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Research Article

Isolating microbes from the surface of an introductory laboratory halite hand sample

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ABSTRACT

Introductory geology labs stress simple physical testing (luster, hardness, etc.) to identify common minerals, using mineral charts to eliminate minerals not exhibiting a particular property. Special properties (magnetism, specific gravity, taste, etc.) for specific minerals narrows mineral identity. Students often express safety concerns about licking minerals, especially when they realize others have previously licked the specimen. As an exercise in medical geology, we cultured microbes from a halite sample used for nearly 25 years and licked by numerous students over that time span (Sample 1), a commercially purchased unused and freshly exposed surface of halite licked by one person (Sample 2), and a disinfected sample repeatedly licked by only one person (Sample 3), to determine microbial presence, especially those potentially harmful to students applying the taste test. From the new crystal licked by one person (Sample 2), we identified sixteen different phenotypic groups of microorganisms after incubation of the crystal in growth medium. 16S ribosomal RNA sequence analysis was performed on one representative from nine of the sixteen groups. From this analysis, we obtained one species of *Bacillus*, four of *Paenibacillus*, and four of *Staphylococcus*, including *Staphylococcus epidermidis*. Comparing our results to published studies of the human tongue biome, we find that all of our cultured microbes occur naturally within a typical person's mouth and do not pose significant health risk as used in lab. Saliva with microbes can be transmitted as the halite is reused, especially if the test is administered quickly after a previous licking, so caution is warranted, but the process is essentially safe under normal conditions.

KEYWORDS

Halite, Medical geology, Microbes, Mineral testing safety, COVID-19

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INTRODUCTION

Halite (NaCl) is one of the primary minerals students are taught to identify in introductory geology laboratories. Halite exhibits perfect cubic crystal growth form and cleavage, easily recognized by students struggling with the concepts of cleavage and symmetry, and halite is familiar to students due to its commonplace occurrence in their homes as a seasoning. It is often pointed out that "salting" is used as a preservative that alters the composition of the microbial community present in food to one that is considered "harmless" to humans (Ingram and Kitchell, 1967, but see Kim et al., 2017, for an alternative view), with the expectation that salt surfaces are relatively free of harmful microbes. Hand-samples are passed from student to student for the "taste test," the definitive technique used for identifying halite.

Some studies have been conducted on inclusions in halite, reporting the presence of ancient microorganisms

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(McGenity et al., 2000; Vítek et al., 2010; Lowenstein et al., 2011). *Haloarchaea* have been recovered within microscopic droplets of brine within the laboratory-grown halite crystal and can be viable for several decades, providing the basis for the hypothesis that ancient microorganisms can be isolated from ancient halite samples (Fish et al., 2002). Adamski and others (2006) reported finding *Pseudomonas aeruginosa* in fluid inclusions in laboratory-grown halite. Using 16S rRNA gene sequence analysis, isolates from the surface of ancient evaporates from different geological formations failed to demonstrate consistent differences in gene sequences despite the potential fracturing and recrystallization events these evaporates may undergo throughout time (McGenity et al., 2000; Fish et al., 2002). Few studies have been conducted on microbes on the surface of halite (see DasSharma, 2007 for a discussion of the potential of *Haloarchaea* for teaching basic microbiology), and none have been conducted to determine bacteria on a halite geology lab hand-sample.

The question arises: is the taste test used in introductory geology lab safe for the students to use? Our goals were to evaluate the safety of the "lick test" used in typical introductory geology laboratory activities by: (1) investigating the diversity of microbes that potentially can be transferred to halite during typical geologic mineral testing; (2) determining if any of these microbes belong to potentially harmful taxa; and (3) investigating the length of time that microbes remain viable on typical lab specimens. Additionally, this study provides a personally relevant model for students of the relatively new geologic field of "medical geology" as an application of basic principles of geology and a possible career path (e.g., Finkelman et al., 2001; Berger, 2003; Bunnell, 2004).

Oral Microbiome

Over 600 taxa inhabit the human mouth, with overall species estimates being closer to ~1200 (Dewhirst et al., 2010; Jenkinson, 2011). The human microbiome varies by individual, no one having the same microbes as another, and varies within the same individual over time depending upon diet, health, and environmental exposure. There is no universal pattern; however, diet, environment, host genetics, and early microbial exposure have all been implicated (Human Microbiome Project Consortium, 2012). This diverse microbiome includes viruses, fungi, protozoa, archaea, and bacteria (Wade, 2013). About half of the bacteria are uncultured in laboratory settings, but the ones that have been cultured have been identified and described through culture-independent methods directly from samples of saliva or oral biofilms (Paster and Dewhirst, 2009; Wade, 2013). As noted above, it is possible to identify cultured microbes through the use of molecular techniques, such as analyzing the 16S rRNA gene, which can then be compared to the human oral microbiome database (HOMD, www.homd.org) (Dewhirst et al., 2010; Wade, 2013).

Bisset and Davis (1960) identified 19 genera within the mouth microbiome belonging to seven families: *Bacillaceae*, *Coccaceae*, *Lactobacteriaceae*, *Mycobacteriaceae*, *Nocardiaceae*, *Streptomycetaceae*, and *Actinomycetaceae*. Genera identified within the mouth included *Bacillus*, *Clostridium*, *Staphylococcus*, *Streptococcus*, *Lactobacillus*, *Mycobacterium*, *Streptomyces*, *Actinomyces*, and *Bacteriodes*. Studies of microbial communities on the tongue reported diverse microbiota with high cell density (Riggo et al., 2008; Papaioannou et al., 2009; Zaura et al., 2009). Zaura and others (2009) reported that the most common bacterial classes found on the tongue are *Actinobacteria*, *Firmicutes*, and *Proteobacteria*. *Bacillaceae* and *Staphylococcaceae* were classified as "exclusive" families, meaning that they were not found in each of the host individuals, but were found in at least one. The highest mean count of bacteria is found in suprangingival plaque and on the tongue, producing very similar bacterial profiles containing high concentrations of *Prevotella malaninogenica* and *Streptococcus salicarius* (Papaioannou et al., 2009). Aerobic, catalase positive, gram-positive cocci, such as *Staphylococci*, are commonly found in oral swabs but usually do not occur in high numbers and inhabit the surface of the tongue (Hardie and Bowden, 1973).

METHODS

Most geology labs provide bulk samples containing many specimens of each mineral for student identification or provide each student, or student group, with their own sample sets. Individual halite samples can be handled by multiple students during a lab session with varying amounts of time between handling. After mineral identification labs are over, these samples may sit for weeks with no human contact. There is no procedure for cleaning halite once it has been used. To simplify the initial procedure and establish a baseline for later studies involving previously used samples, our sampling method in this study was limited to microbial contribution from a single individual using previously unused halite samples. Halite samples used in this study were

obtained from new student packs purchased from Ward's Science along with one large (> 5 cm) "cleavage" specimen that had been used by the class instructor repeatedly for nearly 25 years as a classroom demonstration specimen. As only mineral specimens were sampled, no IRB approval was necessary.

Sample Exposure

Sampling took place within a biological laboratory without the use of a vent hood to simulate typical introductory geology lab conditions in which halite samples are not disinfected before or after use. The 25-year-old halite sample (Sample 1) was swabbed after licking and streaked out onto a TSA plate to determine if growth occurs after licking. To characterize the diversity of microbes that can be transferred to a typical halite sample during the lick test, an unused halite sample from the student pack was broken to expose new surface believed to be uncontaminated by human microbiota; however, it was not disinfected in any other way (Sample 2). Although this control sample was expected to be microbe-free, it was swabbed before licking and tested for microbial presence. Standard microbial sampling procedures used in hospitals and laboratories were used (Madigan and Martinko, 2009; Johnson and Case, 2010). Students lick halite samples within minutes of another student licking the same hand-sample, then move on to other minerals to identify, therefore the licked sample was swabbed every minute for 11 minutes after exposure. Swabs were streaked onto Tryptic Soy Agar (TSA) (Fisher Scientific) plates and incubated at 37 °C for four days. TSA is a general, nonselective, complex medium suitable for growth of most microbial taxa (Johnson and Case, 2010). Colony phenotype was recorded for each swab sample (Table 1). To eliminate potential microbe contamination due to handling the halite that would affect microbe identification, a second unused hand-sample (Sample 3) was subjected to ultraviolet light to disinfect the outside surfaces before conducting any tests. Once disinfected, the halite hand-sample was licked by the same student and stored inside a sterile petri dish.

Microbe Identification

For microbe identification, the sample was crushed within a disinfected bag and pieces of similar size and weight placed in Luria broth (LB) (Fisher Scientific) and Tryptic Soy broth (TSB) (Fisher Scientific) and placed in a New Brunswick Scientific I 24 Incubator Shaker Series at 30 °C for 48 h

Table 1: List of phenotypes cultured from 'sample exposure' halite sample.

Timed Swabs (min)	Phenotypic description
0	Single large white colony with a yellow center
1	Single large white colony, multiple fibrous looking yellow colonies
2	Tiny brown and yellow colonies overtaken by white colonies
3	Single large white colony covering entire section
4	Small white and clear colonies
5	Few white isolated colonies
6	Small white colonies with a single off-white colony
7	White colonies covering section
8	White colonies with orange center dot covered section with a few small all white colonies mixed in
9	Single yellow colony
10	Large white, matte colony covered section
11	Few white colonies

(Table 2). To ensure microbial growth, media with four salt concentrations were used: no increased salt concentration, slightly increased (5 g/L NaCl) salt concentration, moderately increased (10 g/L NaCl) salt concentration, and highly increased salt concentration (50 g/L NaCl). A sample from each broth culture was streaked out onto two types of media plates, Luria broth agar (LB plates) and TSA plates, and placed in aerobic conditions in an upright incubator and anaerobic conditions using an anaerobic jar (McIntosh and Fildes, 1916). After 48 h of incubation at 30 °C, individual colonies were streaked for isolation resulting in a pure culture (one type of microbe per plate). To ensure no colonies were missed during isolation procedures, samples from LB broth were streaked onto TSA and LB plates, and samples from TSB were streaked onto TSA and LB plates. Each strain was tested

Table 2: List of crystal weights and broth types.

Broth	Sample crystal weight
TSB	0.2061 g
TSB	0.1343 g
TSB	0.0705 g
LB	0.2163 g
LB	0.1408 g
LB	0.0645 g

for its ability to grow in all four salt concentrations on LB and TSA plates.

Genomic DNA was extracted from each pure culture sample using the MasterPure Gram Positive DNA Purification Kit (Epicentre). The 16S ribosomal RNA gene in each sample was then amplified by Polymerase Chain Reactions (PCR) using primers 5'-AGAGTTTGATCMTGGCTCAG-3' and 5'-ACGGYTACCTTGTTACGAYT-3' and analyzed by gel electrophoresis. PCR products were gel purified using the QIAquick Gel Extraction kit (QIAGEN) and sent to the Molecular Resource Center at the University of Tennessee Health Science Center in Memphis, TN for sequencing using the same primers used for amplification. Sequence comparisons were performed using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify each isolate.

RESULTS

Sample Exposure and Growth Comparison

Growth to varying degrees occurred on all samples. However, the control (Sample 2; which was swabbed before licking) had a single colony present. For the timed sampling, no single minute had greatly increased growth. Minute 5, minute 8, minute 9, minute 10, and minute 11 all had few or only one colony present after the four day incubation period. Majority of colonies present were small and white or yellow. Few minutes had larger white colonies. Minute 10 had one larger white colony. Minute 11 had five small white colonies.

Microbe Identification

For each of the nine isolates sequenced from the samples, the top five matches in the forward and reverse direction are displayed because full-length sequence for the 16S ribosomal RNA gene was not obtained (TABLE 3). Microbes identified belong to the genera *Bacillus* (KJG4), *Paenibacillus* (KJG15, KJG28, KJG35, KJG63), or *Staphylococcus* (KJG29, KJG31, KJG36). Isolates KJG15 and KJG63 were the cultured in anaerobic conditions.

The BLAST results for isolate KJG4 indicate that is it most similar to *Bacillus circulans*. KJG15 and KJG 28 are most similar to *Paenibacillus provencensis* and *Paenibacillus urinalis*. KJG29, KJG31, and KJG36 are most similar to *Staphylococcus epidermidis*. KJG35 and KJG63 are similar to *Paenibacillus* sp.

DISCUSSION

All of these identifications are considered "likely" but not "conclusive." Regardless of this, enough information exists to determine if the microbes belong to taxa harmful to humans.

Sample Exposure

Growth was more than expected on the 25 year old licked halite sample (Sample 1). The microbial colony present in the control section (Sample 2) could suggest that there are microbes present on the halite sample before licking despite not having been disinfected. However, since only one colony was present, and it did not look phenotypically like other colonies that were found after licking, there are a few viable explanations. First, the colony could be a contaminant from a few different sources (e.g., air borne or breath). Second, the colony could have already been present on the halite surface and unable to grow in the presence of other microbes present after licking. Third, some bacterica have a strong tolerance to high salt stress and cannot be completely eradicated from environments of increased salt concentrations (Feng et al., 2022). Without further testing to determine if this is a recurring phenomenon or a one-time phenomenon, a conclusive explanation as to the origin of this colony or how it got in the specimens is not possible at this point.

The time of 11 minutes was used to simulate the passing of the hand-samples from student to student. Based on the growth that was present during the time frame, the idea that "salt could kill all the microbes present or transferred when licked" is unfounded. In fact, microbes have been found to be able to live for prolonged periods in ancient salt inclusions (Lowenstein et al., 2011).

Bacillus

The genus *Bacillus*, present in one of our isolates, is found in a wide array of environments, including freshwater, saltwater, soil, air, in and on plant and animal tissue, and can survive extreme conditions, such as high temperatures, extreme ranges of salinity, and acidic geochemistry, as well as oxygen poor (anaerobic) environments. These microbes survive extreme environments through the formation of endospores, or dormant cellular structure, which has been recognized as the hardest known form of life on Earth and are characteristic of some halophiles (Nicholson et al., 2000). *Bacillus* is found in the immune systems of animals (Maughan and Van der Auwara, 2011). The *Bacillus* species

Table 3: List of top five possible identifications for each isolate in each direction.

Isolate	Direction	Possible Identification	Sequenced Portion Match
KJG4	Forward	*Bacillus* sp. IMT13	Partial
		Uncultured bacterium clone NS 65	Partial
		Uncultured bacterium clone S 379	Partial
		Bacillus circulans Strain OH3057	Partial
		Bacillus circulans Strain 18.1 KSS	Partial
	Reverse	*Bacillus circulans* Strain 18.1 KSS	Partial
		Bacillus sp. IMT13	Partial
		Bacillus circulans Strain Q11	Partial
		Bacillus circulans	Partial
		Bacillus circulans Strain OH3057	Partial
KJG15	Forward	*Paenibacillus* sp. J16-10	Partial
		Uncultured bacterium clone nbw312e05c1	Partial
		Paenibacillus sp. Cu2	Complete
		Paenibacillus provencensis Strain W03	Partial
		Uncultured bacterium clone nby264d03c1	Partial
	Reverse	*Bacillales* bacterium Cul 0294	Partial
		Paenibacillus urinalis Strain 5402403	Partial
		Paenibacillus sp. J16-10	Partial
		Paenibacillus sp. 7-5	Partial
		Paenibacillus provencensis Strain W03	Partial
KJG28	Forward	*Paenibacillus* sp. J16-10	Partial
		Uncultured bacterium clone nbw312e05c1	Partial
		Paenibacillus sp. Cu2	Complete
		Paenibacillus provencensis Strain W03	Partial
		Uncultured bacterium clone nbw312e05c1	Partial
	Reverse	*Bacillales* bacterium Cul 0294	Partial
		Paenibacillus urinalis Strain 5402403	Partial
		Paenibacillus sp. 7-5	Partial
		Paenibacillus sp. J16-10	Partial
		Paenibacillus provencensis Strain W03	Partial
KJG29	Forward	*Staphylococcus* sp. Isolate O-10	Partial
		Staphylococcus epidermidis Strain APP-10	Partial
		Staphylococcus epidermidis Strain P8	Partial
		Bacterium 7-II	Partial
		Staphylococcus sp. HKG 170	Partial
	Reverse	*Staphylococcus epidermidis* Strain JPR-05	Partial
		Staphylococcus sp. QD53	Partial
		Staphylococcus epidermidis Strain B7 3CO2	Partial
		Staphylococcus epidermidis Isolate OCOB9	Partial
		Staphylococcus epidermidis Isolate OCAT33	Partial

Continued...

Table 3 (*Continued*): List of top five possible identifications for each isolate in each direction.

Isolate	Direction	Possible Identification	Sequenced Portion Match
KJG31	Forward	*Staphylococcus epidermidis* Isolate PN58	Partial
		Staphylococcus epidermidis Isolate PN51	Partial
		Staphylococcus epidermidis Isolate N26	Partial
		Staphylococcus epidermidis Strain MJMG8.1	Partial
		Staphylococcus epidermidis Strain E4.Cd3	Partial
	Reverse	*Staphylococcus epidermidis* Strain JPR-05	Partial
		Staphylococcus sp. QD53	Partial
		Staphylococcus epidermidis Strain B7 3CO2	Partial
		Staphylococcus epidermidis Isolate OCOB9	Partial
		Staphylococcus epidermidis Isolate OCAT33	Partial
KJG35	Forward	*Paenibacillus* sp. J16-10	Partial
		Uncultured bacterium clone nbw312e05c1	Partial
		Paenibacillus sp. Cu2	Complete
		Uncultured bacterium clone nby264d03c1	Partial
		Uncultured bacterium clone nby568g10c1	Partial
	Reverse	*Bacillales* bacterium Cul 0294	Partial
		Paenibacillus urinalis Strain 5402403	Partial
		Paenibacillus sp. J16-10	Partial
		Paenibacillus sp. 7-5	Partial
		Paenibacillus sp. 1	Partial
KJG36	Forward	*Staphylococcus* sp. Isolate O-10	Partial
		Staphylococcus epidermidis Strain APP-10	Partial
		Staphylococcus epidermidis Strain P8	Partial
		Bacterium 7-II	Partial
		Staphylococcus sp. HKG 170	Partial
	Reverse	*Staphylococcus epidermidis* Strain JPR-05	Partial
		Staphylococcus sp. QD53	Partial
		Staphylococcus epidermidis Strain B7	Partial
		Staphylococcus epidermidis Isolate OCOB9	Partial
		Staphylococcus epidermidis Isolate OCAT33	Partial
KJG61	Forward	*Staphylococcus* sp. Isolate O-10	Partial
		Staphylococcus epidermidis Strain I167	Partial
		Staphylococcus epidermidis Strain EH-7	Partial
		Staphylococcus epidermidis Strain EH-6	Partial
		Staphylococcus epidermidis Strain EH-5	Partial
	Reverse	*Staphylococcus epidermidis* Strain JPR-05	Partial
		Staphylococcus sp. QD53	Partial
		Staphylococcus epidermidis B7	Partial
		Staphylococcus epidermidis Isolate OCOB9	Partial
		Staphylococcus epidermidis Isolate OCAT33	Partial

Continued...

TABLE 3 (*Continued*): List of top five possible identifications for each isolate in each direction.

Isolate	Direction	Possible Identification	Sequenced Portion Match
KJG63	Forward	*Paenibacillus* sp. J16-10	Partial
		Uncultured bacterium clone nbw312e05c1	Partial
		Paenibacillus sp. Cu2	Complete
		Uncultured bacterium clone nby264d03c1	Partial
		Uncultured bacterium clone nby568g10c1	Partial
	Reverse	*Bacillales* bacterium Cul 0294	Partial
		Paenibacillus urinalis Strain 5402403	Partial
		Paenibacillus sp. J16-10	Partial
		Paenibacillus sp. 7-5	Partial
		Paenibacillus provencensis Strain W03	Partial

likely found on the halite hand-sample, *B. circulans*, has been found in even more saline environments and has grown on agar with a 7% NaCl concentration (Nakamura and Swezey, 1983). Two species of the *Bacillus* commonly cause infections in humans, *Bacillus cereus* (food-borne illness) and *Bacillus anthracis* (anthrax); the remaining species are perceived as of little clinical significance (Rowan et al., 2001; Maughan and Van der Auwara, 2011) suggesting that the "lick test" is essentially safe with respect to *Bacillus*. However, Griffiths (1990) and Beattie and Williams (1999) reported that *B. circulans* produced toxins to a detectable level when isolated from dairy products, meaning this species may pose a potential hazard, at least in the presence of dairy product. Rowan and others (2001) also reported that when *B. circulans* was isolated from human blood samples, it was associated with diseases such as Sepsis and Lymphoma. Although most published studies were focused on toxins that were detected only when isolated from food products or human blood and not mineral testing, geology students are not likely to transmit blood onto halite samples by licking except under unusual circumstances, so we conclude that the likelihood of B. circulans causing any adverse effect during mineral testing is unlikely, although not impossible.

Paenibacillus

Paenibacillus, meaning "almost Bacillus," became a separate genus in 1993 (Ash et al., 1993) and has over 30 species of facultative anaerobes (Lal and Tabacchioni, 2009). *Paenibacillus* species are commonly found as saprophytes in many environments including soil, water, plant tissue, food, feces and diseased insect larvae and can produce endospores, but are not considered pathogenic to humans (Roux et al., 2008). The two possible matches to our four isolates (KJG15, KJG28, KJG35, KJG36) were *Paenibacillus urinalis*, which was originally isolated from a human urine sample, and *Paenibacillus provencensis*, originally isolated from human cerebrospinal fluid (Roux et al., 2008). Both of these species grow in the presence of 5% NaCl, higher than salt concentrations used in this study. While urine and cerebral fluid contamination of laboratory halite samples is considered unlikely, except under the most extreme of circumstances, there may be some other means of contamination of *Paenibacillus* that has yet to be recognized and documented.

Staphylococcus

The genus *Staphylococcus* currently has 30 species identified within it, and are halotolerant (Komaratat and Kates, 1975; Gill et al., 2005). Only two species are of clinical concern for our study, *Staphylococcus aureus*, an aggressive pathogen, and *Staphylococcus epidermidis*, commonly found on the skin surface (Gill et al., 2005). *Staphylococcus epidermidis*, is now considered to be one of the top five causes of hospital acquired infections, but the virulence differs greatly by strain (Zhang et al., 2003; Gill et al., 2005) and is only of concern with contaminated implanted medical devices, such as indwelling catheters, or a skin puncture (Vuong and Otto, 2002; Von Eiff et al., 2002; Zhang et al., 2003; Gill et al., 2005). Some strains can be found on hospital equipment, such as catheters, and can produce a slime coat that protects the bacterium from antibiotics, thus increasing its virulence (Christensen et al., 1982).

Although we identified *S. epidermidis* on our halite sample, the particular strain of the species remains unknown without more analysis. The lick test for halite did result in the transfer of a strain that produces a slime layer onto the

surface of the halite; however, it is unlikely to pose any danger of infection under normal laboratory exposure to healthy students. For *S. epidermidis* to change from the normal state found on the surface of the skin to an infectious agent, the host must be predisposed or have a compromised immune system (e.g., patients under immunosuppressive therapy, AIDS patients, and premature newborns) (Caputo et al., 1987; Tacconelli et al., 1997; Domingo and Fontanet, 2001; Vuong and Otto, 2002). Caution is warranted because this organism can be viable for extended time due to its resistance to drying and temperature change (Lowy and Hammer, 1983). So, unless the halite is being implanted in a student, the risk of infection is unlikely.

Classroom Application & Implication

Tasting is used primarily to identify halite (NaCl), but also works for borax (sweet alkaline taste), epsomite (bitter), melanterite (sweet, astringent and metallic), and sylvite (bitter). As the special property test of tasting is quick and easy to do, and because salt is a common household item, students readily employ this sense to identify minerals, yet many will feel uncomfortable about the safety of the testing. There is also the potential problem of students contaminating specimens with HCl residue (which also produces a salty taste) by misapplying the taste test to calcite or applying HCl to halite during testing, thus exposing them to low concentrations of that salty acid. We checked over ten currently sold physical geology lab manuals for warnings regarding the use of the taste test for halite, or any other mineral, and found that none contained cautionary statements of any type within either the explanatory text of taste as a physical test or within the mineral identification charts. Ward's mineral kits do come with printed warning labels (FIGURE 1) in the boxes that warn the students that the boxes may contain "small quantities of hazardous substances", specifically lead (galena) and asbestos (talc), but do not list halite as a potentially hazardous mineral. It should be noted that same label also warns against ingesting; however, this warning refers to the listed minerals only.

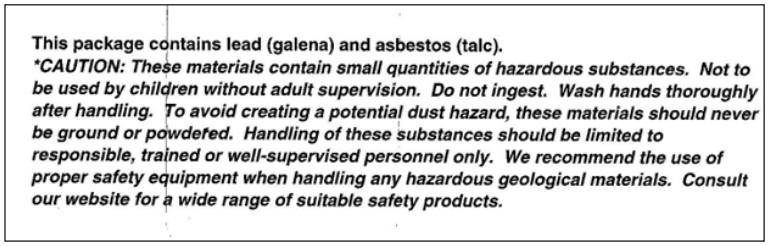

FIGURE 1: Warning label included in Ward's student mineral kits. Hazardous materials such as galena, which contains lead, and talc, which contains asbestos, are clearly labeled with instructions on intended use, proper handling, and suggested supervsion; however, halite is not included by name as the substance itself is not considered hazardous.

Medical Geology and COVID-19

This study is an example of the type of investigations typical of the relatively young field of medical geology (e.g., Bunnell, 2004), only the study was "turned inward" to evaluate the geological community pedagogy itself. Prior to the COVID-19 pandemic, we incorporated the results of our study in the labs when teaching mineral testing to (1) alleviate student concerns over the safety of the taste test itself and (2) provide the students with an example of how geology functions as a truly interdisciplinary science with direct medical applications. This experiment was conducted prior to the COVID-19 pandemic. We now know that the COVID-19 virus can, indeed, be spread via saliva (e.g., Fini, 2020). Although our studies did not include testing for the COVID-19 virus, clearly, the halite taste test is more problematic than we realized from our initial study. We informally polled students in the same courses post-COVID (N = 66) to see if their opinions or concerns had changed. Considering that most students are leery of the taste test in the first place, it is not surprising that there was near unanimous response that the taste test should either never be used, or only by geologists in safe situations.

Medical geology is a topic area within several sections of the introductory level geoscience course that serves to fulfill part of the University's Biological and Physical Systems general education requirement. Inclusion of this study into that course serves as a "personally relatable" experience (experiential learning) for the students laying groundwork for broadening topics to include other medical geology issues such as geophagy (ingestion of loess or clay) and dose-dependent toxicity of minerals (e.g., lead, selenium, arsenic, etc.), and medicinal Earth materials (e.g., Kaopectate®) depending upon exposure and use (Limpitlaw, 2010).

While we have chosen halite to begin a medical geology investigation into our own laboratory exposures and procedures, other minerals could just as easily be tested for the actual interactions with the students during exposures, with the goal of providing scientific evidence as to the degree of hazard testing these minerals pose within the typical geology

laboratory setting. We suggest similar testing of lead transfer from galena samples to student hands and other samples can be studies using standard lead testing kits as class projects in medical geology. Asbestos transfer can be studied by using the same air and surface sampling techniques used in professional remediation studies. Obviously, upper division mineralogy courses would have additional mineral species to consider. Such studies can help alleviate student anxiety of these issues and provide experiential learning opportunities.

CONCLUSION

Introductory geology labs have traditionally stressed the taste test as a routine and viable method to identify the common mineral halite. However, specimens tested in a lab setting are generally "group" specimens used repeated by many students for many classes, thus there is the concern of germ transfer. In this pre-COVID-19 study, cultured microbes from one halite sample that had been used for nearly 25 years in a classroom setting by hundreds of students, a sample repeatedly licked by only one person, and a commercially purchased unused and freshly exposed surface of halite licked by one person resulted in positive identification of microbes on these specimens. The new crystal licked by one person produced sixteen phenotypic groups of microorganisms. 16S ribosomal RNA gene sequence analysis of nine of those groups identified one *Bacillus* sp., four *Paenibacillus* sp., and four *Staphylococcus* sp. (probably *Staphylococcus epidermidis*). When compared to published studies of the human tongue biome, we find that all of our cultured microbes occur naturally within a typical person's mouth. Under normal conditions, they would not pose significant health risk as used in lab. The primary concern should be on the number of people handling the specimens and the time between testing. In a lab setting, microbes in saliva are transmitted as the halite is reused, especially if the test is administered quickly after a previous licking, so caution is warranted. For the geologist working in the field, where samples are not handled by others, the process is essentially safe as the sample is reasonably "isolated." Halite is geologically and chemically stable and is characterized by a low water permeability, suggesting that microbes isolated from the inside of the the mineral are likely to have syndepositional orgins as the mineral (Jaakkola et al., 2016). Microbe species isolated from deposited halite range in the dozens, suggesting a lack of diversity in species that can survive this harsh environment (Kim et al., 2012).

So, what can be done in a geology laboratory setting when teaching the taste test? The simplest solution to reduce this possibility, and further alleviate student apprehension over the test, is for students to be provided new specimens with each lab, which they break before tasting (which will reinforce the recognition of cubic cleavage), or require students to have "personal kits" of minerals. Only very small samples are actually needed for the taste test and these can be discarded. The cost would be minimal. Furthermore, we advocate textbook authors consider adding discussions of the safety of the taste test to their manuals along with suggested procedures for taste testing and that laboratories provide and strongly encourage hand sanitizing immediately after the halite testing. We would generalize these practices to other potentially hazardous geologic materials (e.g., galena, etc.). Additionally, preservation of infectious disease microbes is of interest to agricultural communities using salt blocks and in situations where livestock mix with wildlife (see Kaneene et al., 2017, for examples).

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