This document was prepared in conjunction with work accomplished under Contract No. DE-AC09-96SR18500 with the U.S. Department of Energy.

EVAPORITE MICROBIAL FILMS, MATS, MICROBIALITES AND STROMATOLITES.

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TITLE RUNNING HEAD (Microbial Films).

KEYWORDS (MICROBIAL FILMS, BIOFILMS, ECPS, MATS, MICROBIALITES AND STROMATOLITES).

ABSTRACT

Evaporitic environments are found in a variety of depositional environments as early as the Archean. The depositional settings, microbial community and mineralogical composition vary significantly as no two settings are identical. The common thread linking all of the settings is that evaporation exceeds precipitation resulting in elevated concentrations of cations and anions that are higher than in oceanic systems. The Dead Sea and Storrs Lake are examples of two diverse modern evaporitic settings as the former is below sea level and the latter is a coastal lake on an island in the Caribbean. Each system varies in water chemistry as the Dead Sea dissolved ions originate from surface weathered materials, springs, and aquifers while Storrs Lake dissolved ion concentration is primarily derived from sea water. Consequently some of the ions, i.e., Sr, Ba are found at significantly lower concentrations in Storrs Lake than in the Dead Sea. The origin of the dissolved ions are ultimately responsible for the pH of each system, alkaline versus mildly acidic. Each system exhibits unique biogeochemical properties as the extreme environments select certain microorganisms. Storrs Lake possesses significant biofilms and stromatolitic deposits and the alkalinity varies depending on rainfall and storm activity. The microbial community Storrs Lake is much more diverse and active than those observed in the Dead Sea. The Dead Sea waters are mildly acidic, lack stromatolites, and possess a lower density of microbial populations. The general absence of microbial and biofilm fossilization is due to the depletion of HCO_3 and slightly acidic pH.

1.0 INTRODUCTION

The existence of evaporitic environments can be inferred as early as the Archean (Grozinger 2003), but their depositional setting has varied over time. Some may have been lacustrine, some tidal flats or restricted marine, some possibly cave deposits while others indicate playa settings (Buck 1980, 1992, Lowe 1983, Walter 1983, Olson 1984, Lindsay & Leven 1986, Muir 1987, Martini 1990, Pope & Grozinger 2003). Each system

is influenced by a different set of parameters and these differences can be reflected in the pH, ions in solution, organic and detrital sources.

Evaporitic environments, at least in geological terms, are transitional. However, sedimentary structures of organo-physico-chemical origin from these environments have been useful for historical classification (Eriksson et al. 2007). Changing climates can alter these environments and lead to their demise. For instance, the Dead Sea shoreline is dropping due to a variety of factors which are partly influenced by human activities (Yechieli et al. 1998). Storrs Lake, San Salvador Bahamas could disappear if sea levels continue to rise due to global warming or potential nearby development. Similar scenarios could affect the existence of other sites.

In this work we will compare the microbiology and geochemistry of two diverse hypersaline systems, Storrs Lake in San Salvador, Bahamas, an island sea level lake and the Dead Sea, Israel, an inland evaporite basin. The objectives of this study are to i) compare and contrast the fossilization of microbes and their organic products in environments that differ in salinity and substrate, ii) use field combined with analytical techniques, electron microscopy (EM), and microbiological techniques for identification and characterization of microbial communities in environmental samples, iii) to discuss potential fossilization processes; identify probable microbial fossils, and the metallic ions association with fossilization and iv) document the role importance of both biotic and abiotic processes for biofilm development in evaporite systems.

2.0 Background Information

2.1 *Microbial Communities*

Diverse microbial communities can develop within high salinity environments. The microbes are responsible for diverse biogeochemical and metabolic interactions that also alter any given environment (Krumbein et al. 2003). Temperature, community composition, grazing by eukaryotes and water chemistry (particularly salinity) influence the rate of formation and lithification rates of these communities. Cyanobacteria are

important in these saline environments due to their adaptation to desiccation and other stressors including ultraviolet light (UV) and nutrient limitations (Oren 1993).

These microbial communities, as determined by the substratum and environmental influences, can form simple structures that include the production of microbial films or biofilms, mats, microbialites, or complex stromatolites. A simple structure could be 1-5 µm in thickness and composed mostly of monoculture biofilms (Brigmon et al. 1995) or a stromatolite composed of a highly diverse active microbial community (Farmer & Des Marais 1994). Although all of these structures could be viewed as a continuum of size and complexity due to the interactions of microorganisms, environmental conditions, and organic products including extracellular polymers (ECPS) and inorganic substrates such as sand and dust (Figure 1), certain characteristics define differences between the structural types. For example, biofilms are somewhat less complex, usually thinner, can form on living macrobiota and are more transient than other microbial consortial structures as those observed in cave vents (Brigmon et al, 1995). Abiotic processes including sediment deposition and physical/chemical precipitation can also be important in biofilm or mat formation (Figure 1). These materials can serve as substrates for attachment, potential nutrient sources, as well as an ecological niche necessary for community development.

2.2 *Biofilms*

Biofilms exhibit a wide variation in complexity that is largely dependent on interactions among microorganisms, environmental conditions, and organic products including extracellular polymers (ECPS) and inorganic substrates such as sand and dust (Figure 1). Several classification systems have been developed to classify these unique microbial communities that result in structures (Eriksson et al. 2007). A formal system termed ‘microbially induced sedimentary structures’ (MISS) has been developed to classify the fossilization of biofilms (Noffke et al. 2001). For descriptive purposes that variation can be categorized into three types, subaquatic, subaerial, and biodictyon (Krumbein et al. 2003).

Subaquatic biofilms are, as the name implies, biofilms constantly exposed to water. These aquatic biofilms, both marine and freshwater, demonstrate varying structural spatial and temporal heterogeneity. The marine microbial communities may form uniquely structures mucopolysaccharide layers in combination with other structures including corals for maintenance (Ritchie & Smith 2004). Marine biofilms can be highly diverse and may also contain Archaea (Wegley et al. 2002). Environmental changes, such as tidal (marine) seasonal or episodic (storms) can drastically influence the biofilm structure, particularly when sedimentary deposition rates are altered. In carbonate rich water where sediment trapping, binding, as well as lithification occurs, these components are instrumental in forming stromatolites.

Subaerial biofilms are composed of 99% organic material with minimal amounts of water and can survive extreme environmental conditions such as evaporation or drought. The biofilms can cover rocks, minerals, sand, and other surfaces exposed to the atmosphere. Nutrients sources may include detritus, pollen, dust, animal (i.e. bird) waste, and runoff. Microorganisms in these films will often include phototrophic and nitrogen fixing species as well as chemoautotrophs. Subaerial biofilms can be observed in lichen communities, tidal mats, algal dominated systems, and covering rocks and other dry surfaces (Figure 2). These biofilms are noted for their microbially produced melanins, cartenoids, mineral accumulation, metal precipitation, chlorophyll, and other metabolic byproducts that give them distinct pigmentation. Microbial activity in both sub-aquatic and sub-aerial biofilms can significantly increase the breakdown of silica in the amorphous, sub-crystalline, crystalline and granular forms of quartz (Brehm et al. 2005). This work by Brehm et al. (2005) emphasized weathering-enhancing processes that included effects of microorganisms and biofilms.

The term biodictyon comes from the Greek “bios” for life and “dictyon” net.” These biofilms are characterized by living networks of mostly filamentous organisms imbedded in soil, sediment, or rock and form mats. The networks create an ecological niche for trapping other microorganisms, minerals, sediments, water and other nutrients as they percolate through the matrix. In certain conditions, including

intertidal and mineral springs, the networks can generate ooids or calcispheres. The precipitation of ooids and calcispheres contribute to mat lithification (Dupraz & Visscher 2005). Higher organisms including bryozoans may depend on microorganisms in mats for mutual benefit as in hydrothermal systems (Morris et al. 2002). This interaction may include structural development in the bryozoans (Morris & Soule 2005).

2.3 *Mats:*

Mats are multilayered, multidimensional matrixed microbial communities that incorporate detritus, minerals, and associated geochemical materials including crystals (Krumbein 2003). The interwoven patterns can form laminated or concentric structures. The pigments chlorophyll, phycocyanin and phycoerythrin are frequently detected by chromatographic and spectroscopic techniques. They can produce new minerals and in evaporite environments influence the chemistry and associated microbial ecology (Gerdes et al. 1987, Noffke et al. 2001).

2.4 *Microbialite:*

Microbialites are benthic microbial carbonate deposits that can vary in shape, i.e., columnars, sheet-like, branched, head shaped, depending on the microbial population, environment and the degree of lamination (Reid et al. 2003, Dupraz & Visscher 2005). Microbialites formation results from geochemical interactions combined with exopolymer-mediated calcification of cyanobacteria-dominated microbial mats. The biogeochemical interactions for microbialite formation occurring in a hypersaline lake (Eleuthera, Bahamas) has been described by Dupraz et al. (2004). Partial degradation of microbial produced extracellular polymeric substances (ECPS) by aerobic heterotrophs or UV fuels sulfate-reducing activity and increases alkalinity in mats, inducing $CaCO_3$ precipitation. As a result the ECPS biofilm is calcified and serves as a substrate for physico-chemical precipitation of additional minerals from the alkaline lake water allowing build up of the microbialites.

2.5 *Lithification:*

Lithfication is a microbialite characteristic and can vary from small scale nodules (mm) to larger structures including stromatolites (Krumbein et al. 2003). Microbialites are organosedimentary deposits that have accreted into structures as a result of benthic (prokaryotic and/or eukaryotic) communities or biofilms, trapping and binding detrital sediment in a polysaccharide matrix and / or becoming a niche for mineral precipitation and are more prone to fossilization (Burne & Moore 1987). The interaction of cyanobacteria with other bacteria has shown to be critical in lithification. Experimental data have demonstrated that calcium carbonate precipitation only occurred on cyanobacterial filaments in the presence of active bacteria under specific geochemical conditions (Chafetz & Buczynski 1992). It was also found that dead cyanobacteria were coated with calcium carbonate by bacterial precipitation much more rapidly as compared to live cyanobacteria.

2.6 *Stromatolites:*

Stromatolites are produced by a combination of the accreted products from the dynamic interaction of microorganisms, microbial products including ECPS, and sediment (Decho 2000). Stromatolites have been defined as organosedimentary structures produced by sediment trapping, binding, and/or precipitation activity of microorganisms, primarily cyanobacteria (Awramik 1984). Certain cyanobacteria are known to precipitate, trap, and bind particles of calcium carbonate to form structures and induce lithification (Chafetz & Buczynski 1992).

2.7 *Storrs Lake Microbial Depositional Structures:*

Cyanobacteria have evolved different multiple strategies to adapt to environmental stresses including drying, high salinity, low nutrients, and UV light (Castenholz & Garcia Pichel 2000). The adaptations include pigment production that protects cells from the deleterious effects of UV, desiccation and subsequent cell wall damage. Pigment

production is particularly important in transient evaporite crust environments with wet and dry cycles. The cyanobacteria can dominate in hypersaline aquatic systems with limestone surfaces where they can be endolithic utilizing calcium carbonate that can lead to a permanent increase in travertine in stromatolites (Pentecost & Whitton 2000).

Cyanobacteria may also be the principal nitrogen fixers in a given ecosystem. Recent studies have demonstrated that epilithic cyanobacteria in Holocene beach rock (Heron Island, Great Barrier Reef. Australia) are the main nitrogen fixers (Diez et al. 2007). These cyanobacteria have adapted to a wide range of environments and their key metabolic activities including structural and function in similar hypersaline lakes is of great value to maintenance of microbial-based ecosystems (Dupraz et al. 2004).

The diverse cyanobacteria is the major biofilm producer. The cyanobacteria community is composed predominantly of *Phormidium* as well as *Oscillatoria, Lyngbya* and *Spirulina* (Brigmon et al. 2006). The Storrs Lake cyanobacteria have been observed to be different from those examined at other San Salvador salinity sites as they have a higher proportion of phycobillin pigments (Brigmon et al. 2006). In Storrs Lake evaporite mats, below the photic zone, the cyanobacteria are dead and are often fossilized, but the non-photosynthetic bacteria are dominant and demonstrate high metabolic activity (Brigmon et al. 2006).

The purple sulfur bacteria *Chromatium* spp. is dominant in the Storrs Lake reddish-pigmented layer biofilms that are frequently found on both the shoreline evaporite crusts and stromatolites. Sulfate reducing bacteria (SRBs) including *Desulfovibrio sp.* and the sulfur oxidizing bacteria, *Thiothrix* spp., are components of the stromatolite biofilms (Brigmon et al. 2006). SRBs function in the deeper or oxygen limited layers of the biofilm where lithification may be occurring while the sulfur oxidizers thrive at the oxygen interface near the water surface getting energy from the hydrogen sulfide generated from the deeper anaerobic portions of the biomats (Brigmon et al. 1994).

2.8 *Geology*

Storrs Lake, San Salvador Island, Bahamas (Figure 3): The hypersaline lake is located on the eastern side of San Salvador Island. It is 2.0 meters in depth and about 7.3 km long and 1.3 km to 50 m wide and has well developed stromatolites that are located a few hundred yards from the sea. The lake contains islands which support a diversity of plants including mangroves and sedges (*Carex*). Water is supplied from conduits within the bedrock, which includes the fossil reef, and seepage through Holocene sand that allows limited exchange with the ocean (Davis & Johnson 1990). Additional water sources are rainfall and tropical storms that carry ocean water on shore and deposit it in Storrs and other San Salvador Island coastal lakes. The island, sediment deposition, and its ecosystem are influenced by hurricanes (Yannarell et al. 2007). Fluctuations in Storrs Lake water chemistry have been observed to vary in a 20cm range (Zabielski & Neumann 1990). The water salinities vary from 70 to 100 g L^{-1} and the pH varies between 8 and 9, depending on rainfall and storm activity.

Few plants or animals were observed in or out of the lake along the littoral zone besides the microbial mats, associated stromatolites, microbialites, except the mangrove *Rhizophora* sp. and some sedge (*Carex*) that rim the lake. Microbial mats bordering the lake vary in size and thickness depending on weather conditions. These mats contain *Rhizophora* leaves which are assimilated into the mats (Figure 2). Organisms living in the water column include cyanobacteria, bacteria, diatoms, ostracods, and infrequent gastropods. Most likely halophilic Archaea species could be found in hypersaline Storrs Lake but were not measured here. The current chemical and detrital sedimentary deposits within the lake are usually less than one meter thick and composed of finely laminated organic-rich carbonate mud and evaporite similar to Salt Pond that is adjacent to Storrs Lake (Yannarell et al. 2007). The nitrogen-fixing microbial community in Salt Pond has been shown to change spatially with seasonal salinity changes (Yannarell et al. 2006). The mineralogy of these sediments include argonite, gypsum, and algal derived high-magnesium calcite in the form of clay sized and sand sized particles as well as a variety of stromatolitic structures (Zabielski &Neumann 1990).

During the Pleistocene, large portions of the island were covered by carbonate reefs at levels that were above today's sea levels (Teeter 1995). According to Teeter the lakes are floored by Pleistocene carbonate bedrock covered by approximately 2 M of unconsolidated Holocene sediments. Storrs Lake has been directly connected with the ocean and the paleosalinity history has been reconstructed from MgO content of ostracod carapaces (Teeter 1995). The geology of San Salvador includes three major rock types' eolanites, beachrock, and reefrock (Davis & Johnson 1990). The eolanites are most evident in carbonate dune ridges and found throughout the island including the subsurface. The beachrock consists mostly of cemented shell fragments and ooliths and can be seen covering other rocks on the beaches. Reefrock is made up of fossilized reefs, including various corals, sponges, and cyanobacteria. The reported fossil reefs are found near the current island shoreline, including Storrs Lake. Stromatolites in Storrs Lake have been estimated to range in age 2310+/-70 yrs., growing at the rate of 16 cm/1000 years (Elliot 1994). Storrs Lake, which is probably as old, if not older than the earliest dated modern stromatolites Surveys of the various San Salvador lakes have demonstrated the existence of a diverse water chemistry with some having salinities up to 300g L^{-1} (Teeter 1995).

Dead Sea Israel (Figure 4):

The Dead Sea, a nonmarine evaporite basin, 400 m below sea level with an average pH of 6.3, and salinity of 229.9 g L^{-1}, is located on the northern branch of the African-Levant Rift Systems (Figure 4). The rift system, according to one model, was formed by a series of strike slip faults, initially forming approximately 2 million years ago (Csato, et al. 1997). Over geologic time the rift was occupied by a series of lakes; their existence was controlled by both tectonic activity and climate. Today the remaining lakes are the Sea of Galilee (Lake Kinneret) and the Dead Sea. The precursor of the Dead Sea was another hypersaline body of water, Lake Lisan (Yechieli et al. 1998). The Dead Sea receives its waters from the Jordan River system, runoff from wadis (Arabic for seasonal streams) during the winter months, limited rainwater and from surrounding aquifers both subterraneous and through springs (Yechieli et al. 1996, Ehrlich et al. 1985; Friedman

1998). The detrital and dissolved mineral materials deposited in the Dead Sea are from windblown materials and rock products ranging in age from Triassic to Quaternary and includes gypsum, alkali basalt, chert, conglomerate, sandstone, limestone, clay, sand, sandstone, mudstone, marl, chalk, dolostone (Sneh et al. 1998). Input from a variety of aquatic systems as well as weathering of these rock materials have contributed to the overall dissolved components in the Dead Sea.

During the 20th century the water level dropped and in 1979, after 300 years, the meromictic stratification with an anoxic water mass below 40 m was altered (Herut et al. 1997). Today it experiences mostly annual stratification or a holometric regime (Lensky et al. 2005, Gavrieli et al. 2006). The drop in water, at least in part, is a result of increased fresh water diversion along the Jordan River system. Fresh water diversion may not entirely account for the drop in water level as the balances of evaporation rate and subsurface water flow are not well understood (Lensky et al. 2005). With the drop in surface levels, the Dead Sea was separated into a northern and a southern basin in the 1960's (Steinhorn 1997). The northern basin is ~324 M deep while the southern basin is shallower with a maximum depth of 8 m (Nissenbaum 1975, Steinhorn 1997). The southern basin is divided into evaporation pans for salt and potash production (Anati 1997, Hall 1997, Steinhorn 1997). The residual end brines are depleted in potassium, sodium and enriched in magnesium and chloride; these brines are subsequently pumped back into the Dead Sea. The effects are increased halite precipitation and salinity (Garvrieli 1997). The Dead Sea is expected to reach equilibrium, but not dry up as the unique brine that would be left (Mg, Na, Ca, and Cl) and the low surface area to volume ratio would reduce the evaporation rate (Yechieli et al. 1998).

3.0 METHODS AND MATERIALS

The collecting and processing methods described below for the sites follow procedures that have been described by a number of authors (D'Amelio et al. 1989, Thomas Keprta et al. 1998, Morris et al. 2003, Fratesi et al. 2004, Brigmon et al. 2006)

3.1 *Storrs Lake*

Stromatolites, biofilms, and water samples were collected from Storrs Lake, on the east side of San Salvador, Bahamas. Samples were taken along from the west shore of the lake to the east side of Cactus Island (Figure 3). The coordinates and description of the sampling sites are listed in Table 1. The stromatolites, at all sampling points, had a cauliflower-type appearance, and varied in diameter from 10 cm-1.5 m. The microbial mats, covering the stromatolites in the water column, were 0.5 m thick in some places. Samples were collected with either 15 or 50 ml sterile polycarbonate tube (Fisher Scientific Fairlawn, NJ). Stromatolites were sampled by using the 50 cc sterile tubes to core a 2-3 cm long and 2 cm wide segment from the stromatolites at Sites 1, 2, and & 7. The carbonate material was soft and easily obtained. Samples of microbial mats were obtained with either the 15 or 50 ml tubes. The samples were obtained at depths from 0.1 - 0.5 m and kept cold (~ 3° C) until laboratory processing. Some biofilms were sampled by scraping the edge of the tube on the film to limit perturbation of the stromatolites and liquid samples were collected by sterile 3 ml syringe. Select samples were kept on ice and returned to the US within 48hr for microbial analysis. Samples for microscopy and total microbial densities were fixed in the field with 10% formalin (Fisher Scientific, Fairlawn, NJ). Temperature was measured in the field with a thermometer, dissolved oxygen concentration, nitrate, nitrite, and hydrogen sulfides were determined with field test kits (Chemetrics, Calverton, VA). Salinity was measured with a refractometer and pH with a battery powered test meter (Fisher Scientific). A geochemical water survey of a 1000 M transect through Storrs Lake, was conducted from the western shore to the eastern point of Cactus Island in July, 2001. Nitrate, nitrite, dissolved oxygen, temperature, pH, and salinity measurements were taken at 12 sampling sites with location coordinates determined by GPS (Garmin Model GPS 76). The high salinity of the water required dilution with deionized water (DI) to be examined with a refractometer. Measurements were taken with water samples obtained from a depth of 0.25m. Storrs Lake had a depth of, at the most, 2.0 m during this sampling event.

Three water samples were collected and brought back from Sites 3 (water column) and 5 (stromatolite ridge) on ice for laboratory analysis by ion chromatography

(IC). Chloride, sodium, lithium, manganese, calcium, nitrite, nitrate, phosphate, and sulfate concentrations from Storrs Lake were measured with a Dionex DX500 ion chromatograph equipped with a conductivity detector, and a 250-mm Dionex IonPac AS14 Analytical column (4-mm ID, 16-µm bead; Dionex Corp., Sunnyvale, CA), operated at ambient temperatures. A 3.5 mM sodium carbonate/1 mM sodium bicarbonate buffer solution was used as the eluent (1.2 mL/min) for the IC. Water samples were diluted 100X in deionized water vortexed for 1 minute then centrifuged for 5 minutes at 2500 rpm to prepare for the IC.

Total microbial population densities in the stromatolite samples obtained from Cores at Sites 1, 2, and 7 were determined by the Acridine Orange Direct Count (AODC) technique (Brigmon & De Ridder 1996). Discrete samples were collected aseptically from formalin fixed stromatolite cores, mixed with filter sterilized FA Buffer by Difco Inc. (Detroit, MI), and vortexed for 4 minutes. The resulting dilutions were filtered through Nucleopore, polycarbonate 0.2µm membranes and all microorganisms (prokaryotes, cyanobacteria, Archaea, fungi) were counted using epifluorescent microscopy (Axioskop, Carl Zeiss Inc., Thornwood, NY). Dry weights were determined, and microbial density results were reported in cells/gdw.

Aerobic heterotrophic plate counts provide an estimate of the total number of viable aerobic and facultative bacteria in the stromatolites. Plates were prepared with fresh field samples at the Bahamas field Station Laboratory to determine colony forming units (CFUs). Briefly, fresh 5 gm aliquots of stromatolites from Storrs Lake were weighed, ground, and aseptically mixed with 45mL 0.2 µm filter –sterilized sea water, vortexed for 4 minutes, and plated on non-selective media glycerol artificial seawater agar,(GASW) within hours after collection (Smith & Hayasaka 1982). Five dilutions were made in sterile seawater to determine microbial densities. Each dilution was plated in triplicate and the cultures were incubated at 37°C and CFUs determined after 7 days on a Leica Quebec Darkfield Colony Counter. Dry weights were determined on the stromatolite material tested and density determinations are reported in CFU/gram dry weight (GDW).

3. 2 *Dead Sea*

The samples were collected from seven sites on the western shoreline which included 1 site at Ein Gedi, three at Ein Boqeq, one at Hamme Zohar, and two at Mt. Sedom. The coordinates for the sites, collected sedimentary materials, water temperature and collecting depths are listed in Table 2. The coordinates were determined with a Magellan Model 2500T. Sterile 50 ml tubes (Fisher Scientific, Fairlawn, NJ with screw caps were used to collect the materials and water samples. With the exception of the Ein Gedi halite, all samples were immediately preserved in 5% formalin (Fisher Scientific, Fairlawn, NJ). Halite samples dissolve in formaldehyde and, in order to preserve the relationship of the microbes to their substrates, the samples were kept in sterile plastic bags without preservation. Water samples were collected at each collecting site and analyzed later for salinity, pH and chemical composition. Chemical analysis was done by S. Grasby, Canadian Geological Survey. Carlton C. Allen, NASA Johnson Space Center used a Scintag X-ray powder diffractometer (XRD) for mineral identification.

3.3 *Electron Microscopy Analysis of Storrs Lake and the Dead Sea Samples:*

All preserved samples were initially analyzed with a Philips XL30 environmental electron microscope (ESEM) and subsequently critically point dried, platinum coated for 15 seconds and analyzed with a JEOL 6340F field emission scanning electron microscope (FE-SEM) equipped with a light element electron dispersive X-ray spectrometry system (EDS). Carbonate and evaporite materials are subject to charging by the FE-SEM electron beam and can either destroy or alter thin biofilms and other organic features. The problem can be ameliorated by reducing the kV and adjusting the working distance. The kV for this study, depending on the materials, was varied from 3 to 10 kV with working distances varying from 4-6.

4.0 **RESULTS**

4.1 *Storrs Lake:*

4.1.1 Microbial Analysis: Storr's Lake microbialites are represented by biotically formed stromatolites, microscopically observed as crystals, mineralized filaments, diatom tests, and other microorganisms. Stromatolites are most evident in the shallower areas of Storrs Lake but in this study were observed across the sampling transect. Samples for electron microscopy were taken from the stromatolite ridge described in Table 1.

Filaments: Mineralized filaments were composed of cyanobacteria and were common in the stromatolite biofilm samples. The forms include both continuous and segmented filaments (Figures 5, 6, 7, and 8). In Figures 5 & 6, and 7 the fossilized cyanobacteria filaments with precipitated calcium carbonate are evident in the stromatolite samples. The fossilized filaments were observed to range in length from approximately ten microns to several dozen microns forming a support matrix. Broken mineralized forms indicate the mineralization was initially limited to external "mold" processes with the cellular material now degraded (Figure 7). Note the thickness of the mineralization surrounding the filament is up to a micron in thickness. Some of the filaments shown in Figure 8 do not show the extent of mineralization and evidence of ECPS indicating they are most likely from layers on the exterior of the stromatolite biofilm. These mineralized formations were found associated with all microbial life in the stromatolites from various cyanobacteria, diatoms, spherical bacteria to biofilm. Some filamentous fungi were observed in Storrs Lake biofilms but were not specifically identified. Fungi are known to have an impact on geological processes including stromatolite formation due to their ability to provide physical structure, aggregate particles, and increase reactive areas (Sterflinger 2000)

Spheres: Spherical forms were observed throughout the samples and were found to vary in size. The largest spheres observed averaged 5.3 μm diameter, were relatively uncommon, and ranged from a smooth to rough texture (Figure 9). Medium sized spheres were often observed throughout the samples, usually appearing in clusters from 2-8, were associated with biofilm, averaged 2.0 μm and ranged from a smooth to rough texture (Figure 10). Smallest spheres were imbedded in biofilm, usually associated with

larger microbial features, apparently forming two distinct populations and varied in texture (Figure 11). The smallest spheres were composed of two populations, the larger averaged 0.55 μm in diameter (Figure 11) and the smallest were 0.13μm. The smaller 0.13μm spheres were most likely produced abiotically but the 0.55 μm may have been produced either biotically or abiotically.

Rod-Shaped: Rod-shaped or somewhat dumbell-shaped structures range in size from approximately 1.0-3.0 μm and often were associated with crystals within the biofilm matrix (Figure 12A, B). Some were characterized by small-scale coarse-grained roughness and often flattened in shape when attached to surfaces (Figure12B). Similar mat communities entrapping 'dumbell"-shaped crystals of aragonite have been observed in Asta Springs at Yellow State Nation Park, Wyoming USA (Farmer & Des Marais 1994). The shorter rods may represent sulfate-reducing bacteria (SRB) as they were frequently associated with calcium sulfate crystals (Figs12B).

Diatoms: Diatoms were also observed as integral portions of the biofilm structure (Figures 13A, B). Figure 13A demonstrates a relatively intact diatom being incorporated into the biofilm. Note the rod-shaped bacteria covering the diatom both in clumps as well as single cells indicating colonization. In Figure 13B a partially degraded diatom test is observed within the biofilm structure. Intact diatoms identified in the samples included mainly *Pinnularia* with some *Navicula* and *Achnanthes* species. Again, the active bacteria attached both to the diatom as well as surrounding it with ECPS thus indicating the ongoing nature of the biofilm building process.

Biofilms: Biofilms were found to consist of organic materials (bacteria, including cyanobacteria, fungi, diatoms) and inorganic (sand, limestone) materials enmeshed within the mat structure. Archaea species may have been observed in EM but other tests are necessary to discern them from other prokaryotes (Oren 1993). Close examination with the FE-SEM indicates that binding is due to microbially produced polysaccharide (ECPS) or "slime" (Figure 14 A, B). Examples of the binding can be seen in Figure 14A, as a fossilized cyanobacteria filament is attached to mineralized sand and rock grains. In

Figure 14B ECPS cross-links crystals several µm apart.

Microbial Densities: Total microbial densities (live, dead, aerobic, and anaerobic) in the stromatolites varied from 2.48 X 10^{10} cells/gdw in the Site 1 sample to 1.58 X 10^{10} cells/gdw in Site 2 (Figure 15). These density determinations included all algae, fungi, cyanobacteria, and bacteria and were made from stromatolite material that was fixed in the field to preserve the sample integrity. The aerobic and/or facultative microbial densities or live microorganisms in the stromatolite cores ranged from 4.75 X 10^{4} CFUs/gdw in Site 2 up to 9.46 X 10^{5} CFUs/gdw in Site 1 (Figure 15). While individual bacteria species were not identified from the viable cultures, many diverse morphological colony types and pigmented type variations were observed growing on the GASW medium.

4.1.2 *Geochemical Gradient:* Table 1 describes the Storrs Lake water sampling sites as to their GPS coordinates and physical characteristics. Table 3 contains all the field data that was taken during the survey. The sampling transect range of the coordinates is from the west shore of Storrs Lake (N 24.0549 W 74.45348) to the far end of Cactus Island (N 24.05943 W 74.44016). The results of the nitrate tests for all sampling sites were consistently below the detection limit (1ppm). Dissolved oxygen (DO) levels in the tests ranged from 6 to 9 ppm with a mean of 7.5 ppm (Table 3). There was a gradient of increasing dissolved oxygen concentrations from the Storrs Lake western shore to the middle of the lake (Figure 16A). The DO variation was most likely due to the higher density of biomats closer to the shorelines (Figure 3). Water temperature ranged from 32.5 to 39.5°C with a mean of 36.6°C (Figure 16B). The lake temperature peaked (39°C-39.5°C) at the first and last sampling sites close to the shorelines were also likely to the insulating effect of the biomats. The lake depths varied from 1-2 M over the sampling transect.

The water pH ranged from 8.24 to 9 with a sampling mean of 8.36 (Table 2). There was a pH gradient higher near the island (pH 8.97) that decreased to 8.2 by the western shore (Figure 17A). The pH showed an interesting profile being highest at Site 7

where the water was deepest (around 2 M) and then again elevated by Cactus Island (Site 12) (Figure 17A). The salinity ranged from 66 to 82 g L^{-1} with a mean of 73.2 g L^{-1} (Table 2). Salinity increased steadily as locations were sampled from west to east (Figure 17B). Salinity followed the DO trend of gradually increasing throughout the sampling sites to the middle of Storrs Lake (Figure 17B). Table 4 summarizes the statistical analysis of the water constituents as a function of location as shown from the survey. The positive correlations in Table 3 for the water chemistry demonstrate a significant trend of increasing dissolved oxygen (6-9 ppm), pH (8.2-9), and salinity water concentrations (66-82 g L^{-1}) across the transect moving west to east. No nitrate or nitrite was detected at any of the sampling sites with the field test kits. These findings were similar to previous geochemical analyses at Storrs Lake (Brigmon et al. 2006).

4.2 *Dead Sea*

The Dead Sea (Figure 4), with a salinity of 332.1 g L^{-1}, initially appears devoid of life as it lacks the large, visual stromatolites and mats of Storrs Lake, San Salvador Island, Bahamas, Shark's Bay, Australia or the tufa mounds of Mono Lake, California (Ehrlich 1985, Shearman 1998, Brigmon et al. 2002, Byrne et al. 2002). Microscopic and molecular data analyses indicate the existence of an abundant, albeit not diverse, halophilic species from the Dead Sea (Oren 1997). Many of the isolates belong to the domain Archaea, specifically Halobacteriacea (Arahal et al. 2000, Mack et al. 1993, Oren 1988, 1993) while the domain Bacteria is represented by gram-negative, moderate halophilic species, for example *Bacillus marismortui* (Oren 1988). Other microbial components include the green alga *Duniella*, halophilic ciliate and amoeboid protozoa, fungi and cyanobacteria (Elazari-Volcani 1944, Ehrlich et al. 1985, Huval et al. 1995, Oren 1997). These microorganisms have been classified based on their environmental salt preference ranging from moderately halophilic species able to grow optimally between 0.5 and 2.5 M salt (Ventosa et al. 1998) to extremely halophilic up to 3.4 M and greater salt concentrations (Arahal et al. 2000).

4.2.1 Evaporite Minerals (FE-SEM Analysis): Microorganisms associated with chloride mineral surfaces were limited to rod-shaped structures with filamentous, apical extensions (Figure 18A). The microorganism and the chloride mineral were not stable in the FE-SEM electron beam and as a result subject to charging and deterioration which can occur quickly during the imaging process. Figure 18A is an example of the process as indicated by the unnatural wavy surface of the chloride mineral and the hole in one of the microbial filaments. Microbial fossilization is a cumulative process and begins with the precipitation of a limited number of $CaCO_3$ crystals on the surface of the extant organism with subsequent deposits that enclose the microbe and limited adjacent areas (Figure 18A, B). Thin biofilms extend outward from the microbe as indicated by the folding on the surface that formed during critical point drying (Figure 18B). Gypsum deposits were small, usually in the 5-7 μm range and associated with a chloride mineral. No evidence of microbial remains was found incrusting on the mineral.

4.2.2 Putative Microbes Associated with Sand-Sized Orthoclase and Quartz, Silts, Clays (FE-SEM Analysis): Sand sized orthoclase and quartz fragments were anomalous as to the potential identification of microbes, biofilms or fossilized remains. Rice grain shaped deposits that were in the 2-5 μm size range for microbes were found on these detrital fragments, but there was no evidence of biofilms or other microbial remains (Figure 18C). EDS spectra indicated low to nonexistent levels of carbon or other chemical signals of biotic remains such as potassium.

4.2.3 Microbes Associated with Silts and Clays (ESEM and FE-SEM Analysis) Materials that were preserved in 10% formalin (v/v) in the field were brought back to the laboratory and observed with an environmental scanning electron microscope. This method of analysis proved to be very productive as a wide array of unfossilized microbes and thin or poorly biofilms were detected. Some microbes were elongated with a hammer-like extension that appears to be attached to detrital fragments (Figure 18D, 19A). Others were elongated, appearing to bend around fragments and some were rod shaped (Figures 19B, C, D). The bacillus or rod shaped varied from those with relatively straight walls to those with more or less rounded rods to (Figures 19C, D). The bacillus-shaped microbe

in Figure 19D (Mt. Sedom) appears to have substantial amounts of fossilized ECPS materials surrounding it. Some of the Mt. Sedom observed morphologies may represent the modern population, but some could have been weathered and transported from the surrounding geological deposits in a manner similar to the fossil coccoliths that were found in association with silts and clays (not shown). Figures 19A & C demonstrates different morphologies typically observed in the Dead Sea samples attached to substrate. Figure 19A represents filamentous-shaped microbes encased in a thin biofilms while 19C there can be seen rod-shaped microorganisms.

All of the Dead Sea samples analyzed have lower microbial densities and apparently lower species diversity (Figures 10, 12B, 18A, B, C, D). While some biofilms were observed (Figure 18B), they were generally monolayer or only a few μm in thickness.

4.2.4 Mineralogy: The XRD analysis of the sedimentary materials from Ein Gedi included evaporites, primarily halite, sand-sized orthoclase and quartz sediments. Samples from Ein Boqeq and Mt. Sedom were primarily composed of silts and clays with lesser amounts of evaporites. EDS analysis indicates calcium sulfate (SEM visual analysis indicates gypsum), halite, magnesium chloride, potassium chloride and various silts and clays. In addition to these minerals, other investigators have reported magnesium bromide, carnallite, and calcium chlorite (Nissenbaum 1975, Ehrlich 1985, Zak 1997).

4.3 *Water Chemistry.*

A comparison of the water chemistry analysis for the Dead Sea and Storrs Lake are summarized in Table 5.

4.3.1 Storrs Lake: The water chemistry for Storrs Lake varied, depending on whether the samples were obtained adjacent to the stromatolites or were obtained from the open areas of the lake (Table 5). The levels of ions near the stromatolites in descending order of dominance are Cl> Na> SO_4 > F> Mn> Ca> K. The following were not detected: Li,

Fe, NO_2, NO_3, PO_4. In the open waters their levels, in descending order of dominance are Cl> Na> Ca> SO_4 > Mn. The following were not detected: K, Li, Fe, NO_2 , NO_3 , and PO4. Most of these dissolved components represent major seawater components with the exception of Mn, Li, and F.

4.3.2 Dead Sea*:* The northern basin ions are represented in descending order by Mg> Ca > Cl> Na> K> Sr> HCO_3 >SO_4 >Si> Mn> Li (Table 5). The following were not detected Fe, Ba, and NO_3. The southern basin ions, in descending order were K> Mg> Na> Cl> Ca> SO_4 > HCO_3 > Sr> Mn> Li. The following were not detected: Fe, Ba, Si, and NO_3. Nitrite (NO_2) was not measured for the Dead Sea samples. The levels of the ions were similar in both basins with the exception of SO_4 that was higher in the southern basin and Sr which was higher in the northern basin.

The dissolved ions, originating from surface weathered materials, springs, and aquifers, are more diversified in the Dead Sea because of the long geological time encompassed in its formation. Storrs Lake dissolved ion concentration is primarily derived from sea water and not products of geological processes over millions of years. Consequently some of the ions, i.e., Sr, Ba are found at significantly lower concentrations than in the Dead Sea. The origin of the dissolved ions are ultimately responsible for the pH of each system, alkaline versus mildly acidic.

5. DISCUSSION

5.1 *Nitrogen:*

Storrs Lake: Primary production and the conversion of N_2 to NH_3 (N_2 fixation) by certain cyanobacteria and eubacteria are important metabolic indicators of the potential contribution of microbial mats to carbon and nitrogen budgets of intertidal communities. From the data shown here it is evident that nitrogen is extremely limited Storrs Lake (Table 5. N_2 fixation helps circumvent chronic N-limitation in oligotrophic marine systems. This process may meet mat and community N demands or serve as supplement (rather than exclusive) source of “new” nitrogen for mat growth. The large cyanobacterial component in this system no doubt plays a major role in nitrogen fixation.

In either case, the environmental factors regulating N_2 fixation may subsequently control other mat activities including fixation, primary production, and growth of stromatolites.

Dead Sea: NO_3 (Table 5) was not detected and NO_2 was not measured in any of the samples. Previous researches have measured N and the conclusions are that biological processes have minimal impact on the nitrogen cycles (Stiller and Nissenbaum 1999). Historically the levels of N, particularly in the form of ammonia, increased after the 1979 water overturn. For example in 1960 the recorded levels of N in the form of ammonia was 5.9 mg L^{-1}, but increased in 1991 to 8.9 mg L^{-1}. The ammonia sources were diffusion from bottom sediments and potentially production in oxygenated water by mineralization.

5.2 *Strontium*

Storrs Lake: Strontium levels were not measured in this work. Previous studies on San Salvador have demonstrated that strontium concentrations are elevated in comparison to open marine systems, but are not as concentrated as in the Dead Sea (Swart et al. 1987). The primary reason is that the origin of the waters and the influence of heavy rain and tropical storms due to the proximity to the ocean (Figure 3).

Dead Sea: Strontium levels are elevated in comparison to Storrs Lake and make have had several sources. The sources may have been from the Sedom lagoon, a Pliocene marine evaporite environment that formed the Sedom Formation. The Sedom Formation is exposed at Mt. Sedom (Table 2). Adjacent to the Sedom lagoon there was a Cretaceous limestone and its weathered products provided additional strontium. An alternative method would have been dolomitization of the initial $CaCO_3$ in the Sedom lagoon (Gavrieli & Stein 2006). The earlier depositional sequences contributed to the water chemistry of the Dead Sea's hypersaline Pleistocene precursor, Lake Lisan. Thus the strontium, as well as the elevated concentration of other ions represents the evolution of a sea level evaporite lagoon to an evaporite environment below sea level.

5.3 *Mineralization*

We know that biological activity influences the geological processes of mineral precipitation and stromatolite building, the heterogeneity and temporal impacts of environmental influences makes predictions and all encompassing explanations difficult as biofilms associated with modern marine stromatolites are subjected to constantly changing environmental conditions resulting from tidal, seasonal, diurnal, and depositional events. For instance, the presence of mats or biofilms and organic substrates can provide favorable sites for the nucleations of crystals and contribute directly to biofilm structure (Farmer & De Marais 1994).

Storrs Lake: The process of biofilm formation with biogeochemical interactions is demonstrated in Storrs Lake samples as crystals are covered with bacteria in Figures 12A and B. Figure 14 shows the exopolysaccharide produced by microorganisms and their role in binding inorganic materials including crystals. In hypersaline conditions this biological activity can lead to laminated deposition of minerals and associated microorganisms. For karst or carbonate waters and sediment the cumulative interaction of microorganisms and geochemical conditions in the right environment can results in stromatolite formation (Eriksson et al. 2007).

Dead Sea: There is a paucity of microbially mediated mineralization and biofilm development (Figures 18, 19). A mildly acidic pH and low carbonate levels are counterproductive to calcium carbonate or dolomite precipitation (Table 5). Microbial diversity is restricted to obligate halophilic tolerant groups with periodic blooms of other groups such as the one celled green alga *Dunaliella* after seasonal rains and runoffs from the wadis have diluted the surface waters (Nissenbaum 1975, Oren 1997). Under these conditions microbial groups commonly found in Storrs Lake such as cyanobacteria and fungi are restricted in their development. The halophilic groups produce relatively thin ECPS (Figure 18A, B, 19 C, D).

5.4 *Environmental Influences*

Storrs Lake: Evaporation exceeds precipitation except during the rainy or storm season (Davis & Johnson 1989, Yannarell et al. 2007). The seasonal fluctuations result in large transitions in the water chemistry and microbial activity. Tropical storms, i.e., hurricanes can have even a more dramatic effect on microbial activity due to changes in substrate and sediment and water chemistry (Yannarell et al. 2007). The impact of hurricane disturbance and recovery on microbial community structure and ecosystem functions were studied in a nearby San Salvador hypersaline lagoon, Salt Pond, which is close to Storrs Lake (Yannarell et al. 2007). This environmental microbiology study demonstrated the hurricane related sand deposition on microbial nitrogen fixation rates. The rates were higher in mats re-colonizing sand depositional sites than those re-colonizing sand eroded sites. Microbial population recovery rates were favorably influenced at sites with high diversity which structurally contributed to the rapid recovery of the disturbed ecosystem.

The living ecology in Storr's Lake is limited to a microbial community of mats and no other animals were observed in this work. The microbial mats consisting of bacteria and algae along the shore varied in color from purple to green, brown, with a black bottom layer. There were a few dead fish and crabs observed along the shoreline and seagulls were observed congregating on some of the stromatolites protruding from the lake as well as the island where are nesting. The air around Storrs Lake seeped with a sulfur smell. This sulfur odor was especially evident wading through the lake as the sediments were stirred up. The lake's bottom consisted of areas of stromatolite growth and thick dark anoxic material with a consistency of swamp sediments up to 1 M in depth. An expanding landfill with trash, old vehicles, and other debris several hundred yards Northwest of Storrs Lake could be a threat due to leachate groundwater contamination. Recent anthropogenic activity on San Salvador indicates that the future of Storrs Lake could be in danger due to proposed coastal development (Don Gerace, Personal Communication).

Dead Sea: The Dead Sea is different from sea level evaporite systems similar to Storrs Lake and most nonmarine evaporite systems such as Mono Lake, California and the Great Salt Lake, Utah. For example, the pH level in the upper water mass is slightly acidic, 6.3, rather than basic and the dominant ions are chlorine and magnesium for both the northern and southern basins (Nissenbaum 1975, Ehrlich et al 1985, Domagalski, et al. 1989, MacIntyre et al. 1999). The Dead Sea water levels have dropped over 20 m since the 1950's (Gavrieli & Stein 2006). The drop in water levels is accompanied by a gradual increase in salinity and as the sulfate and bicarbonate are depleted, precipitation of aragonite and gypsum is decreased (Nissenbaum 1975, Gavrieli & Stein 2006. These events have been responsible for the increased halite precipitation. The changes in mineral precipitation may in part be due to the evaporation ponds in the southern basin (Gavrieli & Stein 2006). Nitrate levels have increased, but are still low as the area is arid and lacks significant numbers of cars there are no coal burning industries (Berner & Berner 1996, Stiller et al. 1999).

5.5 *Hydrologic Systems*

Storrs Lake: The hydrologic system on San Salvador has been documented by Davis and Johnson (1989). As is typical of karst areas there are no surface water streams. Potential reasons for the trends demonstrated here are not certain as the water flow patterns can vary. Various vents feeding the water supply were noted on the North end of the Lake. Smaller vents were observed on Cactus Island. Another factor is tidal influence, not directly but maybe through subsurface seeps (Teeter 1995). These seeps may change with changes in sediment deposition due to storm events. One possibility is when rain falls, water flows in towards the middle of the lake, making the hydrogen gradient stronger (pH) in the middle than on the banks. This is evident in its hypersaline characteristic. The ocean side was higher in salinity, suggesting that this could be due to weathering of atmospheric salt deposition. The potential of biogeochemical interactions influencing the water chemistry due to the thick biofilm evident throughout the lake is also an alternative reason.

Dead Sea: The lake is roughly rectangular in shape with no outlet and is separated into a northern and southern basin. The northern basin is 400 M in depth and the southern basin is shallower, varying from 6-8 M. The only fresh water source is the Jordan and limited seasonal rain waters. The Dead Sea is dominated by a variety of salts, most of which are emanating from springs in and around the lake (Nissenbaum 1975, Lensky et al. 2005). Many of the salts and the high levels of strontium are a result of meteoritic water circulating through residual brines deposition beginning with the Sedom Lagoon (Lensky et al. 2005).

5.6 *Biofilms, Mats, Stromatolites:*

Microbial film formation is dependent upon different factors for growth and metabolism. Marine biofilms form on a variety of biotic and abiotic surfaces. In intertidal systems these can be quite extensive (Decho 2000). Development of marine biofilms on abiotic surfaces begins with the attachment of microbes to surfaces and the secretion of extracellular polymers (Decho 2000, Kawaguchi & Decho 2002). Cell to cell signals influence both community profiles and metabolic activities within the polymeric matrix (Davies et al. 1998). These can develop into mats with the addition of photoautotrophs to the community, which then can stimulate the precipitation or trapping of sediment.

The evolution from biofilms to mats to stromatolites is closely linked to water chemistry and the absence of invertebrate herbivores. Stromatolites are found in many different regions of the world in a variety of habitats, including hypersaline environments. All of the stromatolites have a similar microbial ecology including phototrophic microorganisms and photosynthetic microbial mats combined with fossilization (Reid et al. 2003). In alkaline hypersaline conditions both cyanobacteria and algae enable $CaCO_3$ precipitation, thus encouraging extensive microbialite structures.

An example of modern stromatolites growing in a normal, tropical marine, intertidal environment have been described from Stocking Island, Bahamas (Pickney et al.1995). At this site the activities of herbivores are restricted by the physical

environment, i.e., tidal currents and shifting channel sands. The stromatolites described by Pickney et al. 1995 appears to be actively growing due to the association with microbial mats covering the stromatolite surface, an important discovery which explains the ecophysiological properties controlling stromatolite formation. As the organic mass ages and older components die, new growth of cyanobacteria and microbes ensure their continued existence with the open ocean conditions.

Storrs Lake: Hypersalinity, which can over time limit invertebrate grazing biofilms, selects for certain microorganisms (Elliot 1994). The stromatolites at Storrs Lake have been estimated to be around 2500 years old (Zabielski & Neumann, 1990). These San Salvador stromatolites are similar in age to those on Stocking Island, Bahamas.

Total microbial densities in the Storrs Lake stromatolites was as high as 1.58 X 10^{10} cells/gdw in Site 2 and live microorganisms up to in 9.46 X 10^{5} CFUs/gdw in Site 1 (Figure 16) demonstrating high aerobic metabolic activity. While these plates were grown on GASW medium utilizing artificial seawater at 37°C, the resultant densities do not of course represent all viable bacteria present. Certain bacteria that could not grow in these conditions include obligate anaerobic or extreme halophilic microorganisms (Oren 1993). In addition, the salt requirement and tolerance of many bacteria vary according to other growth conditions including temperature and medium composition (Ventosa et al. 1998). A majority of environmental bacteria are often nonculturable when using one medium in any given location. The geochemical gradient observed in this study could yield a related gradient in microbial speciation due to the different available nutrients.

Symbiotic sulfur oxidizing bacteria including *Thiothrix* spp. forms laminated mats around detrital particles and builds nodules in the hindgut caecum of marine spatangoids (De Ridder & Brigmon 2003) were identified in Storrs Lake biofilms. Anaerobic conditions prevail in these small microbialites and hence the mutualism because *Thiothrix* spp bacteria remove sulfide. It is thought that the symbiosis provides a detoxifying effect with nutritional consequences to the echinoid (Brigmon & DeRidder 1996). Sulfur nodules, (i.e., a nucleus wrapped by a layered microbial mat), are structurally similar to oncoids, i.e., a type of biolaminated particle in sedimentary structures. No other algal types including green algae or red algae were observed in the

stromatolite samples. This could have been to due their low densities, nutrient deficiencies (i.e. lack of nitrogen), or the high salinity.

Marine Systems: Corals form a similar biofilm except much of the polymeric material is produced by mucus cells in the coral polyp (Harvell et al. 2007). The microbial community develops within the surface mucopolysaccharide layer, but does not appear to adhere to the epidermal cells (Ritchie 2006). Microbial cell to cell communication occurs by the production of homoserine lactones in marine systems to maintain biofilm structure (Johnson 2005). The microbial community profile is determined, in part, by the production of antibiotics and other allelopathic compounds (Ritchie 2006), and by carbon source availability. Distinct differences in biofilm species composition appear to be structural and functional. For example, in contrast to biofilms formed on microbialites and stromatolites, establishment of significant numbers of cyanobacteria only seems to occur on corals during black-band disease (Weil et al. 2006).

Dead Sea: The Dead Sea lacks obvious stromatolites, microbialites or biofilms and cursory investigation would not produce any evidence of life or microbially induced calcium carbonate precipitation in the form of stromatolites. Microscopic investigations negates the visual finding as there are a number of microbes present that are adapted to aquatic saline environments (Nissenbaum 1975, Oren and Anati 1996, Oren 1997).

Biofilm development was observed to be minimal in the Dead Sea samples. This is in contrast to Storrs Lake which produces thick, viscous biofilms as well as stromatolites. The stromatolites in Storrs Lake are similar to those described in Stouts Lake, San Salvador Bahamas with active microbial mats (Elliot 1992). The Dead Sea biofilms were primarily detected with an environmental scanning electron microscope and in contrast to Storrs Lake there is minimal fossilization (Figure 19A, B, C and 21B). Fossilization in the biofilms and microbes is being controlled by the depletion of HCO_3 and the slightly acidic pH.

5.7 *Nutrient Systems*

Studies of the nutrient distribution, ie carbon and nitrogen, within ecosystems can provide useful insights into the structure and function of those ecosystems. While N-fixation was not measured in Storr's Lake, the extreme N limitation in the system as demonstrated by both field (Test Kits) and laboratory techniques (IC) makes it apparent that this analysis needs to be addressed. A walk down of the surrounding area makes it apparent that local sources of nitrogen are negligible. While not measured in this study, the uptake of dissolved organic carbon (DIC) from the water column is essential for photosynthesizing microbial mats as those at Storrs Lake. DIC that comes in contact with the biofilms are either fixed into organic matter abiotically or biotically or diffused out to the water (Des Marais & Canfield 1994).

In a closed evaporite system like Storrs Lake the movement of DIC would not be as dynamic as in an open water system. Studies that compare extreme environments such as evaporite systems will enable us to better analyze the data as it is apparent in comparing Storrs Lake and the Dead Sea that visual identification of organic activity can be difficult. Microscopic analysis, if they are directed towards stromatolitic or microbialite remains can also fail as life may be sparse and the chemistry may limit fossilization.

Biofilms can form under a wide range of conditions including extreme environments. For example, acidic streams from mines can contain metal-containing leachate can contain iron, sulfur, and arsenic that select for certain microorganisms. These materials have been shown to be accumulated as ferric arsenate and arsenate-sulphate precipitates in rapidly growing bacteria-made microbialite structures (Leblanc et al. 1996). The ongoing development of bacterial biofilms alternating with sand deposition, drying, and and erosive cycles results in the formation of As-rich ferruginous accretions. These laminated and dome-shaped bacterial structures are similar to those of stromatolites but lack the abundance of cyanobacteria. This incorporation of minerals and metals into biofilms layered with sand and sediment is similar to those processes in natural systems (Krumbein 1978). Biofilms as well as microbialites and stromatolites are organosedimentary structures that can grow in a wide range of environments, where

water temperature, nutrients, geology, and pH vary widely. While they are most often observed in neutral and alkaline waters, stromatolites can form in acid-sulfate springs and geyser systems including geothermal areas in New Zealand (Jones et al. 2000) and the United Sates (Farmer & De Marais 1994). As might be expected the growth and development of stromatolites from acidic thermal waters compared with those from neutral and alkali waters have been found to be microbiologically and geochemically distinct (Jones et al. 2000).

While biofilms of varying quality and quantity can be observed worldwide, stromatolites are only seen where environmental conditions are conducive to growth and development of these unique structures. The formation of stromatolites that are basically laminated microbial mats constructed from layers of filamentous and other microorganisms that may become fossilized requires certain environmental conditions. These conditions include the geochemistry, temperatures, water flow, depth, and microbiology similar to those found at Storrs Lake. Storrs Lake due to its proximity to the ocean, shallower depth, and environmental factors is more dynamic in terms of spatial and temporal changes that have an obvious impact on the geomicrobiology of the site. Microbial diversity and densities were observed to be much greater in Storrs Lake compared to samples from the Dead Sea. The Dead Sea microorganisms, although in lower densities and apparent diversity, were observed actively attached to abiotic substrates.

Recent research on the microbiology of hypersaline systems (Yannarell et al. 2007) will likely provide valuable information in a number of fields. Much of the interest in microbiology of hypersaline systems is limited due to problems with isolation of organisms from environmental samples (Brigmon et al. 1994). Here we extensively employed electron microscopy to allow direct examination of samples from those sites. Both culture and microscopy techniques demonstrated an extremely active microbial community in Storrs Lake's stromatolites. However, the methods used to determine aerobic and heterotrophic microbial densities here were performed with GASW medium made with artificial seawater. Some extreme halophilic species may not grow at this salinity hence this method may have underestimated the actual population.

In this work we discussed and compared microbiology and geochemistry from two distinct hypersaline environments. We compared the two systems although the Storrs Lake ecosystem was found to be much more biologically active than the Dead Sea. This ecological range does show the diversity and adaptability of microorganisms to their extreme environments. The diversity of the microbial community demonstrated in this study gives rise to multiple functions (i.e. incorporation of diatom tests), products (i.e. ECPS for cross-linking crystals and fossilized bacteria) and functions (i.e. uv-resistant cyanobacteria pigments in evaporite crusts) that allow these resilient biological materials

6.0 Conclusions

In this review, we described the development of microbialites from biofilms to microbial mats to more complex structures like stromatolites in hypersaline Storrs Lake, San Salvador Bahamas. At the same time we can conclude that the Dead Sea, also a hypersaline ecosystem, has a very different, but limited, microbial ecology. We also point out the importance of biofilm establishment due to biotic and abiotic processes in evaporite systems. The microbiology of these evaporite systems is highly dependent on the geology and other key associated environmental influences. The development of stromatolites in Storrs Lake was related to oxygen, water flow, pH, and associated geochemistry for the evaporite system. The characteristics of hypersaline microbialites in the Bahamas were compared with those in the Dead Sea.

In this work we observed unique interactions between geochemical (ie. crystals) and possible microbiological activity. Future work on identifying specific microbial processes involved (ie. redox coupled reactions) is needed to understand the function of those interactions in extreme geochemical environments. Here we have shown how the diversity of the geological settings and microbial communities rise to unique ecosystems and structures. The incorporation of diatom tests adds to the strength and structure of the stromatolite biofilms. Bacterial products including ECPS were shown cross-linking various crystal-types and fossilized bacteria to “cement” the structure together.

As shown here with the use of EM and microbiological techniques biofilms are also excellent environments for stabilization of fossilized filaments, diatoms, and inorganic detritus. These conclusions are supported by other research indicating the important of microbial –substrate interactions due to precipitation of carbonate "cement" as recently described in other tropical environments (Diez et al. 2007). Cyanobacteria in Storrs Lake make up most of the evaporite crust as well as the fossilized material below and some of other functions including UV-resistant pigments. These microbially produced pigments in evaporite most likely allow these resilient microorganisms to survive under a wide range of conditions. The interaction of an extremely active bacterial population with cyanobacteria evident here most likely accelerates calcium carbonate precipitation and fossilizations as documented in microbial mats in other sites by Chafetz and Buczynski (1992). We hypothesize that the nitrogen limitation is made up by the nitrogen fixing cyanobacteria and associated mat bacteria in the Storrs Lake biofilms.

6. ACKNOWLEDGEMENTS

We would like to thank Dr. Donald T. Gerace, Chief Executive Officer, and Vincent Voegeli, Executive Director of the Gerace Research Center, San Salvador, Bahamas, for their support. We thank Christopher Berry and Victoria Stewart of the Savannah River National Laboratory for their analytical support. L. Hulse and Mayra Nelman, NASA-Johnson Space Center for Scanning and Environmental Electron Microscope access, Dr. Carlton C. Allen, NASA-Johnson Space Center for XRD analysis, Monica Byrne for many of the Storrs Lake images, coccoid measurements, Dawn Smith for field support at the Dead Sea, and Dr. S. Grasby, Canadian Geological Survey, Calgary for the Dead Sea water analysis. This paper was prepared in connection with work done under a subcontract to Contract No. DE-AC09-76SR00001 with the U.S. Dept. of Energy. The investigation of P. Morris was supported by NASA Astrobiology grant NAG9-1113, NAG9-1114. G. Smith was supported by NSF 0516347 and The World Bank Global Environmental Fund to the Coral Disease Working Group.

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8. **Figures Captions**

Figure 1. A diagram illustrating the formation of biofilms because of the interactions of microorganisms, environmental conditions, and organic products, including extracellular polymers (ECPS) and inorganic substrates such as sand and dust. A. Bacteria initially colonize the sediment or surface and begin to produce ECPS. B. Subsequent colonizers may include phototrophs including cyanobacteria where near sunlight as well as mineral precipitates and other microorganisms. C. Lithification occurs overtime through a combination of ECPS, microbial biofilm, and minerals interacting to create a hardened matrix.

Figure 2. Storrs Lake, San Salvador Island. *Rhizophora mangle* leaves being incorporated into biomats on the north end of Storrs Lake. Arrows indicate leaves.

Figure 3. Storr's Lake (Inset) is on the east side of San Salvador, Bahamas. The sampling transect is shown between the west side of Storrs Lake and Cactus Island.

Figure 4. Dead Sea, Israel. The arrows and numbers indicate the north and south basin sampling sites.

Figure 5. Storrs Lake, San Salvador Island. FE-SEM images. Intact mineralized filaments composed of cyanobacteria are common in the Storrs Lake stromatolites. Arrows indicate small ooid shaped mineral precipitates. .

Figure 6. Storrs Lake, San Salvador Island. FE-SEM images. Segmented mineralized filaments with fossilized biofilms and smallest spheres.

Figure 7. Storrs Lake, San Salvador Island. FE-SEM images. Broken fossilized filaments that are similar to those in Figure 5. The filament is hollow indicating that initial mineralized formed an external mold with small ooid shaped mineral deposits.

Figure 8. Storrs Lake, San Salvador Island, FE-SEM images. Cyanobacteria-like fossilized filaments also appeared as structural elements in biofilm formation covering

stromatolites**.** The filaments are layered and intertwined and are indicative of microbial network building activities.

Figure 9. Storrs Lake, San Salvador Island, FE-SEM images. Example of the largest spherical structures with sizes averaging 5.3 µm diameter and ranging from a smooth to a rough texture.

Figure 10. Storrs Lake, San Salvador Island, FE-SEM images. Medium sized spheres averaging 2.0 µ are common, usually appearing in clusters from 2-8, frequently associated with biofilm, and ranging from a smooth to a rough texture.

Figure 11. Storrs Lake, San Salvador Island, FE-SEM images. The smallest spheres are found imbedded in biofilms, occur in clusters and are usually associated with larger microbial features. The spheres form two distinct population sizes averaging .55µm (imaged in this figure) and .13µm.

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Figure 12. A. Storrs Lake, San Salvador Island, FE-SEM images. The arrow indicates a rod-shaped bacterium attached to the surface of a calcium sulfate crystal and associated with biofilms matrix. Other microbial examples were found, ranging in size from approximately 1.0-3.0 µm and often associated with crystals within the biofilm matrix **B.** Shorter rods typical, sometimes appearing as rough textured dumbbells were also observed, usually associated with calcium sulfate crystals and probably representing sulfate reducing microbes.

Figure 13. Storrs Lake, San Salvador Island, FE-SEM images. Diatoms formed integral portions of the biofilm structure. **A.** A relatively intact diatom being incorporated into the biofilm and partially covered by bacteria similar to those imaged in Figure 12B (arrow). **B.** A partially degraded diatom test is observed in the biofilm structure, and similar to Figure 13A, there are bacteria similar to Figure 12 B and calcium sulfate crystals (arrows).

Figure 14. Storrs Lake, San Salvador Island, FE-SEM images. **A** demonstrates a fossilized cyanobacterial filament held to sand and rock grains by biofilms (ECPS). Arrow indicates cross-linked biofilm **B** Higher magnification of Figure 14A, arrow indicates the ECPS binding calcium sulfate crystals.

Figure 15. Storrs Lake, San Salvador Island microbiology. Total and viable aerobic microbial densities in stromatolite samples.

Figure 16. Storrs Lake, San Salvador Island. **A**. Dissolved oxygen concentrations in the water column. **B.** Temperature changes from the shoreline across the lake to Cactus Island.

Figure 17. Storrs Lake, San Salvador Island. **A**. Sampling sites pH measurements. **B**. Salinity measurements across the lake.

Figure 18. Dead Sea, Israel. FE-SEM images. **A.** Halotolerant microorganisms associated with chloride mineral surfaces were limited to rod-shaped structures with filamentous, apical extensions. **B.** Arrow indicates thin, wrinkled biofilms extending outward from a fossilized microbe. **C.** Sand sized orthoclase and quartz fragments with rice-grain shaped potential microbes or fossilized remains. **D.** ESEM images. Filamentous microorganism with hammer-like extension (arrow) that appears to be attached to detrital filaments.

Figure 19. Dead Sea, Israel. ESEM images. **A.** Higher magnification of Figure 18D. Filamentous microorganism with hammer-like extension (arrow), attached to detrital fragments. **B.** Elongated filament bending around fragments and rocks. **C**. Bacillus or rod shaped structures, inhabiting the surfaces of silts and clays. **D**. Bacillus or rod-shaped, but with relatively straight walls in comparison with Figure 19C. Substantial amounts of EPS materials are observed.

9. Table Captions

Table 1. Storrs Lake, San Salvador Island. Site descriptions.

Table 2. Dead Sea Israel. Sum data of Dead Sea water and substrate survey. The Ein Gedi site is located in the northern basin and the other sites are in the southern basin.

Table 3. Storrs Lake, San Salvador Island. August 9, 2001, real time water chemistry survey.

Table 4. Storrs Lake, San Salvador Island. Statistical analysis of real time ground water survey.

Table 5. Comparison of anion and cation concentrations, pH and average temperatures at select Storrs Lake and the Dead Sea locations.

10. Tables.

Table 1. Storrs Lake Site Description

Site	Coordinates	Description
1	N 24.0549 W 74.45348	Storrs Lake western shore
2	N 24.0590 W 74.45247	20m from shore
3	N 24.05901 W 74.45211	Past stromatolite mantle
4	N 24.05811 W 74.45132	Beginning of stromatolitic ridge between Cactus Island and shore
5	N 24.5893 W 74.4506	Middle of stromatolitic ridge
6	N 24.05957 W 74.45019	10 meters from Cactus Island
7	N 24.06025 W 74.44879	Shore of Cactus Island
8	N 24.06038 W 74.44738	Western shore of Cactus Island
9	N 24.05973 W 74.44651	Northern shore of Cactus Island
10	N 24.06018 W 74.44373	Eastern shore (ocean side) of Cactus Island
11	N 24.05943 W 74.44016	Southern shore of Cactus Island
12	N 24.05973 W 74.44783	20m from Eastern shore of Cactus Island (ocean side)

Table 2. Sum Data of Dead Sea Water and Substrate Survey

Site	Name	Coordinates	Water Temp.	Description Sediment Sample	Depth of collection	Water Sample*
1	Ein Gedi	31°27'09"N 35°23'57"E	31° C	sand, evaporites, primarily halite	.61 m	yes
2	Ein Boqeq (by Gardens Hotel)	31°11' 48"N 35°21'45"E	32° C	sand mixed with silt	.61 m	yes
3	Ein Boqeq, 183 m. north of site 2		33° C	gravel mixed with sand, silt	.61 m	yes
4	Ein Boqeq, 805 m. north of site 2		30° C	silt	.13 m	yes
5	Hamme Zohar	31°10'12"N 35°22'02E	33.5° C	gravel and sand	.61 m	yes
6	Mt Sedom	31°05'23"N 35°23'35E		sand, silt with halite	surface sample	no
7	Mt Sedom	31°03'50"N 35°23'43"E		sand, silt with halite	surface sample	no
*all water collected approximately .1524 m below the surface						

Table 3. Sum Data of 8/9/01 Storrs Lake Water Chemistry Survey

Site	*Coordinates*	N (ppm)	DO (ppm)	T (°C)	pH	Salinity (ppt)
1	N 24.0549 W 74.45348	**nd****	**6**	**39**	**8.24**	**68**
2	N 24.0590 W 74.45247	**nd**	**7**	**38.25**	**8.3**	**66**
3	N 24.05901 W 74.45211	**nd**	**7**	**37.8**	**8.36**	**66**
4	N 24.05811 W 74.45132	**nd**	**8**	**35**	**8.48**	**72**
5	N 24.5893 W 74.4506	**nd**	**8**	**36**	**8.51**	**74**
6	N 24.05957 W 74.45019	**nd**	**9**	**35**	**8.63**	**75**
7	N 24.06025 W 74.44879	**nd**	**not meas***	**36.5**	**9**	**74**
8	N 24.06038 W 74.44738	**nd**	**not meas**	**37.5**	**8.9**	**75**
9	N 24.05973 W 74.44651	**nd**	**not meas**	**36**	**8.74**	**73**
10	N 24.06018 W 74.44373	**nd**	**not meas**	**32.5**	**8.72**	**77**
11	N 24.05943 W 74.44016	**nd**	**not meas**	**35.5**	**8.52**	**76**
12	N 24.05973 W 74.44783	**nd**	**not meas**	**39.5**	**8.97**	**82**

*not meas=not measured

**nd=not detected

Table 4. Statistical Analysis of Storrs Lake Water Survey

Chemistry Parameter	mean (μ) ± standard deviation (σ)	Correlation (r)
Nitrate & Nitrite (ppm)	nd*	NA
Dissolved Oxygen (ppm)	7.5 ± 1.05	.9683
Temperature (°C)	36.55 ± 1.98	-.2892
pH	8.36 ± 0.899	.7425
Salinity (ppt)	73.17± 4.67	.8859

*nd=not detected

Table 5. Comparison of anion & cation concentrations, pH, average temperatures at select locations in Storrs Lake and the Dead Sea.

Measurements		LOCATION			
		Dead Sea		Storrs Lake	
Chemical	Parameter	Dead Sea-Evaporite Ponds (N=4)	Dead Sea-Ein Gedi (N=1)	Storrs Lake-Stromatolites (N=3)	Storrs Lake Water (N=3)
CATIONS ($Mg\ L^{-1}$)	Na	23327	21010	25300	6670
	Ca	19017	23930	1230	5330
	K	8351.3	8351.3	640	nd
	Mg	45280	45280	not meas.**	not meas.
	Mn	23.21	23.21	1500	433
	Li	8.69	8.69	nd	nd
	Fe	nd*	nd	nd	nd
	Ba	nd	nd	not meas.	not meas.
	Sr	299.7	364.6	not meas.	not meas.
	Si	nd	52.16	not meas.	not meas.
ANIONS ($Mg\ L^{-1}$)	Cl	222133	235150	52300	17700
	SO_4	464.9	195.6	5670	2300
	HCO_3	300.47	344.5	not meas.	not meas.
	NO_2	not meas.	not meas.	nd	nd
	NO_3	nd	nd	nd	nd
	Fl	not meas.	not meas.	3440	nd
	PO_4	not meas.	not meas.	nd	nd
Temperature	(C)	31.5	32	36.3	36.8
	pH	6.3	6.3	8.8	8.4

nd* =not detected
not meas** =not measured

Figure 1. Biotic biofilm formation (A) Initial attachment of bacteria and production of extracellular polymers (ECPS) (B) Photrophic bacteria & detritus including precipitatesaccumulate on biofilm surface (C) lithification occurs over time within biofilm

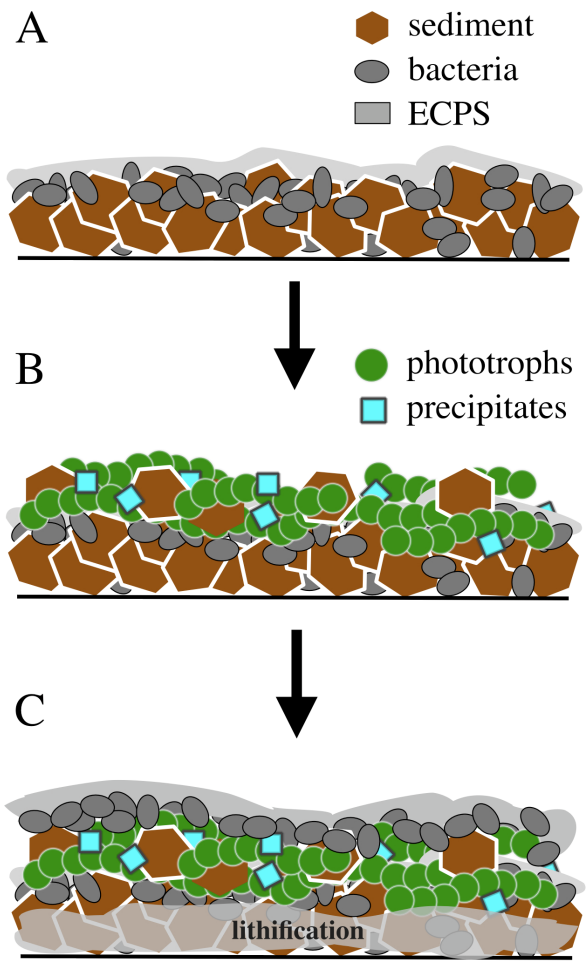


Figure 2. Leaves being incorporated in biomats in Storrs Lake.

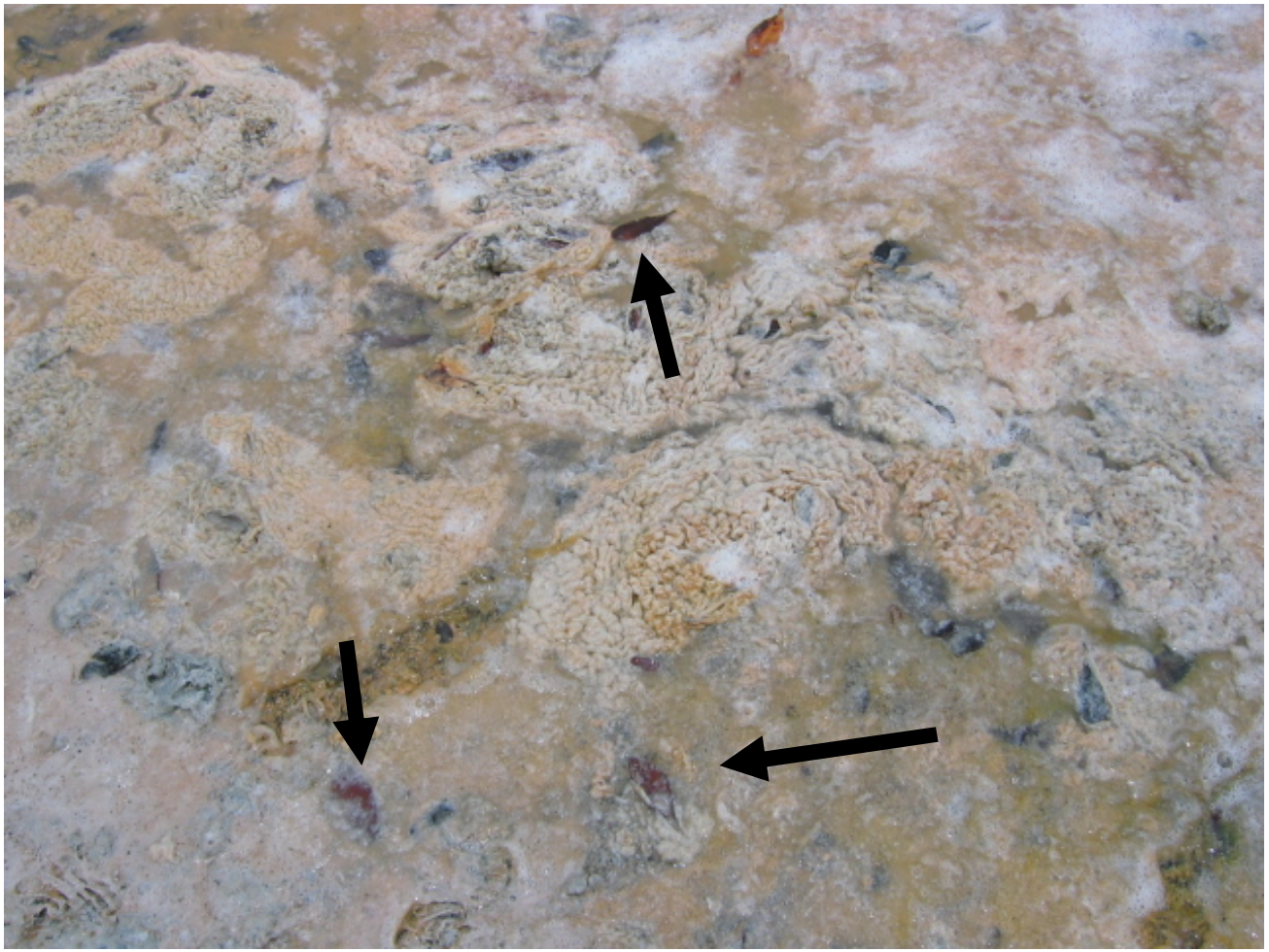


Figure 3. San Salvador Island, Bahamas & sampling locations

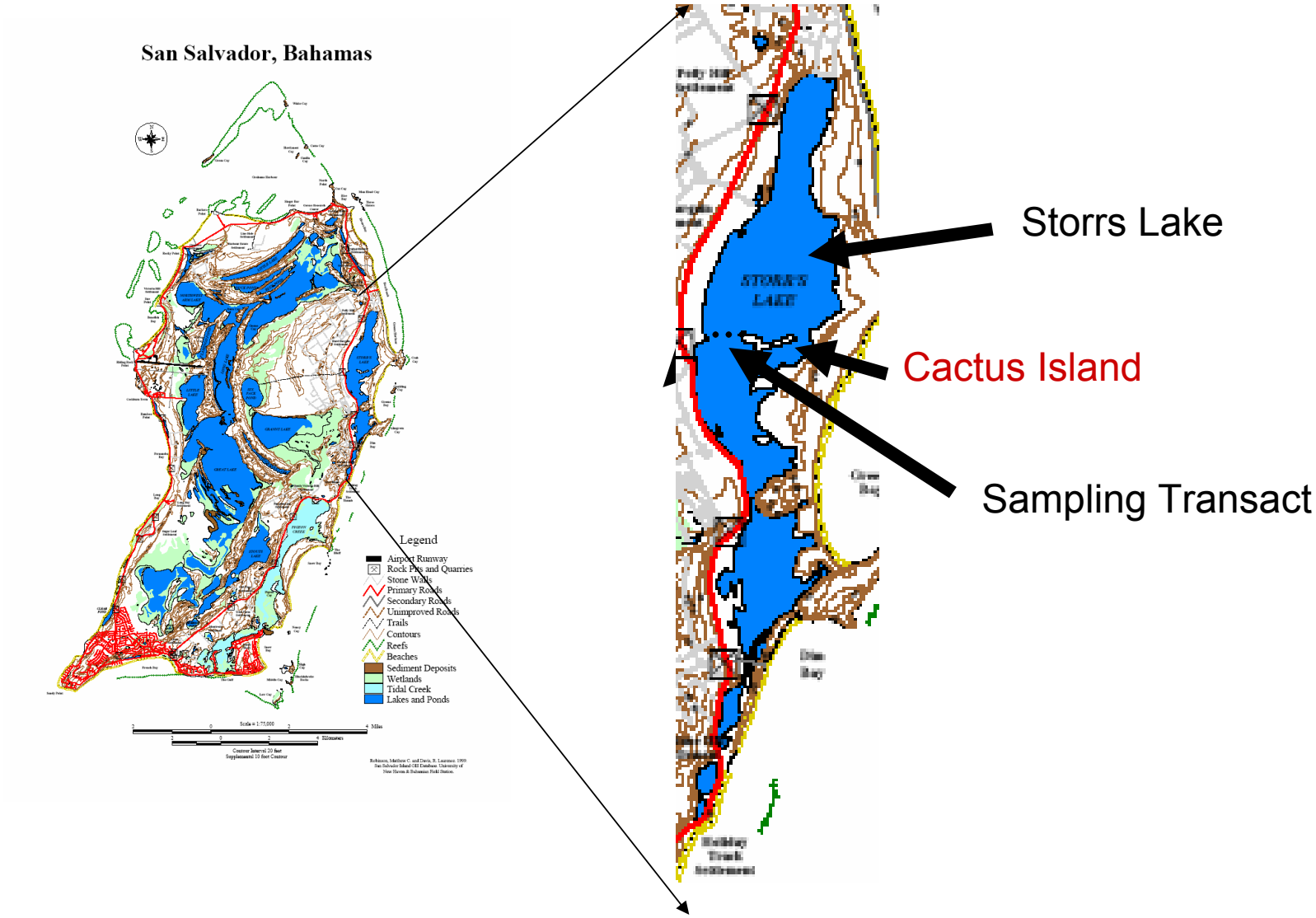


Figure 4. Dead Sea and sampling locations

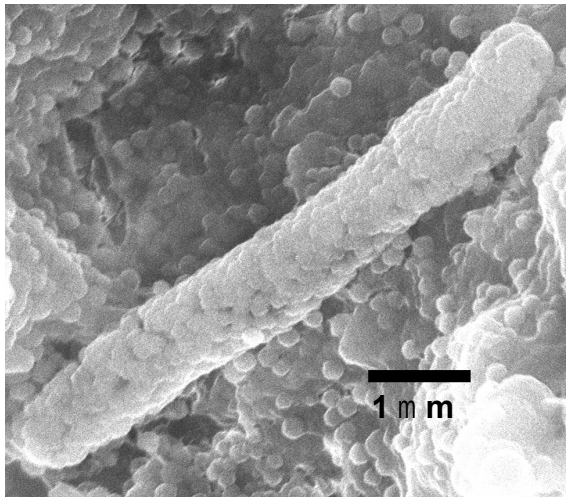


Figure 5. Intact fossilized cyanobacteria

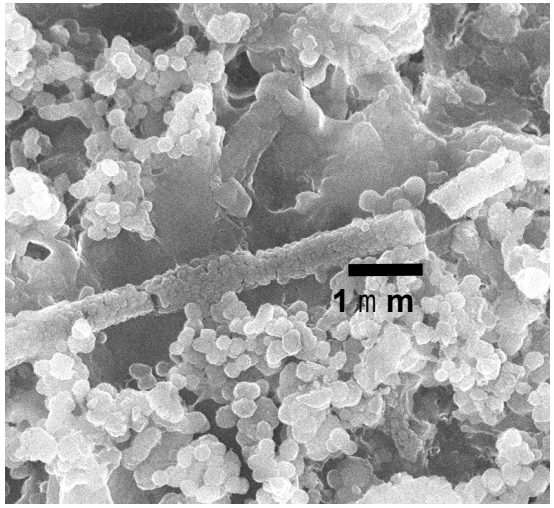


Figure 6. Segmented fossilized cyanobacteria

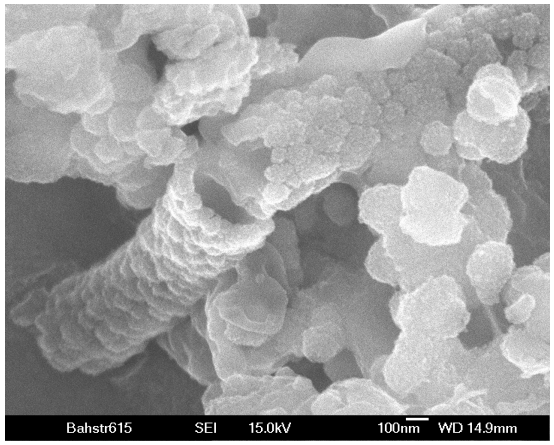


Figure 7. Fossilized Cyanobacteria mold

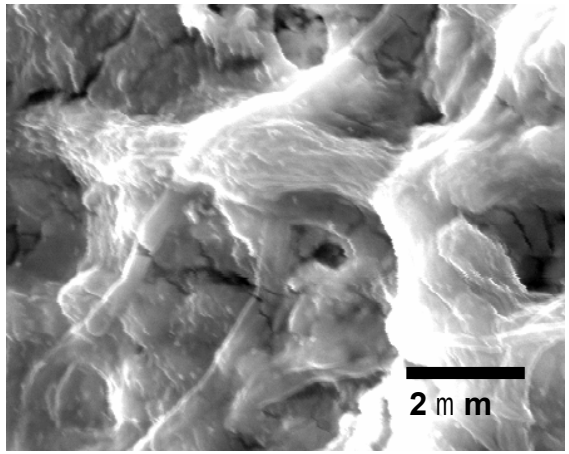


Figure 8. Cyanobacteria incorporated in biofilm

Figures 9., 10., 11, & 12. Spherical structures of varying sizes from Storrs Lake Stromatolites

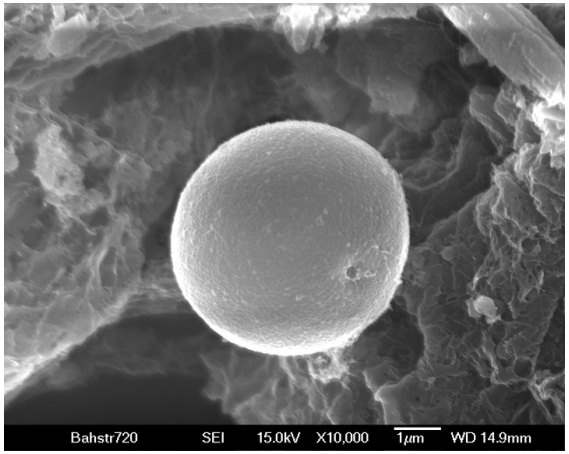


Figure 9

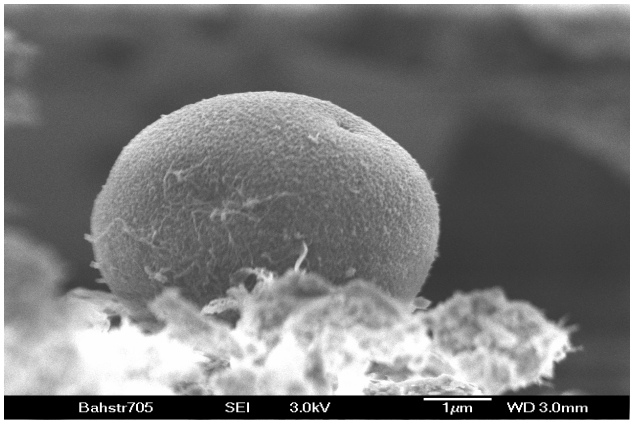


Figure 10

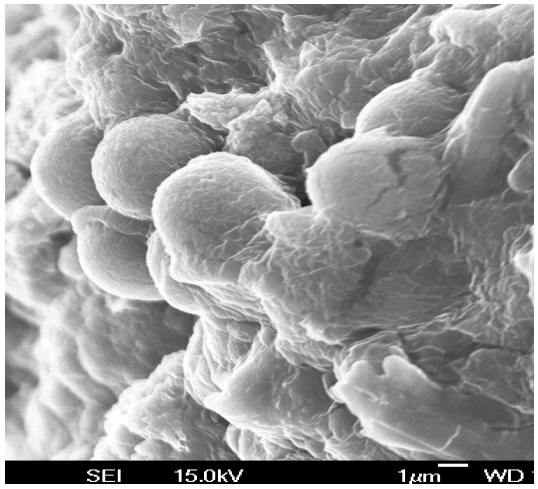


Figure 11

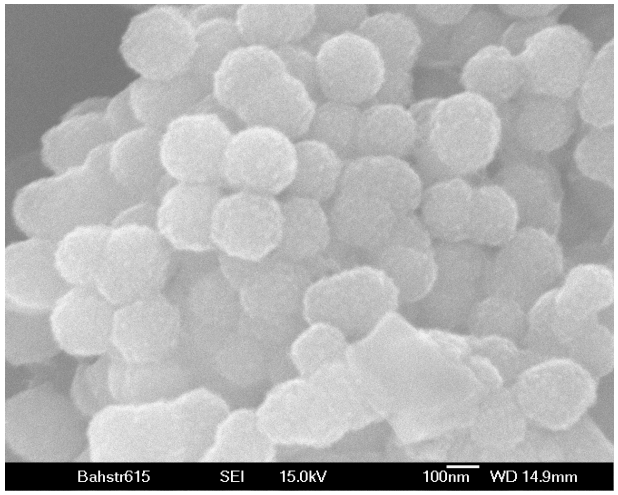
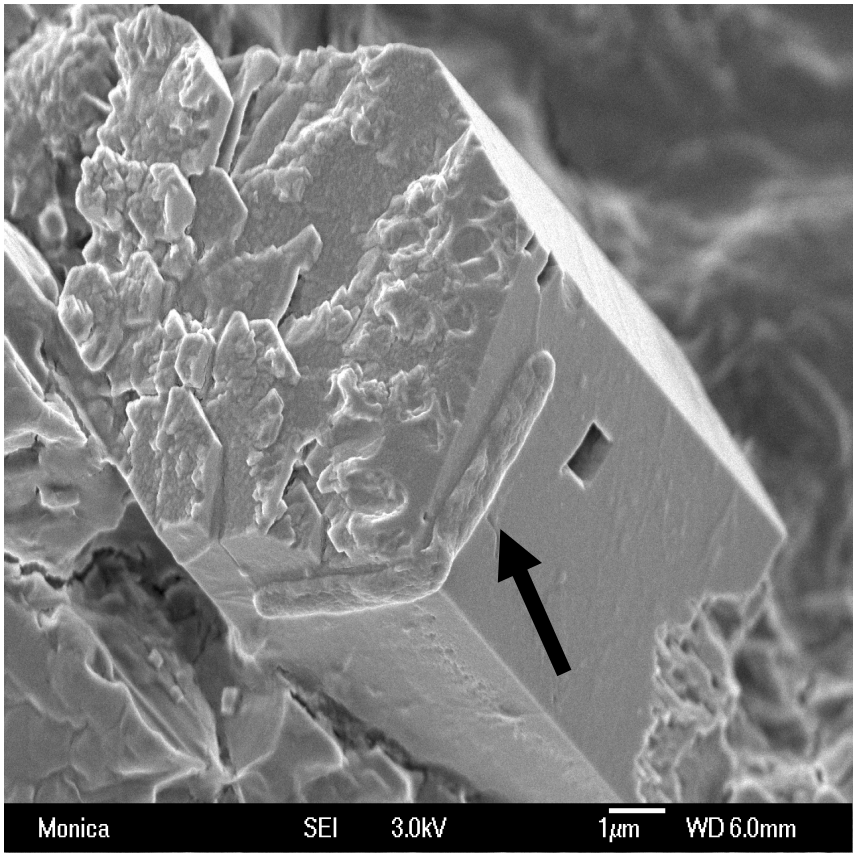
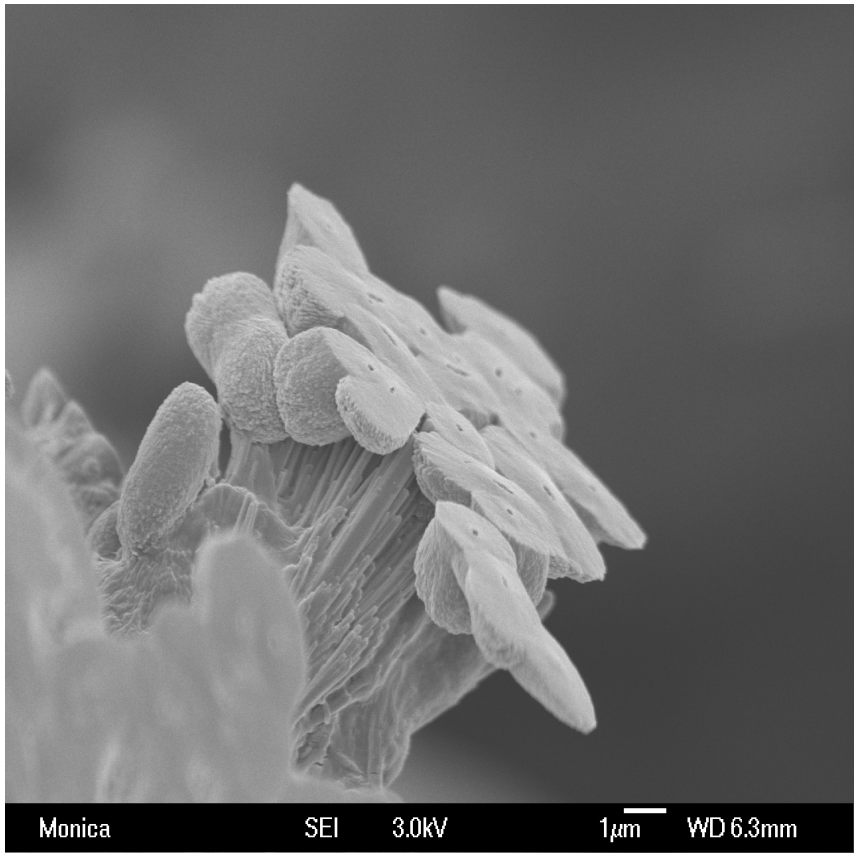


Figure 12

Figure 13. Bacteria filaments (A) and rods (B) attached to crystals in biofilm matrix

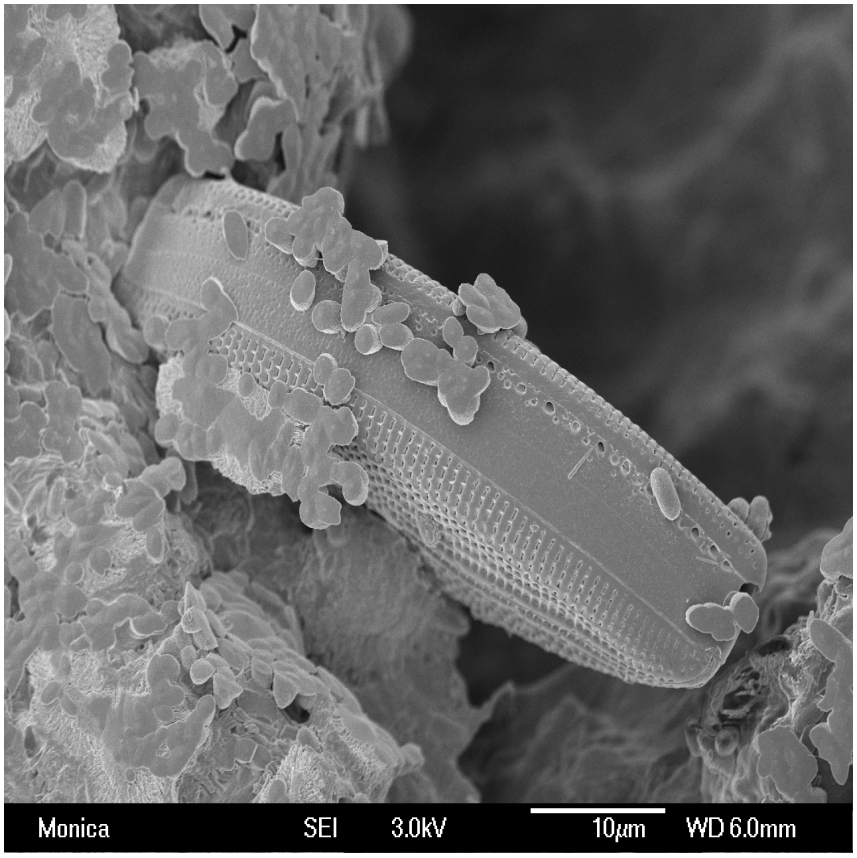


14A

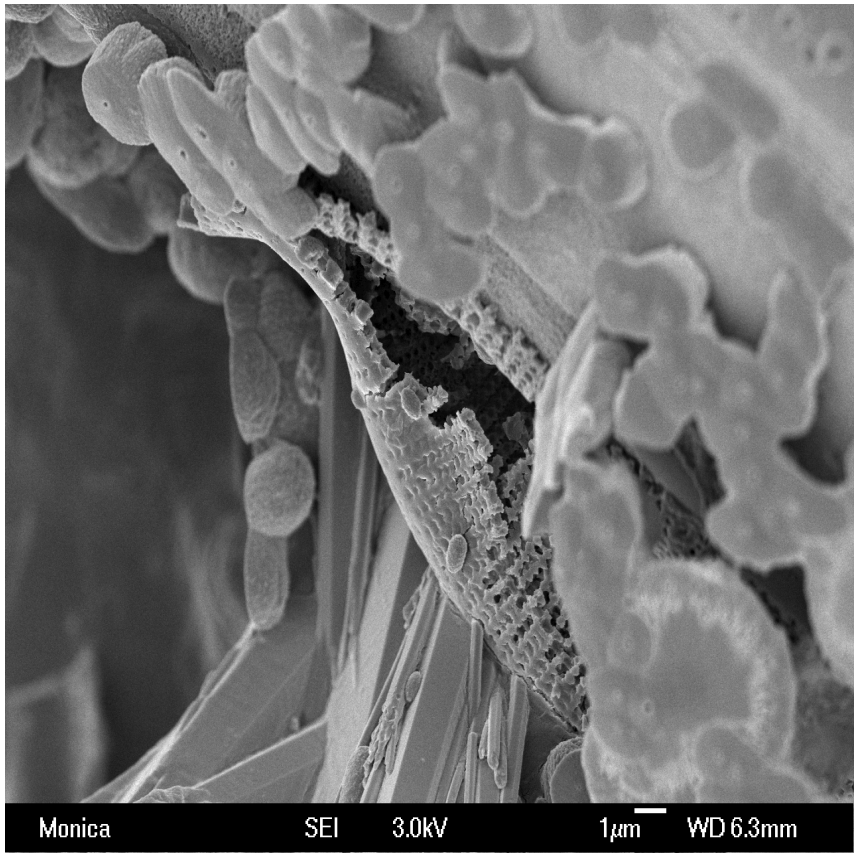


14B

Figure 14. Diatoms incorporated in biofilm matrix intact (A) and tests (B) by active bacteria.

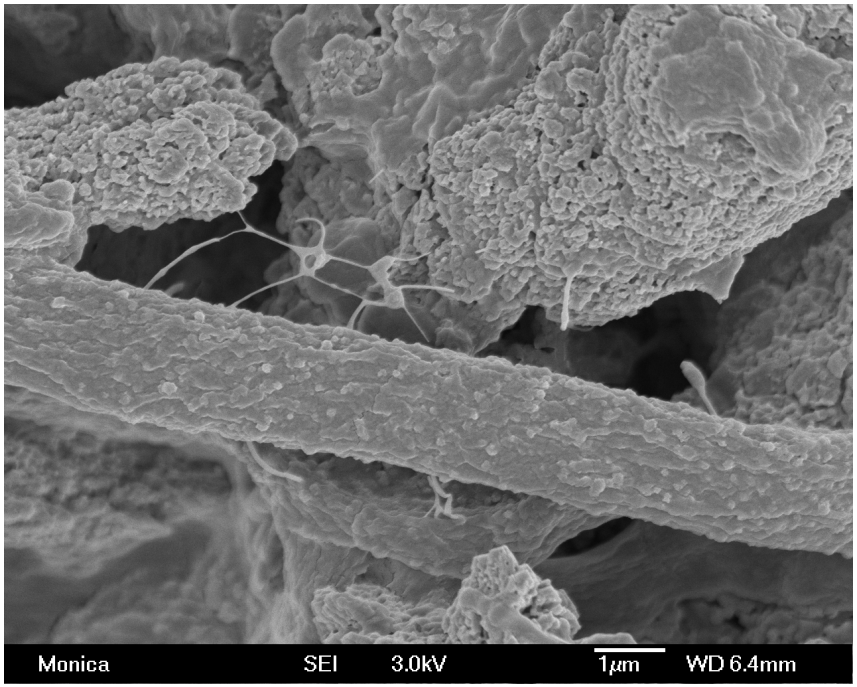


15A

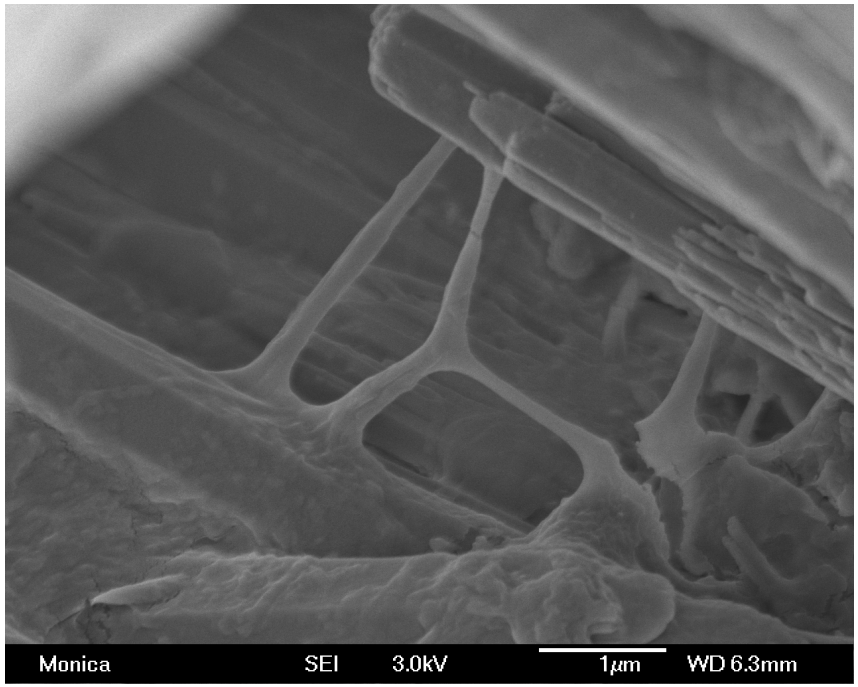


15B

Figure 15. Extracellular polymers (ECPS) in Biofilm Matrix Crosslinking Fossilized Cyanobacteria (A) and Crystals (B).



A



B

Figure 16. Total and viable aerobic microbial densities in Storrs Lake stromatolite samples.

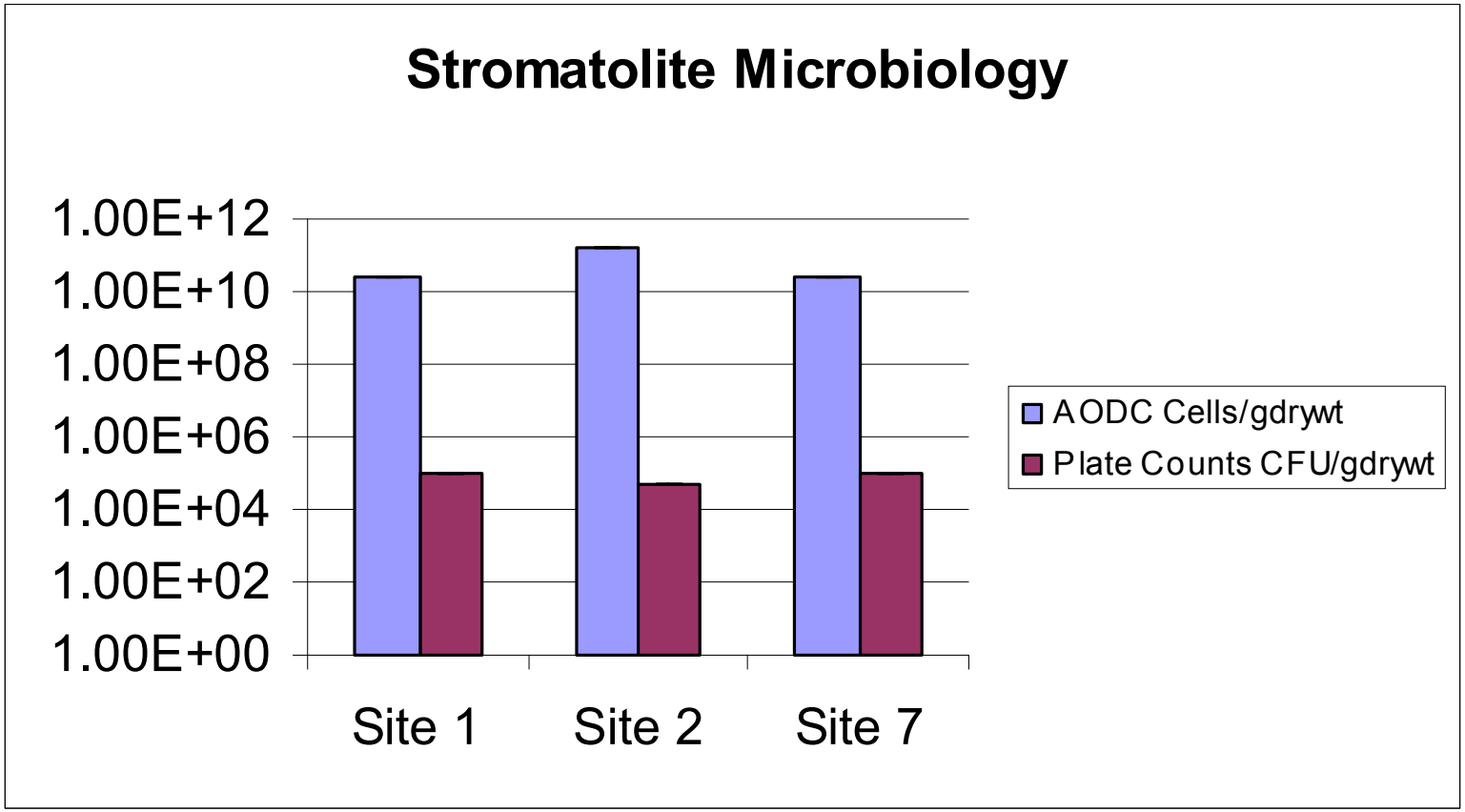
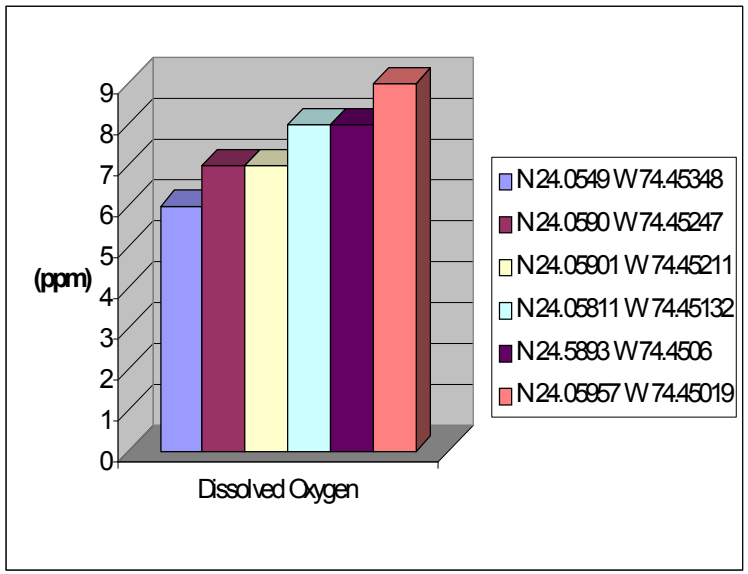
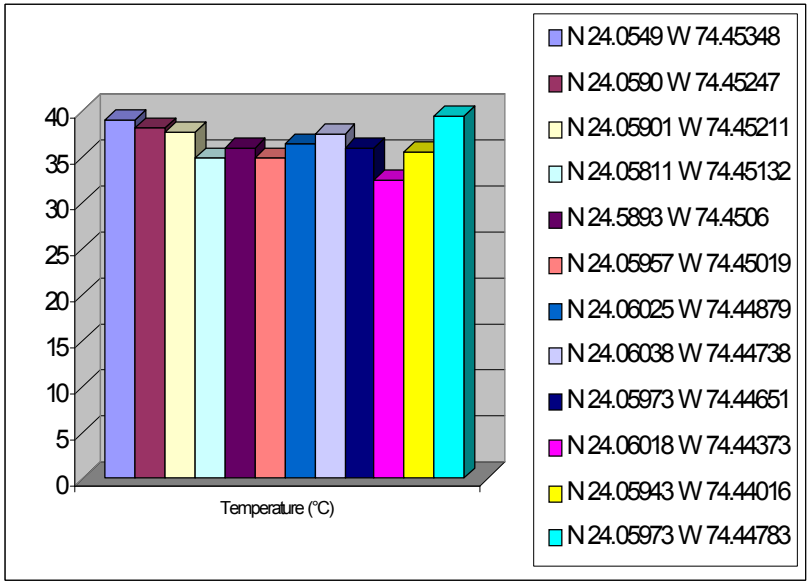


Figure 17. Storrs Lake Water Dissolved Oxygen Versus Sampling Sites (A) and Temperature Versus Sampling Sites (B)

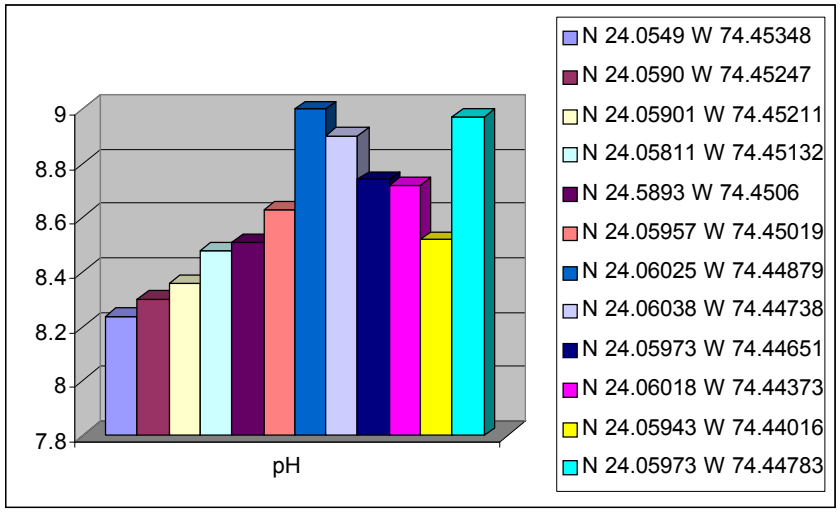


16A

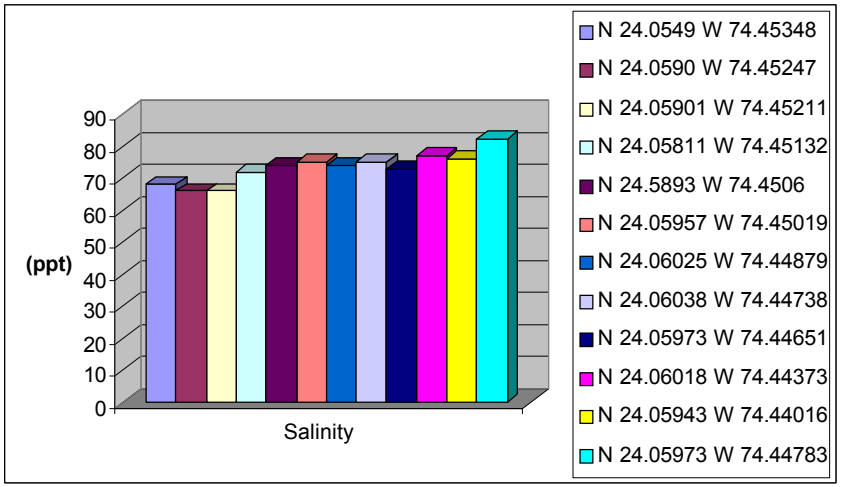


16B

Figure 18. Storrs Lake water pH vs Sampling Sites (A). And Salinity vs Sampling Sites (B)



17A



17B

Figure 19. Bacteria colony (A) extracellular polymers (ECPS) (B) attached rods (C) and filaments (D) in Dead Sea Samples

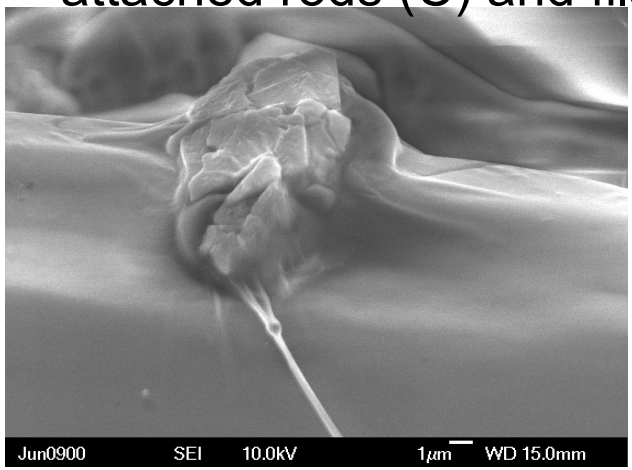
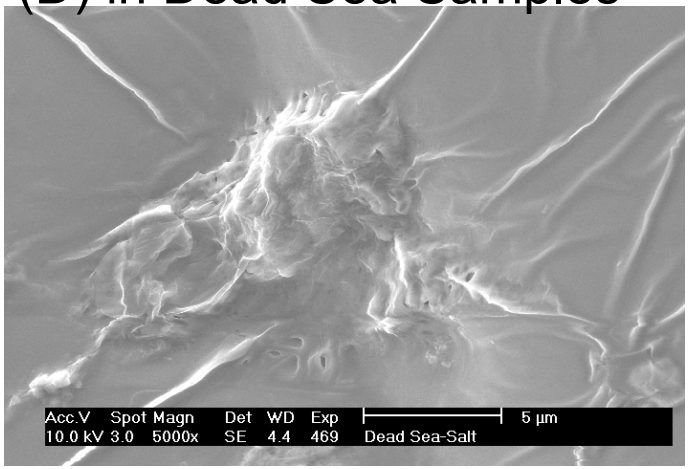
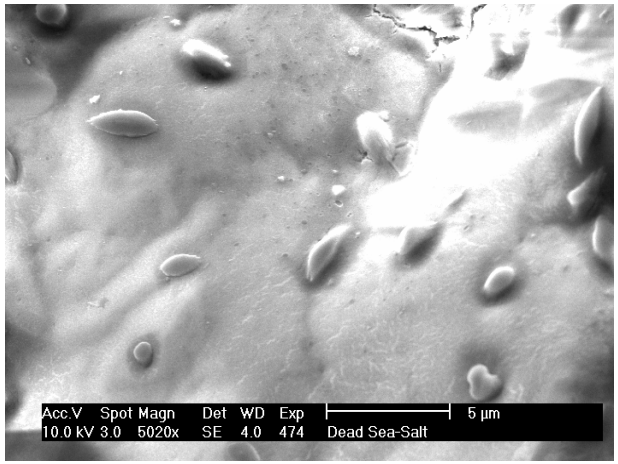


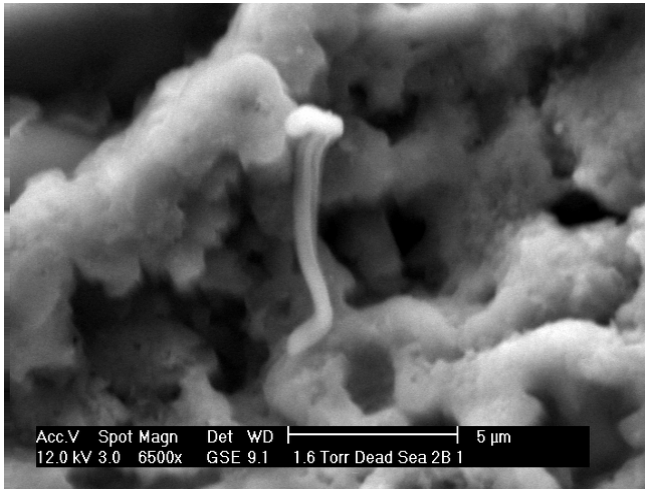
Fig 19A



19B



19C



19D

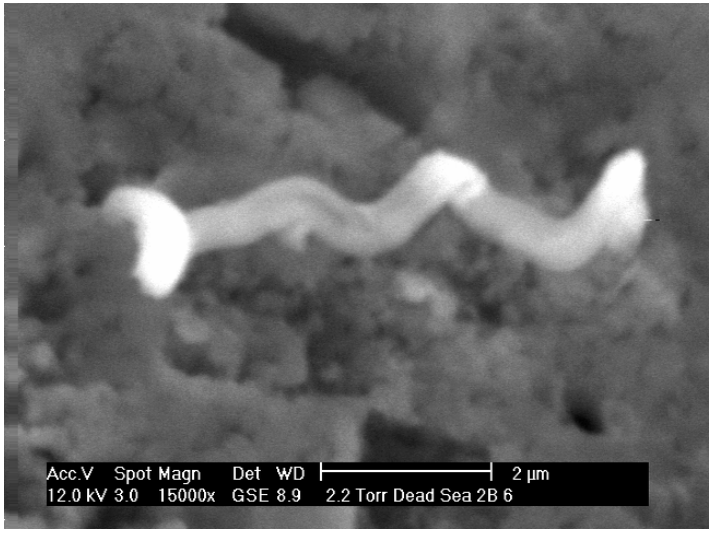


Fig 20A

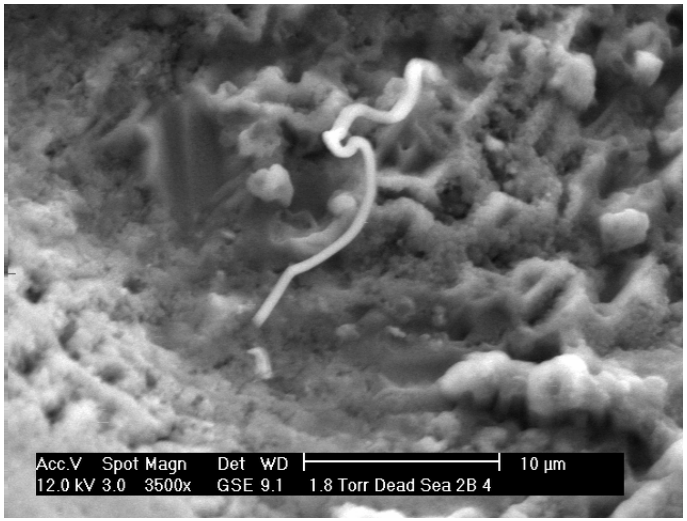
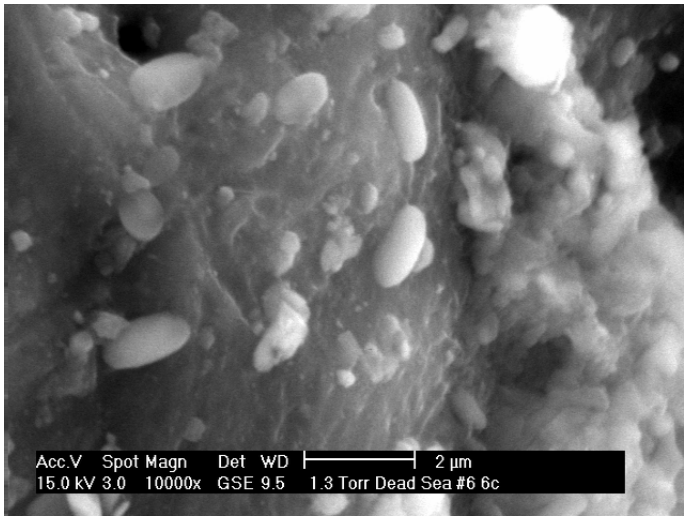


Fig 20B



20C

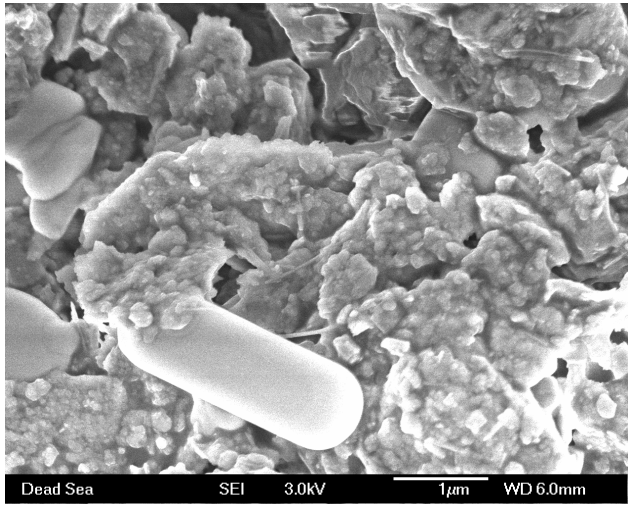


Fig 20D

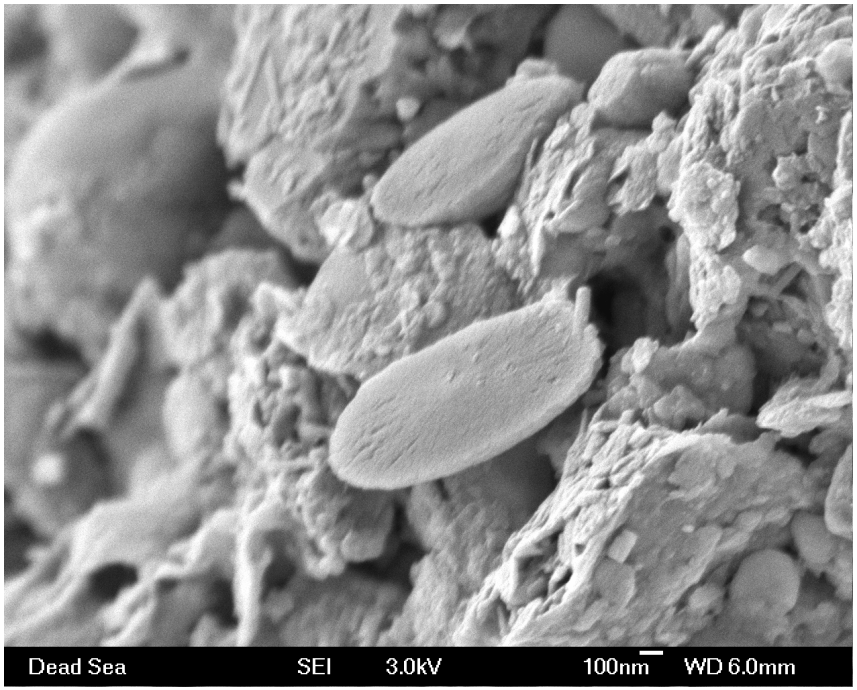


Figure 21A

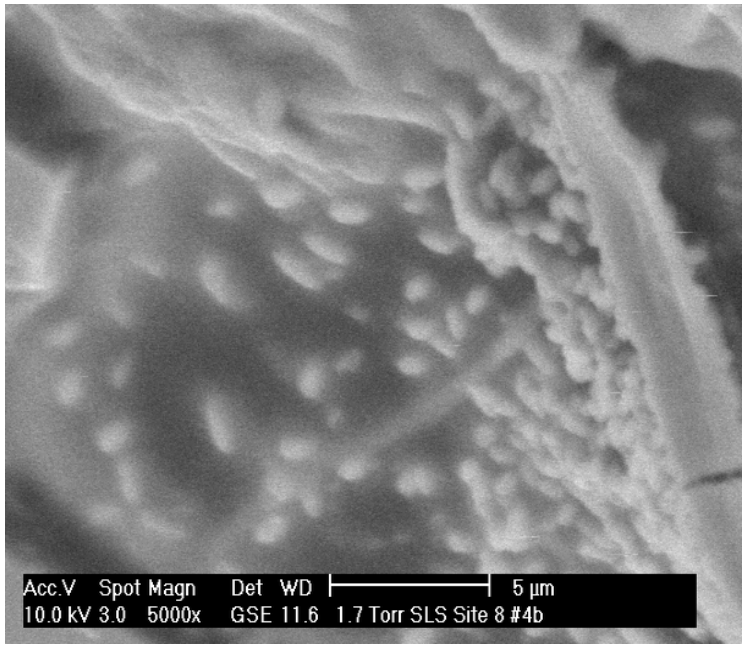


Figure 21B