani et al. 2018). Finally, the nominal reaction route states that phosphate (HPO$_4^{2-}$) is reduced to phosphite (HPO$_3^{2-}$) by the concurrent oxidation of iron (II) to iron (III), which is directly related with the presence of ferric oxides in the rock varnish. This suggests that the capacity to employ reduced P molecules like phosphite as a single P source is common in microbiological systems (White and Metcalf 2007; Kamat et al. 2011), and the presence of phosphite in the layer contributes to the survival and growth of microbiota in the varnish layer (Schink and Friedrich 2000). Since it has long been believed that clay minerals in general may have played a significant part in the origin and evolution of genetic molecules, the presence of these molecules in clay-rich rock varnish supports the microbial growth theory of their development (Gallori and Branciamore 2012).

5. Conclusion

The dark brown, glossy varnish layer covering the rocks of Ladakh cold desert contains a robust microbiome with many organic metabolites. Researches have always been intrigued by the existence of microspecies in the varnish layer because of their connection to the global evolution of life in arid conditions. The current work offers the first account of an as-yet unreported surface characteristic of the rock varnish layer supported by thriving microbial community. By giving these multi-microbial species like fungus and bacteria a hydrophobic property, rock varnish acts as both a host and a barrier, protecting them from harmful environmental influences. Presence of organic molecules and the isotopic values of the varnish layer supports the idea of biotic mediation playing a part in varnish production. Overall, this unique surface feature of the varnish layer confirms the theory that the patina may have protected the microbial populations from harsh environmental conditions, particularly the UV irradiations and weathering processes. It may also answer questions about
the microbial source and its role, in varnish formation, a long-standing question. The best examples of rock varnish surviving for thousands of years are petroglyphs, which are found all across the world. This is probably because of its insulating function - The Hydrophobic Character of the Rock Varnish.

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Data Availability

The data generated during and/or analysed during the current study are available in the main manuscript and additionally provided in supplementary file.
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