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The American University in Cairo

School of Sciences and Engineering

THE MICROBIOME OF THE EGYPTIAN RED SEA PROPER AND
GULF OF AQABA

A Thesis Submitted to

The Applied Sciences Graduate Program

in partial fulfillment of the requirements for

the degree of Doctorate in Applied Sciences (Biotechnology)

By Ghada Alaa El-Din Kamal Mustafa

Masters of Science- American University in Cairo

Bachelor of Science - Ain Shams University

Under the supervision of

Professor Rania Siam

Chair of the Biology Department

Fall 2015

The American University in Cairo

The Microbiome of the Egyptian Red Sea Proper and Gulf of Aqaba

A Thesis Submitted by Ghada Alaa El-Din Kamal Mustafa

To the Biotechnology Graduate Program, Fall 2015

in partial fulfillment of the requirements for the degree of Doctorate of Applied Sciences in
Biotechnology has been approved by

Dr. Rania Siam

Thesis Committee Chair / Adviser -----

Affiliation -----

Dr.

Thesis Committee Reader / examiner

Affiliation-----

Dr.

Thesis Committee Reader / examiner

Affiliation-----

Dr.

Thesis Committee Reader / examiner

Affiliation-----

Dr.

Thesis Committee Reader / examiner

Affiliation-----

Dr.

Thesis Committee Reader / examiner

Affiliation-----

Program Director

Date

Dean

Date

DEDICATION

This Thesis is dedicated to my Family

My adored Mother: Mrs. Malaka Abd El-Fatah

I dedicate my dissertation work to my mother. Without her teaching me how to fight, how to always be positive and insistent, without her constant love, concerns, support and encouragement I would not be able to sustain all the efforts to reach my goals.

My Father: Dr. Alaa El-Din Kamal Hamouda

I dedicate this thesis also to my father who has keenly watched my progress in this field of science and give me the opportunity to excel and achieve my master

My sisters: Dr. Abeer & Dr. Yasmine

I dedicate this thesis to my lovely sweetest ever sisters, Abeer and Yasmine, who supported me a lot and were proud of me all the time, giving me the confidence that I can always achieve my goals.

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ABSTRACT

The Microbiome of the Egyptian Red Sea Proper and Gulf of Aqaba

The Red Sea is one of the most unique environments worldwide. It possesses a unique geography, physical, chemical and biological characteristics. It encounters several ecosystems articulating with each other, these include, corals, mangroves, algae, fisheries, invertebrates and microbiota of each one of these along with microbiota of the Red Sea waters and sediments. Studying the collective microbial communities of the Egyptian Red Sea coastal sediments have not been reported before. In regards to the severe pollution impacting the different Red Sea ecosystems, sediments samples have been collected from different impacted sites. The selected sites included 1- four ports for shipping aluminum, ilmenite and phosphate, 2-a site previously reported to have suffered extensive oil spills, 3-a reported tourism impacted site 4- two mangrove sites and 5-two lakes. Bacterial communities for each site have been studied through two different approaches, Culture-Dependent and Culture-Independent approaches. Pyrosequencing of V6-V4 hypervariable regions of 16S rDNA, isolated through the two approaches, has been used to assess the microbial community of each site. Physical parameters, Chemical analysis for 29 elements, selected semi-volatile oil contents, along with Carbon, Hydrogen, Nitrogen and Sulfur (CHNS) contents have been measured for each site. 131,402 and 136,314 significant reads have been generated through the Culture-Dependent and Independent approaches, respectively. Generally, Proteobacteria, Firmicutes, Actinobacteria, Fusobacteria, Gemmatimonadetes and Bacteroidetes are the major bacterial groups detected through the two approaches. The Culture-Dependent datasets distinctive analysis revealed three main patterns (1) marine *Vibrio spp.*-suggesting a "marine Vibrio phenomenon"; (2) potential human pathogens; and (3) oil-degrading bacteria. While the Culture-Independent datasets analysis reported (1) an Egyptian Red Sea Coastal Microbiome, taxa detected in all the sites and (2) Hydrocarbon biodegrading bacteria predominance to the majority of the sites; particularly in two ports. On the other hand, the two lakes, through the two approaches, showed unique bacterial patterns, which generally grouped into anaerobic, halophilic and sulfur metabolizing bacteria. Individually, sites showed unique evolution of their microbial communities based on minor intrinsic and imposed variation per sites. Our results draw

attention to the effects of different sources of pollution on the Red Sea and suggest the need for further analysis to overcome the hazardous effects observed at the impacted sites.

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**INTRODUCTION: THE ENVIRONMENT
OF THE RED SEA AND ITS
ARTICULATING ECOSYSTEMS**

1. INTRODUCTION

Red Sea is one of the warmest waterbodies on earth creating a favorable condition for the growth of several marine ecosystems. This gives the Red Sea one of its unique characteristics; “high biodiversity and endemism” (Stehli and Wells 1971, Ormond and Edwards 1987). The Red Sea has several other unique characteristics that attracts researchers from different disciplines to study. Climate scientists see the Red Sea as an ideal model to study the case of global warming on different ecosystems, mainly reefs. One of the most interesting debates about the Red Sea is the fact that it is the warmest and most saline ecosystem in the world where extensive reef formation occurs (Berumen, Hoey et al. 2013). The Red Sea is 2000 km long with a surface area of 458,620 km²; and almost entirely locked by land (Rasul, Stewart et al. 2015). It has three depth’ levels; a shallow zone of less than 50m depth, a deep zone of depth range between 500m and 1000m, and the deepest area of the Red Sea is the central axis which has a depth ranging between 1000 and 2,900m. The areas that caught the attention of research are, mainly, the both extremities; the shallow and coastal areas (from 40% to 25% of the Red Sea) and the deepest areas of the central rift (15% of the Red Sea). The Red Sea possess different biological ecosystems that orchestrate together in a direct or indirect form along with the Red Sea waters and sediments environments. Apart from the water and sediments, as two different ecosystems harboring their own organisms, other ecosystems are engulfed in the Red Sea environment too. Coral reefs’ ecosystem is the most abundant shallow ecosystem of the Red Sea (Sawall and Al-Sofyani 2015). Red Sea possess 3.8% of the world’s coral reefs (Berumen, Hoey et al. 2013), represented by over than 50 different genera. Corals of the Red Sea not only present a model example of acclimate ecosystem (not affected by climatic changes), they also present one of the complex and diverse ecosystems. They harbor and unify with different other ecosystems, including coral microbial communities resembling the food source and unicellular algae known to be the reason for the high sustainability of corals till now, in addition to fisheries, sponges and other ecosystems. All the inhabitants of the corals are referred to as the “coral holobiont”, and this character of corals are showing their wide range physioecological flexibility (Sawall and Al-Sofyani 2015). Now this rich ecosystem is being greatly endangered due to several factors including mainly the anthropogenic effects, to the point that their future survival is thought to be directly linked to the degree of the anthropogenic effects (Bruckner and Dempsey 2015).

Another nourished Red Sea ecosystem is the Red Sea sponges which have been known since two decades for their production of natural compounds with pharmaceutical values. Red Sea sponges are also known for their ecological importance to the corals' ecosystem (Lee, Wang et al. 2011), however, sponges' overgrowth represents one of the threats endangering coral ecosystems (Klaus 2015).

Mangrove forests of the Red Sea forms the boundary for the mangroves of the Indo-pacific regions which gives it an environmental and biogeographical significance. Mangroves are of enormous valuable significance from several aspects. They represent a shelter and food for different marine biota, a factory for medicinal products, fuels and building materials. They also provide stabilization for the shorelines, and more importantly, they serve as a store and sequester for considerable amounts of carbon to the whole ecosystem; and they provide the marine ecosystem with the needed organic nutrients; they are very well articulating with the Red Sea ecosystems, such as coral reefs, sea-grasses and fisheries, to the point that Mangroves' degradations in the region shows possible adverse effects on these ecosystems (Khalil 2015).

All of the aforementioned ecosystems are harboring their microbial symbionts by which they are primarily dependent on producing their nutrients and other metabolic needed compounds. Even water and sediments of the Red Sea are considered among those ecosystems that harbor their own biota mainly dominated by the microbial communities. The dominant players in the whole Red Sea biological systems, mainly sediments and waters, are the phytoplanktons that resemble the primary producer in the food chain. Microbiologically, cyanobacteria plays a major role in Red Sea nutrient production, and any complex ecosystem of the Red Sea, has been studied in terms of its cyanobacterial members. Consequently, the emergence of the field "omics" triggered the research of the different ecosystems/niches' microbiota. On the other hand, most of the discussed ecosystems are being endangered by several factors, the common to all of them are the pollution and the anthropogenic activities. Lack of studies along with the dramatic status of the Red Sea are hindering the conservation efforts especially in the Egyptian Red Sea. Different polluting activities have been reported severely from the Egyptian Red Sea, and on the other hand, novel bioactive compounds have been reported from microbes harboring different ecosystems of Red Sea in Egypt. However, the research on the Red Sea microbiology, especially within the Egyptian borders, is very scarce and underestimated in terms of the wealth of its natural characteristics. Despite the high biodiversity of the Red Sea and the early history of scientific research, still, almost all the Red Sea ecosystems

are not well understood yet. This review summarizes two different areas of research on the Egyptian Red Sea. It describes the current status of the deteriorating health of the Egyptian Red Sea, represented by reviewing research reporting anthropogenic activities, pathogenicity and oil pollution there. While the other aspect, is reviewing the research describing the microbiota of different Egyptian Red Sea ecosystems. This review aims to shed the light upon the dramatic status threatening the different biodiverse, unique and endemic ecosystems of the Egyptian Red Sea.

2. THE STATUS OF THE RED SEA EGYPTIAN COAST:

2.1. Anthropogenic activities affecting Red Sea Egyptian coast water quality:

The status of the Red Sea Egyptian coast due to human activities, tourism and other anthropogenic sources, has been studied and reported several times. According to global standardized water quality parameters, *E. coli* (EC), Intestinal Enterococci, Fecal Streptococci (FS), Total Coliforms (TC), Saprophytic bacteria (SB), and Slat Tolerant Saprophytic Bacteria (STSB) are being used as indicators for water pollution due to human activities, and different anthropogenic impacts (Organization 2003, Directive 2006, McGarrigle, Lucey et al. 2010). Several studies in Egypt were concerned of examining the levels of these indicators along the coast including, bathing, recreational, other touristic important sites and ports, In 2005, a one year intensive study of 40 coastal sites visited seasonally; 200 duplicate surface water samples were collected along the Red Sea coast starting from Taba till Shalateen (El-Shenawy and Farag 2005). In this study, SB, STSB, EC and FS, were used to assess the water quality in these areas. At Aqaba Gulf, Sharm El-Sheikh showed the maximal detection of these indicators; where SB and STSB at a sport club there, and FS, EC, and TC at Marina Sharm (an increased recreational site); the two sites receive a lot of litters and garbage from ships, obvious sewage discharge and fecal contamination. In the Gulf of Suez, the city of Ras Gharib, showed a significantly increased concentrations of all used bacterial indicators SB, STSB, TC, EC and FS. This city is known for its high inputs of organic wastes and nutrient matters to the sea. While on the Red Sea proper, a primitive fishing port at Bir Shalateen (south of the coast); showed pronouncing concentrations of the five bacteria indicators, as this native port is known to receive a lot of fecal and organic wastes. Based on that, the authors attributed the environmental changes in the Red Sea coast, which consequently affected and altered the bacterial communities, to either organic inputs or

anthropogenic effects (Seyfried, Tobin et al. 1985, El-Shenawy and Farag 2005). On the other hand, from the sanitary perspectives, occurrence of fecal coliforms is used as an indication for the water quality and also known that the occurrence of these coliforms is associated with the presence of disease-causing species which is of concern to the public health, reviewed in (El-Shenawy and Farag 2005). Interestingly, the incidence of several disease causing bacteria has been reported recently (Mustafa, Abd-Elgawad et al. 2014). In 2006, El-Shenawy et al group, analyzed 1400 water samples collected during the time period of seven years seasonal field trips to the same 40 coastal sites mentioned in the previous study (El-Shenawy, Farag et al. 2006). The microbiological analysis for these sites, TC, EC and FS contents, proved that 80% of the sites showed the acceptable levels of water quality and approved as safe for recreational activities. In this study, the relation of bacteria with salinity and temperature have been assessed, as salinity showed a reverse relationship with TC and EC in the Gulf of Suez, while the temperature showed a reverse relationship with FS in the Red Sea Proper. Expectedly, increasing the activities of shipping phosphate, coastal oil processing and shipping, fishing activities, recreational and different touristic carryings-on caused the increase in the bacterial pollution indicators at the same sites analyzed before, to the point that 23% of the studied sites exceeded the standard values and were not acceptable for recreational activities (Ibrahim, Farag et al. 2011).

Staphylococcus aureus levels have been counted along with TC, EC and FS in another study to also assess the water quality of the Egyptian Red Sea coast (El-Shenawy 2005). In this study, *Staphylococcus aureus* exceeded the national and the international guide levels for the sanitary water quality in 53% of the sites and this was associated with the intensified recreational activities at these sites.

In general, since 2000, bacteriological water quality shown to be very poor in terms of high detection of pollution indicators, and even this showed a remarkably deterioration status in regards to 1999 and 1998 status; which has been attributed to the increase of touristic activities since that time (EIMP 2002).

2.2. Possible pathogenic taxa of the Red Sea:

Several pathogenic bacterial members have been reported from the Egyptian coasts of the Red Sea. Strangely, *Listeria monocytogenes*, has been detected along the Red Sea Egyptian coast waters (El-Shenawy and El-Shenawy 2006); which is a serious pathogen that cause listeriosis. This

was not the only incidence to detect severe human pathogens at the Red Sea coast. In 2014, a study of the Egyptian coastal sediments' cultures have reported the detection of several human pathogens in the coastal sediments of the Egyptian Red Sea; including *Clostridium botulinum*, *Vibrio alginolyticus*, *Vibrio harveyi*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus* (Mustafa, Abd-Elgawad et al. 2014). In this collective study 29 different species of *Vibrio* have been reported and this was described by the author as “Red Sea Vibriosis Phenomenon”. However, before this study, other studies, separately, reported infections from *Vibrio* species. Fish Vibriosis in the Egyptian Red Sea has been reported severally; In 2010, a study ran on a seasonally collected 300 fishes from Gulf of Suez showed that *Vibrio anguillarum* and *Vibrio alginolyticus*, respectively, were the dominant cause for the naturally infected collected fishes (Moustafa, Mohamed et al. 2010). Also, 80.4% of randomly collected 225 shrimps from Suez Bay showed infections with *V. alginolyticus*, *V. parahaemolyticus* and *V. fluvialis* (Abd El-baky 2012, El-baky 2012). Interestingly, the first isolation of *V. alginolyticus* from marine ornamental Bird wrasse fish happened in 2012 from the indoor aquarium of National Institute of Oceanography (NIOF) in Hurghada (El-Galil and Mohamed 2012). However, this was not the only incidence of detecting vibriosis in this aquarium; in 2013, *Vibrio harveyi* infections to marine ornamental Arabian surgeon fish has been reported (Hashem and El-Barbary 2013). Also, *Vibrio parahaemolyticus* has been found in 45% of shrimps' samples collected from fish markets; and those shrimps originally came from the Gulf of Suez waters (Abd-Elghany and Sallam 2013). *Vibrio parahaemolyticus* is a human pathogen that infects human through raw, cooked and processed seafood, causing gastroenteritis which in some cases develops to septicemia and became a life-threatening pathogen (CDC 2005). More importantly, *Vibrio parahaemolyticus*, have been shown to be the second most dominant bacteria in mangrove environment in Safaga (Abou-Elela, El-Sersy et al.). Similarly, *Vibrio parahaemolyticus* and *Vibrio vulnificus* were detected in significant concentrations in Suez Canal outlet to Lake Timsah (Bahgat 2011). In the same lake, which lies on Suez Canal, *Vibrio cholera*, *Staphylococcus aureus*, *Salmonella sp.*, *Shigella sp.*, and *Aeromonas sp.*, in addition to other contaminating indicators, were all detected in all the stations tested in the four seasons (Khalil, Beltagy et al. 2014). Based on all aforementioned studies, vibriosis has long history with the Red Sea and presents a serious concern to the health and quality of the Red Sea. On the other hand, pathogenesis of the Red Sea, in general, is proven to be a communal problem which represent hazards not only on the marine life of the sea but also to human as well.

2.3. Oil pollution of the Red Sea:

Red Sea is receiving one of the most drastic pollution sources due to the massive petroleum industry and other heavy metals shipping along its coasts, in Egypt and other countries such as Saudi Arabia. Several studies assessed the levels and numbers of hydrocarbons in the water, sediments and organisms of the Red Sea. In 2006, a study revealed that there are 16 different Poly Aromatic Hydrocarbons (PAHs), in waters collected from the Egyptian Gulf of Suez, Gulf of Aqaba, and Red Sea proper. The results showed that Gulf of Suez is the most hydrocarbon polluted area, due to the massive industry there, followed by Gulf of Aqaba, where the main source of pollution there was the atmospheric deposition from oil fields along the Saudi Arabian coasts. Also, the study showed that the most polluted areas in the Red Sea proper are Safaga and Quesseir, and both of them have intense shipping activities (Said and Hamed 2006). Also, several studies were interested in assessing the levels of hydrocarbons, (PAHs, THC_s, and Aliphatic hydrocarbons), in the corals of the Red Sea. A study sampled corals along the Egyptian Red Sea coast, from Taba till Marsa Alam, and assessed the levels of aromatic and aliphatic hydrocarbons. Based on their analyses and the dominance of certain hydrocarbons, they have attributed the sources of pollution in each area to certain origin. For instance, Hurghada corals pollution has been attributed mainly to sewage from cities and touristic resorts, while Sharm El-Sheikh, Na'ama Bay, Dahab, Ras Nasrani, Nweibaa and Taba' corals hydrocarbon contamination were attributed to land-filling, dredging and siltation. Meanwhile, Safaga, Quesseir and Hamrawein, have been proven by several studies, including this lastly mentioned corals' study, to be drastically affected by the heavy metals shipping ports, and mainly the phosphate industry and shipping there. Northern of the Red Sea, represented in Aqaba in Jordan and Eilat in Israel, the major source for hydrocarbon pollution there is the onshore and offshore oil production, processing and transportation (El-Sikaily, Khaled et al. 2003).

Egyptian Red Sea coast has several oil companies and onshore and offshore drilling facilities, from which several successive oil spills' accidents happened. The fact that oil spills is one of the chronic and major pollution sources in the Red Sea (Hanna 1995), the microbial communities there are being altered to combat or adapt with this disturbance. Correspondingly, scientists started to isolate oil/hydrocarbon degrading bacteria from polluted sites. Water samples have been collected from a chronically oil polluted site, Gemsa Bay, for microbiological analysis (El-Sheshtawy, Khalil et al. 2014). The oil contents at this site reached 496.4 ppm while the

standards levels based on the environmental law is only 15ppm, which made this site considerably highly polluted site. Using culture-dependent approaches, fifteen bacterial strains were identified from Gemsa Bay, two of them belonging to *Pseudomonas xanthomarina* KMM 1447 and *Pseudomonas stutzeri* ATCC 17588, have shown higher growth rate on crude oil, where they managed to change the oil color, turbidity and dispersion in only one day incubation. Addition of biosurfactants and certain nanoparticles helped those two bacterial strains to do three things: 1- degrade more iso-paraffin, 2- complete degradation of certain rings of polyaromatics, 3- increase in the percentage of degradation of other polyaromatics. All of these made those bacterial strains potential for oil bioremediation (El-Sheshtawy, Khalil et al. 2014). In a collective study based on sequencing DNA of cultured microbiota from the Red Sea Egyptian coast, several oil degrading bacteria were identified; *Propionigenium maris*, *Psychrilyobacter sp.*, *Tepidibacter sp.*, *Photobacterium sp.*, *Marinobacter sp.*, *Bacillus sp.*, and *Anaerophaga sp.* (Mustafa, Abd-Elgawad et al. 2014). In Ras Gharib at the Red Sea, two phenol-degrading bacteria, *Ochrobactrum sp.* and *Kocurica camiphilia*, have been isolated and a new strain suggested to be potential bioremediation tools for their biodegradation activities (Hamedo, El-Shamy et al. 2015). Recently, a study managed to assess the levels of 16 different PAHs in fishes collected from El-Sokhna area. Data revealed that all the fishes were critically affected and that all of the tested PAHs are high enough to a lethal level (Ali, Ahmed et al. 2014).

3. RED SEA BACTERIAL ECOSYSTEMS/NICHES:

3.1. Red Sea water and sediments' microbiota:

Egyptian Red Sea microbial communities has been studied from different aspects, however, the “free goal-studies” about the microbiology of the Egyptian coast of the Red Sea are uncommon, in which scientists should study the microbiota of the Red Sea without certain questions to be answered. Of this type of studies, two studies have managed to isolate and identify two novel halophilic and haloalkaliphilic bacteria from a salt lake connected to the Red Sea in Ras Muhammad protected area in Sharm El-Sheikh, *Halomonas sinaiensis* and *Salinivibrio sharmensis*, respectively (Romano, Lama et al. 2007, Romano, Orlando et al. 2011). Another collective study, analyzed bacterial communities harboring the sediments of ten different coastal sites from Taba till Marsa Alam. Using Pyrotag-sequencing technology, this study reported the

detection of 211 different genera, of which 75 were of significant values in their cultures. These data were dominantly members of Gammaproteobacteria, followed by Firmicutes, Fusobacteria and Bacteroidetes. Although this study was aimed to assess the effects of industry and different sources of pollution on sediments bacterial consortia, it reported the detection of other bacteria of marine ecological significance; such as *Photoacterium sp.* and several halophilic bacteria (*Halomonas sp.* and *Salinivibrio sp.*) (Mustafa, Abd-Elgawad et al. 2014). Generally, studying the microbial profiles of the Egyptian Red Sea waters and sediments are the most underestimated research done till now.

3.2. Red Sea Cyanobacteria:

The Red Sea has a long history and strong relationship with Cyanobacteria. Firstly, its name has been attributed to the phenomenon of the blooming of the Cyanobacteria *Trichodesmium erythraceum* seasonally, which gives it its seasonal red hue color (Rasul, Stewart et al. 2015). Also one of the most well studied cyanobacterial mats in the world are in the Solar Lake connected through a seepage to the Red Sea in Egypt (Clark, Dunlap et al. 2012). The story of cyanobacterial mats formation, activities and unique characteristics have been thoroughly described since 70s (Cohen, Padan et al. 1975, Jørgensen and Cohen 1977, Walsby, Van Rijn et al. 1983). Also, the fact that Red Sea, especially Gulf of Aqaba and northern Red Sea, is an ideal example for an ultra-oligotrophic marine ecosystem made it important to understand how organisms sustain their life there (Berninger and Wickham 2005), which drove the prevalent attention to cyanobacteria, the main player of this mechanism in these scarce-nutrient Red Sea environment. Several debates about whether the low biomass of the bacterio/phytoplanktons there are due to the scarcity in the inorganic nutrients, especially during the stratification season (Bottom-Up control), or due to the grazing effects from larger organisms (Top-down control) (Berninger and Wickham 2005). From this point of view, several studies tried to understand this mechanism in the northern Red Sea and Aqaba Gulf mainly. Researchers collected samples seasonally (especially during the summer blooming season) from the Red Sea to detect the availability of different sized-planktons, including bacterioplanktons. Of the bacteria identified to sustain this oligotrophic conditions, and are repeatedly reported, are the two cyanobacteria; *Synechococcus spp.* and *Prochlorococcus spp.*, which their abundance is shown to be affected by the water column conditions. They both showed equal abundance and uniform distribution till 300m depth in Gulf of Aqaba and 60m depth in

northern Red Sea during the stratified summer (Sommer, Berninger et al. 2002). An important finding of this study was that the food web in this oligotrophic sea is being dominated by the “microbial loop”, in which the picoplanktons including photosynthetic bacteria (cyanobacteria) and heterotrophic flagellates starts the food chain as being nutrient sources for ciliates then copepods; and this opposes the classical food chain which starts by phytoplanktons then copepods then fishes. This data shows that the microbial loop dominates over the classical food chain in the energy and carbon flow. However, another study on the central and south of the Red Sea, started from south of the Egyptian Red Sea, demonstrated that, not only photosynthetic bacteria are the predominates of the microbial loop but also heterotrophic bacteria are important grazers in the food chain as they consume the phytoplanctonics nutrient products in the organic matter transfer (Weisse 1989, Weisse 1991). By this heterotrophic bacteria are considered the secondary producers while heterotrophic flagellates are their main consumers (Karrasch, Mehrens et al. 2001).

Also, one more reason for the importance of the cyanobacterial mats studied in the Solar Lake of the Red Sea to the environment is harboring other active microbes contributing to the global geochemical cycling such as sulfate-reducing bacteria and several archaeal groups, mainly methanogens (Teske, Ramsing et al. 1998, Cytryn, Minz et al. 2000)

Moreover, these cyanobacteria were of great interest to scientists for their produced novel secondary metabolites that shows promising pharmaceutical activities. For instance; the cyanobacteria *Moorea producens*, cultivated from mangroves of the Red Sea near Sharm El-Sheikh; scientists were able to isolate from it two new potent cytotoxins (apratoxin H and apratoxin A sulfoxide), in addition to other known cytotoxins have been isolated from (Thornburg, Cowley et al. 2013). More interestingly, from the same cyanobacteria, *Moorea producens*, in another study, many anti-proliferative compounds showing potent and modest activities against HeLa cancer cells have been isolated (Youssef, Shaala et al. 2015). Also, the cyanobacterium *Leptolyngbya* sp. collected from the *SS Thistlegorm* shipwreck near Ras Muhammed, have shown to produce two new grassypeptolides that showed promising activities against HeLa cells and mouse neuro-2a blastoma cells (Thornburg, Thimmaiah et al. 2011).

3.3. Red Sea Sponges:

Although the “omics” new technology initiated the emergence of the field of studying the microbiology of higher organisms, still there were some primitive trials before that. In 1999, a group of scientists collected samples of the sponge *Theonella swinhoei* from Hurghada, Egypt, to study the different endobionts harboring this sponge. They found filamentous photosynthetic prokaryotes constitutes (15%) of the sponge tissues, which then have been attributed to unicellular chroococcoid cyanobacteria. In addition to 40% owed to other filamentous non-photosynthetic prokaryotic cells, (which suggested to be the sulfur bacterial group Beggiatoa). Also, unicellular heterotrophic bacteria constituted 20% of the sponge tissues (Magnino, Sarà et al. 1999). Authors of this research concluded their work by the fact that coral/ sponges ecosystems are more complex than what it shows. Now after the advancement in the science technologies, not only scientists were able to accurately define the micro-species harboring the macro-species ecosystems, but were able to extract and predict pharmacological activities of different natural compounds released due to this symbiont relationships between those species. Red Sea sponges have been known to harbor bacterial communities that produce remarkable bioactive compounds. Four different strains of *Bacillus* species have been isolated from the sponge *Amphimedon ochracea*, collected from El-Gouna, at the Egyptian Red Sea coast; these strains have proven promising cytotoxic activities against hepatocellular carcinoma cell lines (HepG2), colon carcinoma cell lines (HCT), and breast carcinoma cell lines (MCF-7) (Aboul-Ela, Shreadah et al. 2012). Similar to the sponges that encounter valuable bacteria, Red Sea algae represents a rich microbial ecosystem too. For instance, the known intermittent marine bacteria *Methylophaga*; all of its species were known to be “Vitamin B12 auxotrophs. In 2006, two new strains, that are vitamin B12- independent in their growth and does not require any other factors for their growth, and even the vitamin addition does not promote their growth; have been isolated from algae at the Egyptian shores of the Red Sea, and yet they still produce auxins (Li, Doronina et al. 2007). Although, data from the Egyptian Red Sea about microbial communities of the Red Sea sponges are considered, other scientists have presented an intense detailed study about microbial communities of the Red Sea sponges but from Saudi Arabia waters. They have shown two main findings, the first is that the microbial communities of the sponges of the Red Sea have more similarities with the microbial communities of the sponges from other marine environments than to the microbial communities of the water samples surrounding the Red Sea sponges. And if this tells anything, it confirms the phenomenon

of the presence of sponge-specific microbes. The other finding is that the microbial groups detected in these sponges versus those detected in the nearby water samples. The sponges show more bacterial diversity than the water samples, as only 9-12 bacterial phyla were detected in the water samples, while from 19-24 phyla were detected in the sponges' tissues. Also, the water samples were dominated by Alphaproteobacteria (Rhodospirillales) and Cyanobacteria Family II; and those groups largely diminished in the sponges' samples. While the sponges phyla were mainly Gammaproteobacteria (Oceanospirillales and Sphingobacteria) and Anaerolineae, Clostridia and Deltaproteobacteria. Archaeal species were detected also in both the water and the sponges' samples (Lee, Wang et al. 2011).

3.4. Red Sea Mangroves

Another fruitful ecosystem engulfed among the Red Sea environment which is being greatly underestimated in the research studies, is the dense mangrove forests. Mangroves plays a vital role to other marine ecosystems surrounding it, to the environment by sequestering and storing large amounts of carbon, to the economy by producing fuel and building materials (Khalil 2015). However, one of the main values of these forests are the microbial communities harboring the roots, stems and leaves of the mangrove, and even those harboring waters and sediments surrounding the plant itself. As mangroves are type of plants with unique characteristics and values to the environment, its microbiota plays unique roles too to the environment. A study on oil-polluted mangrove forests in both Abu Monkar Island and Safaga Mangrove forests have shown the isolation of 28 oil-utilizing bacteria, of which, four most potent bio-degraders and producers for bio-surfactants are belonging to *Pseudomonas pseudomallei* SAS1; *Bacillus subtilis*-SBS53 and *Bacillus polymyxa*-SR, *Bacillus licheniformis*-SAS2, along with their produced natural bio surfactants have been isolated from sediments, waters and respiratory roots of the two oil-polluted mangrove forests (Bayoumi and El-Nagar 2009). Apart from the pollution, normally the Red Sea coastal mangroves in Egypt are known to harbor ten different species of purple non-sulfur bacteria, dominated by the genus *Rhodopseudomonas* represented in four species; *Rhodopseudomonas marina*, *Rhodopseudomonas acidophila*, *Rhodopseudomonas rutila* and *Rhodopseudomonas palustris*. The second most dominant genus is *Rhodobacter* represented by *Rhodobacter sulfidophilus* and *Rhodobacter sphaeroides*. This was followed by *Rhodospirillum photometricum*,

Rhodospirillum salexigens, *Rhodospirillum molischianum* and *Rhodocyclus tenuis*. (Shoreit, El-Kady et al. 1992, Zahran 1997).

3.5. Red Sea Corals:

Another rich endangered ecosystem engulfed among the Red Sea environment is the soft and hard corals of the Red Sea. Red Sea is considered one of the richest corals habitat, however, research on Red Sea corals is greatly underestimated in regards to similar biodiverse corals' ecosystems. Only 41 studies has been conducted on Red Sea corals related contexts, till 2013. Of these 41 studies, 33 studies (80%), were on the northern part of Gulf of Aqaba, which resembles only 2% of the Red Sea size; and only 8 studies on the rest of Red Sea (Berumen, Hoey et al. 2013). Marine invertebrates are known to either directly or indirectly produce secondary metabolites and bioactive compounds. Although studying bacterial reef communities is an emerging field, Red Sea corals, as one of the marine invertebrates, are known to harbor bacterial communities that play the major roles in producing antimicrobial, cytotoxic and antagonistic compounds that the host also can benefit from it through protecting themselves from other marine pathogens (Isnansetyo, Cui et al. 2003, Chellaram, Sreenivasan et al. 2011, Chen, Kuo et al. 2012). A recent study in 2015, managed to identify 20 bacterial isolates, members of Gammaproteobacteria, Firmicutes, and Actinobacteria, from a soft coral, *Sarcophyton glaucum*, collected from the Red Sea. These isolates showed an affiliation to *Pseudomonas aeruginosa*, *Pantoea endophytica*, *Klebsiella variicola*, *Enterobacter asburiae*, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus pumilus*, *Bacillus sporothermodurans*, *Brevibacillus borstelensis*, *Bacillus licheniformis*, *Streptomyces sp.*. These isolates revealed a broad antimicrobial activities against several known pathogenic indicators; which suggest that they protect the host, the soft coral, from marine pathogens (ElAhwany, Ghozlan et al. 2015).

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**CHAPTER 1: EGYPT'S RED SEA COAST:
PHYLOGENETIC ANALYSIS OF
CULTURED MICROBIAL CONSORTIA
IN INDUSTRIALIZED SITES**

ABSTRACT

The Red Sea possesses a unique geography, and its shores are rich in mangrove, macroalgal and coral reef ecosystems. Various sources of pollution affect Red Sea biota, including microbial life. We assessed the effects of industrialization on microbes along the Egyptian Red Sea coast at eight coastal sites and two lakes. The bacterial communities of sediment samples were analyzed using bacterial 16S rDNA pyrosequencing of V6-V4 hypervariable regions. The taxonomic assignment of 131,402 significant reads to major bacterial taxa revealed five main bacterial phyla dominating the sampled sites: Proteobacteria (68%), Firmicutes (13%), Fusobacteria (12%), Bacteroidetes (6%), and Spirochetes (0.03%). Further analysis revealed distinct bacterial consortia that primarily included (1) marine *Vibrio* spp.—suggesting a “marine *Vibriophenomenon*”; (2) potential human pathogens; and (3) oil-degrading bacteria. We discuss two divergent microbial consortia that were sampled from Solar Lake West near Taba/Eilat and Saline Lake in Ras Muhammad; these consortia contained the highest abundance of human pathogens and no pathogens, respectively. Our results draw attention to the effects of industrialization on the Red Sea and suggest the need for further analysis to overcome the hazardous effects observed at the impacted sites.

1. INTRODUCTION

The Red Sea possesses a unique geography, as it is almost entirely locked by land, and its ecosystems are diverse, including mangrove, macro-algae and coral reefs (Alkershi and Menon 2011). The Red Sea encompasses two gulfs, the Gulf of Suez and the Gulf of Aqaba, in addition to the Red Sea proper. The Gulf of Suez is entirely bordered by Egypt, while the borders of the Gulf of Aqaba are shared among four countries: Egypt, Israel, Jordan and Saudi Arabia. The Red Sea proper is bordered by six countries: Egypt, Sudan, Eritrea and Djibouti on the western shore and Saudi Arabia and Yemen on the eastern shore.

Severe pollution of the Red Sea has been reported multiple times from Egypt (Riegl and Velimirov 1991, Ibrahim, Farag et al. 2011, El-Sorogy, El-Kammar et al. 2013, El-Taher and Madkour 2013), Israel (Loya and Rinkevich 1980, Riegl and Velimirov 1991, Loya 2004, Abelson, Olinky et al. 2005), Yemen (Alkershi and Menon 2011), Saudi Arabia (Hanna and Muir 1990, Badr, El-Fiky et al. 2009, Montaser, Mahfouz et al. 2010, Ali, Elazein et al. 2011), Jordan (Al-Najjar, Rasheed et al. 2011), Sudan (Idrisa, Eltayebb et al. 2007) and Eritrea (United Nations Development Programme 2007). Pollution issues in the Red Sea are increasing in severity for several reasons: the Red Sea's small size (458,620 km²); the fact that it is bordered by eight countries; and its slow rate of water turnover (Medio, Sheppard et al. 2000).

The sources of pollution in the Red Sea include land-based sources (including urban development, industrial activities, dredging and filling, tourism and agriculture activities), oceanic sources (shipping, fishing, marine traffic and petroleum industries), and atmospheric sources (industries or port activities). Such severe pollution is likely to affect biological life and disturb the Red Sea's natural ecosystems (Regional Organization for the Conservation of the Environment of the Red Sea and Gulf of Aden 2001). One of the major pollution threats studied is the health of the coral reefs and their ecosystem (Pandolfi, Bradbury et al. 2003, El-Sorogy, Mohamed et al. 2012). Reports on coral reef degradation and the impairment of coral growth and reproduction through algal overgrowth, increased sedimentation and coral disease, have been reported worldwide. Red Sea coral reefs constitute 3.8% of the world's coral reefs (PERSGA/GEF 2004). In an assessment of 21 mangrove sites along the Gulf of Aqaba and the Egyptian Red Sea coastlines, covering ~ 550 hectares, mangrove degradation was reported from Egypt as a result of oil pollution, tourism, camel grazing and browsing and solid waste accumulation, primarily of

plastics (PERSGA/GEF 2004).

However, equally important is the effect of pollution on microbial life, a topic that has not been well studied in the Red Sea. Two studies have addressed human pathogens along the Red Sea coast (El-Shenawy and Farag 2005, Ibrahim, Farag et al. 2011). In one study, the researchers measured the abundance of saprophytic (SB), salt-tolerant saprophytic (STSB), total coliforms (TC), *Escherichia coli* (EC) and fecal streptococci (FS) in 40 sites along the Egyptian Red Sea coastal waters and in the Aqaba and Suez Gulfs. Of these samples, 91.5% met “European and Egyptian current standards” (El-Shenawy and Farag 2005). In 2011, TC, EC and FS in water samples were used as indicators of microbial pollution. This study reported that the Suez Gulf is the most polluted among 194 sites in the Red Sea and Gulf of Aqaba (El-Shenawy and Farag 2005, Ibrahim, Farag et al. 2011)

Conversely, the Red Sea’s Egyptian coast has been better studied from perspectives other than the effect of pollution on the microbial life. For example, in 2005, the geochemistry of the sediments and seawater of four Red Sea lagoons were analyzed. The fauna of the Abu Ghoson lagoon was degraded due to the excessive shipping of phosphates, illmenites and feldspars through the port. Safaga Port encounters more than one pollution source causing heavy metal accumulation, including phosphate shipping, adjacent cement industry, land filling, navigation and construction activities, and shipyards (Abd El-Wahab, Dar et al. 2005, Mohamed 2005). Safaga was shown to have the highest concentration of Fe, Pb, Mn and Zn among the analyzed sites (Abd El-Wahab, Dar et al. 2005). The highest concentrations of ‘P’ and ‘V’ were detected in Qusseir Port, and phosphates were detected in high concentrations in its sediments (Mohamed 2005). Similarly, Hamrawein Port sediments were reported to show distinctive brown coloration, which is characteristic of ‘P’ presence. This is not surprising because Hamrawein Port is one of the oldest harbors for shipping phosphate (Mohamed, Madkour et al. 2011). Sharm El-Maya is a shallow bay (approximately 6 meters deep) that is located in the southern suburb of Sharm El-Sheikh, and its southern reaches are connected to the Red Sea. This bay has been exposed to several oilspill accidents, including the accidental spill of 700 tons of fuel from a cargo ship in 1983 (Roberts and Sheppard 1988, Khattab, Temraz et al. 2006), the 1994 oil spill in Sharm El-Sheikh (Pilcher and Abou Zaid 2000) and the 1999 oil spill accident in Sharm El-Maya (Morsy, Soliman et al. 2010). All oil spills represent a major threat to the bay ecosystem, including sea grass and coral patches.

Additionally, the bay acts as a nursery for commercially valuable fish (Morsy, Soliman et al. 2010). The combination of the oil spills with the structure and ecosystem of this bay resulted in the entrapment of sediments and oil particles, which caused deleterious effects on coral reef reproduction and the photosynthetic cycle (Loya and Rinkevich 1980, Al-Halasa and Ammary 2007).

In addition to the coastal sites of the Red Sea, lakes near the sea can be affected by industrialization. In this study, we analyzed two lakes: Solar Lake at the Gulf of Aqaba (Solar Lake-W) and Saline Lake inside the Ras Muhammed National Park protected area (Saline Lake-RM). Solar Lake-W was selected for this study because it is the only Egyptian Red Sea site where the microbial community has been thoroughly studied. Saline Lake-RM is a petroleum-impacted site that has limited impact from tourism, which allowed us to assess one pollution impact in isolation.

Solar Lake was discovered in 1967 by the workers of the Eilat Nature Reserve (Por 1968, Eckstein 1970). They reported the presence of hot water at the bottom of the lake, which was interpreted as hot brines. Later, it was attributed to solar radiation (Eckstein 1970). When the lake was discovered, it was reported to have the dimensions 80 x 40 m and to be 30 meters away from the Red Sea coast, and it was characterized as a meromictic lake (Por 1968).

Solar Lake is rich in H₂S and was thought to release high concentrations of H₂S compared with the production ever reported from non-polluted waters (Cohen, Padan et al. 1975). This has been attributed to the activity of cyanobacterial mats (Cohen, Padan et al. 1975). Several strains of cyanobacteria have been isolated from Solar Lake. In 1975, *Oscillatoria limnetica* was isolated from the H₂S-rich layer of the lake (Cohen, Padan et al. 1975). *Cyanobacterial laminites* was detected in different layers of the lake and aided in tracking the age and history of the lake (Cohen Y., Krumbcin W. E. et al. 1977). Cyanobacteria control the diurnal cycle of the lake through photosynthesis and O₂-H₂S regulation (Jorgensen, Revsbech et al. 1979). Cyanobacteria anoxygenic photosynthesis is a major contributor to elemental sulfur production and sulfur cycling in the lake (Jorgensen, Kuenen et al. 1979). *Dactylococcopsis salina*, a gas vacuolated cyanobacterium, was also isolated from the Solar Lake (Walsby, Van Rijn et al. 1983). Additionally, a novel species of *Desulfovibrio oxyclinae* was isolated from the Solar Lake cyanobacterial mat. This species was demonstrated to adapt to wide variation in oxygen and sulfide

concentrations(Krekeler, Sigalevich et al. 1997). Archaeal 16S rDNA analysis demonstrated that halobacteria dominate the archaeal community of the lake and halophilic methanogens were identified in the sulfide- and methane-rich layer (Cytryn, Minz et al. 2000).

Few studies have addressed microbial life in Saline Lake-RM. A Gram-negative, haloalkaliphilic and facultative anaerobic bacteria, *Salinivibrio sharmensis*, and *Halomonos sinaiensis* were isolated from Saline Lake (Romano, Lama et al. 2007, Romano, Orlando et al. 2011).

Much work in the Red Sea has focused on visible marine pollution, its various sources and its effects on coral reefs, mangroves and fisheries. However, neglected topics include research on microbial life in Red Sea sediments or water and how pollution affects the distribution and abundance of the microbial communities. Here, we taxonomically identify microbial communities cultured from sediment samples collected at sites that have been impacted by petroleum, industry and tourism.

2. MATERIALS AND METHODS

2.1. Site description and Samples' collection:

Eight coastal sites and two lakes on the Red Sea Egyptian coast were selected for sampling based on the industrial impacts affecting each (Table 1, Figure 1). Six of the ten sites lie on the Red Sea proper, two are on the Sinai Peninsula and two are on the Gulf of Aqaba. The ten analyzed sites included four ports for shipping aluminum (Safaga Aluminum Port), ilmenite (S-Abu GhosonPort) and phosphate (QusseirPort and HamraweinPort), a site previously reported to have suffered oil spills (Sharm El-Maya) and a tourism impacted site (Assala Dahab). Two sites were considered to be non-impacted sites: a protected site (Abu Monkar Island) and a protected mangrove area (Safaga Port-mangrove), which showed oil deposits. The two lakes (Saline Lake-RM and Solar Lake-W) showed different impacts. Saline Lake, which is inside Ras Muhammed, was thought to be a protected area; however, we found extensive oil deposits evident in the soft sediments of its dense salt marches. Solar Lake-W is the west shore of the Lake and lies in the direction of the mountains, not toward the rift. The water on the west side of the lake was characterized by green coloration and H₂S odor, and salt deposits surrounded the lake. Core sampling on the western side of the lake released black fumes (data not shown). Surface sediment samples were collected from the eight sites along the Egypt Red Sea coast in addition to the two lakes in Sinai Peninsula (Table 1, Figure1). The samples were collected using a basic homemade stainless steel core (5 cm diameter/0.5 m length) and an AMS Multi-Stage Sludge and Sediment Sampler (using one 12'' plastic liner; cat. no. 403.31). The samples were collected near the shore at depths ranging from 0.5 to 1 meter from the sea surface. The middle part of the core (~0.25 m depth) was taken for further analysis to minimize the contamination from the seawater or the shore sand during the on-site handling process.

2.2. Bacterial culturing and DNA Preparations:

A few grams (~5 grams) of the collected sediments was inoculated, on site, directly in 20-mL of freshly prepared Difco™ Marine Broth 2216, using 50 ml falcon tubes to allow for aeration. The cultures were incubated with random mixing for 3 days at room temperature. After delivering the cultures to the lab, the cultures were inverted several times, and 1 mL was taken from each culture for bacterial DNA extraction. The DNA was extracted using QIAamp® DNA Blood Mini

Kit (cat no. 51106) following the Protocol for Bacteria in the kit's mini-handbook. The prepared DNA was kept at -80°C for sequencing.

2.3. PCR amplification and 454 Pyrotag sequencing and 16S rDNA analysis:

For bacterial taxonomic assignment, prepared genomic bacterial DNA was used to amplify the bacterial 16S rDNA hyper-variable regions V6 and V4 as previously described (Sogin ML, Morrison HG et al. 2006). The bacterial primers utilized in this study have been described (Siam, Mustafa et al. 2012). The amplicons recovered were subjected to pyrosequencing by 454 GS FLX Titanium technology (454 Life Sciences). V6-V4 reads were deposited in NCBI SRA under the accessions SRR1437688-SRR1437697.

The resources on the Visualization and Analysis of Microbial Population Structures (VAMPS) website, hosted by the Josephine Bay Paul Center, MBL, Woods Hole (<http://vamps.mbl.edu/resources/databases.php>) were used for the phylogenetic analysis and taxonomic assignment of the reads to major bacterial taxa. Fisher's exact test was used to determine the species/genera that differed significantly in abundance across the different sites ($p < 0.05$, Bonferroni-corrected). The total number of raw reads (no significance test applied) and the number of assigned taxa are presented in Table 2. The significant reads are the reads that passed the cut-off value of the former test. We selected known taxa from these significant reads (significant taxa).

3. RESULTS

3.1. Samples and Physical Parameters

We attempted to characterize the microbial community along the Egyptian Red Sea coast and two lakes, as illustrated in Figure 1. The coordinates, temperature gradients, pH and dissolved oxygen of the sampling sites are illustrated in Table 1. We started in Solar Lake-W, which is situated near the border of Egypt and Israel on the Gulf of Aqaba (northeastern Egypt), and ended with S-Abu Ghoson Port at the south coast of the Red Sea (southern Egypt). We observed the highest temperatures in the two lakes investigated, Solar Lake-W and Saline Lake-RM, measuring 34.7 and 31.7°C, respectively. The lowest temperature (20.6°C) and highest pH (9.00) were detected in Safaga Port (Aluminum). The remaining sites showed pH ranges of 8.5 ± 0.085 . Solar Lake-W and Saline Lake-RM had higher salinity than did the coastal sites (Edwards and Head 1987, Thompson, Field et al. 2013); the lakes' measured 107.9 and 149.8 ppt, respectively. More variation in the dissolved oxygen was observed in our samples, with Assala-Dahab showing the highest dissolved oxygen saturation (8.42 mg/L), followed by Safaga Port (Aluminum) and Abu-Monkar Island, measuring 6.00 and 5.75 mg/L, respectively (Table 1). Aside from Assala-Dahab, in which the oxygen saturation is considered to be greater than the saturation level of the Red Sea, the dissolved oxygen saturation level in the remaining sites was within the previously reported range (4.8 to 6.5 ml/L; (Institute of Marine Research 2012).

3.2. Pyrotag 16S rDNA Diversity and Taxonomic assignments

A total of 131,916 reads were generated from the cultured Red Sea coastal sediments and the two lakes using pyrotag sequencing. The taxonomic assignment of the reads to major bacterial genera detected 211 different genera. Following significance testing, we concluded that only 75 genera were significantly detected; these were represented by a total of 131,402 reads. Only 1.3% of these were unassigned reads, including both unassigned bacteria and unassigned organisms. The label “unassigned organism” indicates a taxon of unknown bacteria, archaea or eukarya (Table 2). We compared the diversity of bacterial phyla reads across the sites. Using the media and conditions described in the materials and methods section, five major bacterial phyla were cultured from the 10 sites sampled, predominantly Proteobacteria (68%), followed by Firmicutes (13%),

Fusobacteria (12%), Bacterioidetes (6%) and Spirochetes (0.03%)(Figure 2). Members of Proteobacteria included γ -proteobacteria (92%), followed by δ -proteobacteria (7%) and ϵ -proteobacteria (1%) (Figure 2, Table 3). Very low proportions of unassigned organisms were detected across sites (0.9% collectively).

Proteobacteria-assigned reads predominated at all of the sampled sites. No other phyla were significantly detected in S-Abu Ghoson Port. Fusobacteria were not detected in the two lakes. Firmicutes represent the second most abundant phylum in the two lakes and Qusseir Port. Bacterioidetes, Firmicutes and Fusobacteria were detected in the remaining coastal samples, but in varying abundances. For example, Assala-Dahab showed the highest incidence of Bacterioidetes (43/23.6% total Bacterioidetes/total culture), followed by Sharm El-Maya (30/14.1% total Bacterioidetes/total culture). Sharm-El-Maya showed the highest incidence of Fusobacteria (23.7%), followed by Assala-Dahab (21.3%). Fusobacteria was the second most abundant phylum in all of the sites, except Qusseir Port, S-Abu Ghoson Port and the two lakes. Unassigned organisms were the second most abundant phylum in S-Abu Ghoson Port. Interestingly, all reads for Spirochetes were detected in Solar Lake-W. A significant number of reads (424) representing 0.3% of the total analyzed reads were assigned as unknown bacterial phylum; these were only detected in Safaga Port (Aluminum) (Table 3).

3.3. Bacterial consortium along the Red Sea Coast

An average of 34 ± 1.6 reads were assigned to the remaining seven Red Sea Coast samples, with rare reads constituting 28 ± 1.5 . The S-Abu Ghoson Port reads were unique from the other coastal sites: 15 bacterial reads were identified, of which 11 were considered rare. Unknown species of *Photobacterium* (50%) and *Photobacterium halotolerans* (24%) predominated among the bacterial-assigned reads in S-Abu Ghoson Port. Unassigned *Vibrio* spp. predominated among the reads in the remaining Red Sea Coast samples (53.4%), followed by *Propionigenium maris* (15.4%). Qusseir Port and S-Abu Ghoson Port were exceptions (Supplemental Table 1).

Table 4a shows a preliminary taxonomic assignment of V6-V4 reads to the previously reported pathogenic bacteria (strictly infecting humans). Note that the V6-V4 reads are relatively short for assigning taxa at the species level. However, we observed that *Clostridium botulinum*

and three assigned species of *Vibrio* represented the pathogenic bacteria detected in the cultures of these Red Sea sites. In total, *Vibrio parahaemolyticus* was the most abundant pathogenic bacterium (48.5/0.67% of the total pathogenic bacteria/total reads), followed by *Clostridium botulinum* (35.8/0.5% of the total pathogenic bacteria/total reads). The pattern of the pathogenic bacteria (distribution and abundance) in the Solar Lake-W was different from the remaining sites, as *Clostridium botulinum* was detected only at this site. As expected, the taxonomic assignment of Saline Lake-RM reads did not match any known pathogenic bacteria, followed by Abu-Monkar Island and Assala-Dahab, which showed the lowest abundance of reads to pathogenic bacteria (0.8/0.1% and 2.2/0.3% of the total detected pathogenic bacteria/the site's total cultured bacteria, respectively).

We also detected reads for which previously identified genera/species are reported to include both pathogenic and non-pathogenic members. We refer to these as potential pathogens (Table 4b). Our culture approach has detected these potentially pathogenic bacteria, including five unknown families of Lachnospiraceae, Ruminococcaceae, Peptostreptococcaceae, Clostridiaceae and Vibrionaceae. Additionally, eight unknown species of unassigned *Anaerovorax*, *Fusobacterium*, *Bacillus*, *Clostridium*, *Sedimentibacter*, *Desulfovibrio*, *Arcobacter* and *Vibrio*. Additionally, two unknown orders of Sphingobacteriales and Clostridiales were detected, and we categorized them as potentially pathogenic bacteria.

3.4. Solar lake-W and Saline lake-RM Bacterial Consortia

In total, 36 and 28 bacterial reads were cultured and amplified from the Solar Lake-W and Saline Lake-RM, respectively. Four bacterial reads were unique to these two lakes, including reads assigned to *Orenia marismortui* and unknown species of *Caloranaerobacter*, *Clostridiisalibacter* and *Halomonas*. Eight and nine reads were unique to Solar Lake-W and Saline Lake-RM, respectively (Table 5). Of the 36 bacterial reads cultured from Solar Lake-W, 21 are considered rare bacterial reads (less than 1%). Unknown species of *Vibrio* dominated the cultured community (55%). The remaining reads constituted six species of *Vibrio* (*Vibrio parahaemolyticus*-2.5%; Figure 3). Surprisingly, 9% of the culture was assigned to the genus *Clostridium*, with 4.1% assigned as *Clostridium botulinum*. Seven percent of the culture was assigned to *Desulfovibrio* and

4% to *Clostridibacter*. Conversely, an unknown genus of Marinlabiaceae and *Geosporobacter* genus represented 7% and 5% of the total bacterial reads of this lake, respectively (Figure 3).

Of the 28 bacterial reads in the Saline Lake-RM, 17 were considered to be rare bacterial taxa (less than 1%). Saline Lake-RM was dominated by an unknown species of *Clostridium* (29%), followed by an unknown species of *Marinobacter* (24%), an unknown species of *Halomonas* (18%), and an unknown species of *Idiomarina* (5.7%). Only six bacterial reads were assigned at the species level: *Clostridiisalibacter paucivorans* (4.3%), *Desulfovibrio halophilus* (4.3%), *Bacillus chandigarhensis* (1.8%), *Oreniamarismortui* (0.5%), *Paraliobacillus quinghaiensis* (0.12%) and *Clostridium caminithermale* (0.01%).

Four more unknown species were significantly detected: unknown species of *Clostridiisalibacter*, *Bacillus*, *Alteromonas* and *Anaerophaga* account for 3.3%, 2.8%, 2.5% and 1.8% of assigned reads, respectively. The rare bacterial taxa (below 1%) include two species, nine genera, two families and two orders (Figure 4).

4. DISCUSSION

We analyzed the microbial community in sites that have been impacted by, land-based, oceanic and atmospheric pollution sources along the Red Sea coast. Additionally, we assessed the microbial community in two lakes on the Sinai Peninsula: Solar Lake-W and the Saline Lake-RM, which are believed to have seeps from the Red Sea (Aharon, Kolodny et al. 1977). Following sediment cultures, we used the V6-V4 hypervariable region and amplified a significant number of pyrotags; 131,916 16S rDNA reads were obtained, of which 211 were assigned to major bacterial taxa. We analyzed the 75 significant taxa detected (131,402 reads) and grouped them into two major categories: human pathogens and oil-degrading bacteria.

We used a culture media that allows the cultivation of heterotrophic marine bacteria. Therefore, this study examines a portion of the bacterial community in these environments. Our cultured marine surface sediment samples had several taxa in common. For example, γ -proteobacteria dominated all of the cultured bacteria in the 10 sampled sites. Several previous culture-independent approaches showed a significant dominance of γ -proteobacteria in marine sites (Liao, Xu et al. 2009), followed by Firmicutes, Fusobacteria and Bacteroidetes. A previous study identified Firmicutes and Bacteroidetes from Ras Muhammed sponges using a culturing approach (Radwan, Hanora et al. 2010, Aboul-Ela, Shreadah et al. 2012). Fusobacteria have previously been detected in the Red Sea; however, they were detected in deep sediments of a brine pool (Siam, Mustafa et al. 2012). Most species of Fusobacteria and Bacteroidetes are anaerobes. Fusobacteria have been cultured from surface sediments samples of the Wadden Sea, Germany, under strict anaerobic conditions (Köppke, Wilms et al. 2005). Note that strict anaerobic conditions were not implemented in our culture conditions; however, anaerobic bacteria such as Fusobacteria, Bacteroidetes and Clostridium were detected. Because our culture conditions are most likely enriched for aerobes, it is likely that such anaerobes are more abundant *in situ*. This finding suggests that we may have cultured the predominant phyla in our studied sites; however, rare taxa are as important in the microbial community. This imposes a limitation on our study, in that it strictly focuses on the numerically dominant bacterial taxa. A culture-independent approach would allow the study of the entire bacterial community.

Several human pathogens were detected in our Red Sea samples, including known *Vibrio* and *Clostridium* species. *Vibrio* species are naturally detected in marine environments (Johnson, Flowers et al. 2010). *Vibrios* pp. have been found to dominate ‘plastisphere’ (i.e., plastic marine debris; (Zettler, Mincer et al. 2013). In this culture-independent study, 10 different *Vibrio* species were detected and were dominated by an unknown species of *Vibrio*. Surprisingly, our culture-

dependent approach detected 28 *Vibrio* species; of these, 12 were significantly detected in our cultures, which were dominated by an unknown species of *Vibrio*. This “marine *Vibrio* phenomenon” was detected at all of our sites except Saline Lake-RM. In addition to the striking dominance of this unknown species of *Vibrio*, three of the remaining 11 known *Vibrio* species were human pathogens. Several *Vibrio* species have been reported to cause gastrointestinal, skin and other infections (Thompson, Iida et al. 2004), including *V. parahemolyticus*, *V. vulnificus* and *V. alginolyticus*. Note that *V. vulnificus*, *V. splendinus* and *V. sinaloensis* were not detected in the ‘plastisphere’ *Vibrio* community (Zettler, Mincer et al. 2013). *V. shilonii*, which was detected in our cultures, is known to cause coral bleaching (Banin, Israel et al. 2000, Thompson, Iida et al. 2004). *V. fortis* and *V. harveyi* may contribute to coral bleaching (Thompson, Iida et al. 2004). Solar Lake-W was most dominated by pathogenic bacteria, which constituted 7.3% of the Solar Lake-W bacterial culture; these were primarily *Vibrio parahaemolyticus*. One limitation in our study is the short read length of the V6-V4 region, which does not provide optimal resolution at the species level. We therefore cannot assign taxa to the species level based solely on V6-V4 16S rRNA. However, our results imply that the “marine *Vibrio* phenomenon” may pose a pathogenicity risk for human and/or marine life.

Similarly, other human pathogens were detected in our sediment samples. Most importantly, *Clostridium botulinum* was uniquely and significantly detected in Solar Lake-W. Clostridial members are known for their ability to survive under harsh conditions through spore formation. *Clostridium botulinum* outbreak detection includes the detection of spores from the contaminated environment, which may include soil and aquatic environments (e.g., marine sediments; (Neimanis and Speck 2012)). This may explain the detection of strict anaerobes under our aerobic culture conditions: we could have isolated bacterial DNA from bacterial spores. Additionally, it is not uncommon to detect *Clostridium* in marine sediments, as this is one of its natural habitats (Neimanis and Speck 2012). However, *C. botulinum* is a serious human pathogen (Neimanis and Speck 2012). Botulism was first reported in 1991 from a traditional Egyptian salted raw fish, known as “faseikh” (Weber, Hibbs et al. 1993). Since then, no botulism cases have been reported in Egypt (Horowitz 2010). In a 2011 study *C. botulinum* was isolated from food samples in Assiut, Egypt, but no botulism cases were reported (Ahmed, Badary et al. 2011).

In contrast to all of the other sites, Saline Lake-RM had no cultured/detected known pathogenic *Vibrio* or *Clostridium*. The bacterial community in Saline Lake-RM is predominated by a different and unknown bacterial community that is more likely to play a role in hydrocarbon metabolism. Other unknown bacterial families, genera and species were detected in different samples along the Red Sea coast (Table 4b). Related members of these bacterial groups were shown to be human pathogens. Due to the lack of assignments of bacterial reads to known families, genera or species, we may consider them potential pathogens. Further analysis on these groups should be performed to identify their pathogenic potential.

It was not surprising to detect oil-degrading bacteria along the Red Sea, particularly at industrialized sites. However, the oil-degrading bacterial consortia detected in the Red Sea coastal samples were distinct from those detected in the two lakes. *Propionigenium maris*, *Psychrilyobacter*, *Tepidibacter* and *Photobacterium* were mainly detected in the Red Sea coastal samples. *Propionigenium maris* is a marine debrominating bacteria (Watson, Matsui et al. 2000). This species was one of the most abundant species in most of the analyzed sites. *Psychrilyobacter* produces H₂ and acetate and has the ability to degrade hexahydro-1,3,5-trinitro-1,3,5-triazine and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine, two nitramine explosives (Zhao, Manno et al. 2009). *Tepidibacter* was isolated from an oil field in China (Tan, Wu et al. 2012). *Photobacterium* species show mercuric resistance (Reyes, Frischer et al. 1999), are stable in biodiesel production at high methanol concentrations (Yang, Sohn et al. 2009) and have high oil emulsification activity (Ryu, Kim et al. 2006). A bacterial consortium constituting 21 taxa was unique to the two lakes (Table 5). Additionally, three unknown species of *Marinobacter* and *Bacillus* were detected in Saline Lake-W only, and *Anaerophaga* was detected in both lakes. The unknown species of *Marinobacter* was the second most abundant taxa in Saline Lake-RM (24%). Previous studies have identified several strains of *Marinobacter* as oil-degrading bacteria that can degrade aliphatic hydrocarbons under oxic conditions (Cohen 2002, Duran 2010). An unknown species of *Idiomarina* was also uniquely identified in Saline Lake-W (5.7%). A strain of crude oil-degrading bacteria *Idiomarina xiamenensis* was isolated from surface water enriched in crude oil (Wang, Wang et al. 2010, Wang, Lai et al. 2011). Interestingly, the sulfate-reducing bacterium *Desulfovibrio halophilus* was isolated for the first time from the Solar Lake (Caumette, Cohen et al. 1991). However, in our study, we have only detected it in Saline Lake-RM. This species was also detected in brine stratal water of an oil field (Welsh, Lindsay et al. 1996). This bacterium is known to accumulate organic solutes under high salt conditions (Welsh, Lindsay et al. 1996), such as those in Saline Lake-RM. We identified an oil-degrading bacterial consortium in Saline Lake-RM. *Anaerophaga* (detected in the two lakes only) was present in blackish-oily sedimentary residues in an oil separation tank (Denger, Warthmann et al. 2002, Schink 2010). It is worth noting that all of the previous

studies on Solar Lake isolated specific cyanobacteria and analyzed the cyanobacterial mats (Krumbein, Cohen et al. 1977, Jorgensen, Revsbech et al. 1979, Teske, Ramsing et al. 1998, Wieland and Kühl 2000). Strikingly, our approach did not detect or assign any of the reads to cyanobacteria in the Solar Lake culture. Note that cyanobacteria were not detected in uncultured sediments (data not shown). Taken together with our results, the studies conducted on Solar Lake West near Taba/Eilat between its discovery in 1967 and the last reported study in 1983 suggest that the microbial community in this lake has varied greatly during the past 20 years. This finding draws attention to the importance of microbial studies in monitoring and conserving marine environments.

This study molecularly characterized cultured microbial consortia along Egypt's Red Sea coast, with a focus on industrialized sites. Our results demonstrate the dominance of *Vibrio* spp. (human pathogens, coral pathogens and predominantly unknown species), common marine bacteria, hydrocarbon-degrading bacteria and other human pathogenic bacteria. The oil-degrading bacterial consortia were distinctly unique in the Red Sea coast compared with the two lakes sampled, suggesting different hydrocarbon exposures in these two ecosystem types. Additionally, the human pathogen consortia were dominated by *Vibrio* spp., which is different from the Saline Lake-W, that shows 0% of any human pathogenic bacteria. This study provides preliminary evidence for the use of bacterial consortia to assess the impact of industrialization on marine environments.

TABLES

Table 1: Samples, Sampling locations and Physical Parameters

Sites	Coordinates		Physical Parameters		
			Temperature (°C)	pH	Dissolved Oxygen (mg/L)
Solar Lake-W	N 29.42266	E 34.82969	34.70	8.17	2.77
Assala Dahab	N 28.51285	E 34.51566	26.52	8.52	8.42
Saline Lake-RM	N 27.74368	E 34.24069	31.51	8.24	1.94
Sharm El-Maya	N 27.85449	E 34.27381	27.40	8.35	4.46
Abu Monkar Island	N27.21401	E033.88068	23.60	8.53	5.75
Safaga Aluminum Port	N26.73757	E33.94269	20.60	9.00	6.00
Safaga Port (Mangrove)	N 26.61669	E 34.01048	24.10	8.46	4.30
Hamrawein Port	N 26.25083	E 34.20295	27.70	8.53	3.50
Quesser Port	N 26.10335	E 34.28542	30.8	8.76	3.4
S-Abu Ghoson Port	N 24.45249	E 35.20274	23.51	8.12	5.13

Table 2: Pyrotag 16S rDNA data set

	Solar Lake-W	Assala-Dahab	Saline Lake-RM	Sharm El-Maya	Abu Monkar Island	Safaga Aluminum Port	Safaga Port (Mangrove)	Hamawareen Port	Qusseir Port	S-Abu Ghoson Port	Totals
Number of total Taxa	56	66	54	47	55	57	56	48	50	26	211
Significant Taxa	64.30%	65.20%	52%	72.30%	54.50%	66.70%	60.70%	62.50%	66%	57.70%	75
Total Reads	16053	14720	18577	16895	12430	10438	10932	10580	10709	10582	131916
Significant assigned Reads	98.25%	98.90%	98.85%	98.87%	98.60%	94.60%	98.63%	98.76%	98.63%	98.40%	129736
Unknown Reads	1.26%	0.70%	0.80%	0.90%	0.89%	4.83%	0.91%	0.95%	0.96%	1.36%	1666
Total analyzed Reads	15975	14655	18510	16860	12369	10379	10881	10550	10665	10558	131402

Table 3: Total number of reads assigned at the phylum level in Red Sea coastal samples and two lakes

Phylum	Solar Lake-W	Assala-Dahab	Saline Lake-RM	Sharm El-Maya	Abu Monkar Island	Safaga Aluminum Port	Safaga Port (Mangrove)	Hamrawein Port	Qusseir Port	S-Abu Ghoson Port	Totals
Bacteroidetes	1561	3453	346	2377	104	49	75	63	9	0	8037
Firmicutes	3285	1076	7900	809	1541	824	324	269	1410	2	17440
Fusobacteria	0	3256	0	3636	2197	1558	2806	1655	183	1	15292
Proteobacteria	10881	6771	10118	9882	8416	7444	7577	8462	8960	10411	88922
Spirochetes	45	0	0	0	0	0	0	0	0	0	45
Unknown bacteria	0	0	0	0	0	424	0	0	0	0	424

Table 4: a) Preliminary assignment of total number of reads to known human pathogens, b) Total number of reads assigned to potential pathogens (previously reported genera/species included human pathogens and non-pathogens)

a)

Pathogenic bacterium	Solar Lake-W	Assala-Dahab	Saline Lake-RM	Sharm El-Maya	Abu Monkar Island	Safaga Aluminum Port	Safaga Port (Mangrove)	Hamrawein Port	Qusseir Port	S-Abu Ghoson Port	Totals
<i>Clostridium botulinum</i>	651	0	0	0	0	0	0	0	0	0	651
<i>Vibrio alginolyticus</i>	29	2	0	3	0	2	7	4	6	4	57
<i>Vibrio harveyi</i>	24	2	0	2	0	4	12	5	8	7	64
<i>Vibrio parahaemolyticus</i>	400	26	0	61	12	70	96	71	73	72	881
<i>Vibrio vulnificus</i>	68	10	0	6	3	10	21	6	29	12	165
Total	1172	40	0	72	15	86	136	86	116	95	1818
	7.30%	0.30%	0%	0.40%	0.24%	0.95%	1.30%	0.91%	1.50%	0.90%	

b)

Potentially Pathogenic members (Unknowns)	Solar Lake-W	Assala-Dahab	Saline Lake-RM	Sharm El-Maya	Abu Monkar Island	Safaga Aluminum Port	Safaga Port (Mangrove)	Hamrawein Port	Qusseir Port	S-Abu Ghoson Port	Totals
Unknownn genus of Lachnospiraceae	36	24	60	16	0	4	2	0	5	0	147
Unknown genus of Ruminococcaceae	0	22	0	25	0	0	0	0	0	0	47
Unknown species of an unassigned <i>Anaerovorax</i>	1	66	0	0	0	0	0	0	0	0	67
Unknown species of <i>Fusobacterium</i>	0	134	0	0	0	0	0	0	0	0	134
Unknown genus of Peptostreptococcaceae	0	0	0	0	196	376	34	2	10	0	618
Unknown Species of <i>Bacillus</i>	0	0	514	0	0	0	0	0	0	2	516
Unknown Species of <i>Clostridium</i>	347	103	5310	256	4	77	15	0	165	0	6277
Unknown species of <i>Sedimentibacter</i>	0	84	0	0	0	0	0	0	0	0	84
Unknown Species of <i>Desulfovibrio</i>	1153	1305	11	2474	0	17	0	0	0	0	4960
Unknown Species of <i>Arcobacter</i>	0	128	0	411	0	28	95	0	6	0	668
Unknown family of Sphingobacteriales	4	174	5	271	0	0	0	0	0	0	454
Unknown genus of Clostridiaceae	29	22	17	179	2	3	10	0	388	0	650
Unknown family of Clostridiales	4	18	3	42	3	76	21	19	541	0	727
Unknown Species of <i>Vibrio</i>	8761	5034	0	6501	7775	6739	5810	7479	8521	2402	59022
unknown genus of Vibrionaceae	20	0	0	2	2	6	14	2	6	21	73
Totals	10355	7114	5920	10177	7982	7326	6001	7502	9642	2425	74444
	64.80%	48.50%	31.90%	60.40%	64.50%	70.60%	55.15%	71.10%	90.40%	23%	

Table 5: Bacteria identified only in the two lakes

Bacteria	Saline Lake-RM (%/number of reads)	Solar Lake-W (%/number of reads)
Unknown species of <i>Halomonas</i>	18.33%/ 3405	0.01%/ 1
Unknown species of <i>Caloranaerobacter</i>	0.15%/ 27	0.01%/ 2
<i>Orenia marismortui</i>	0.5%/ 97	0.1%/ 13
Unknown species of <i>Clostridiisalibacter</i>	3.33%/ 618	0.42% / 670
Unknown species of <i>Paraliobacillus</i>	0.1%/ 16	0
<i>Paraliobacillus quinghaiensis</i>	0.12%/ 22	0
Unknown species of <i>Halocella</i>	0.12%/ 23	0
Unknown species of <i>Halanaerobium</i>	0.16%/ 29	0
<i>Bacillus chandigarhensis</i>	1.8%/ 326	0
Unknown species of <i>Alteromonas</i>	2.5%/ 460	0
<i>Desulfovibrio halophilus</i>	4.35%/ 808	0
<i>Clostridiisalibacter paucivora</i>	4.4%/ 812	0
Unknown species of <i>Idiomarina</i>	5.7% 1061	0
<i>Nesiotobacter exalbescens</i>	0	0.12%/ 19
<i>Salinivibrio proteolyticus</i>	0	0.12%/ 19
<i>Salinivibrio costicola</i>	0	0.15%/ 24
<i>Spirochaeta bajacaliforniensis</i>	0	0.3%/ 45
Unknown species of <i>Desulfocella</i>	0	1%/ 161
Unknown species of <i>Salinivibrio</i>	0	1%/ 161
Unknown genus of <i>Porphyromonadaceae</i>	0	1.3%/ 205
<i>Clostridium botulinum</i>	0	4.1%/ 651

FIGURES

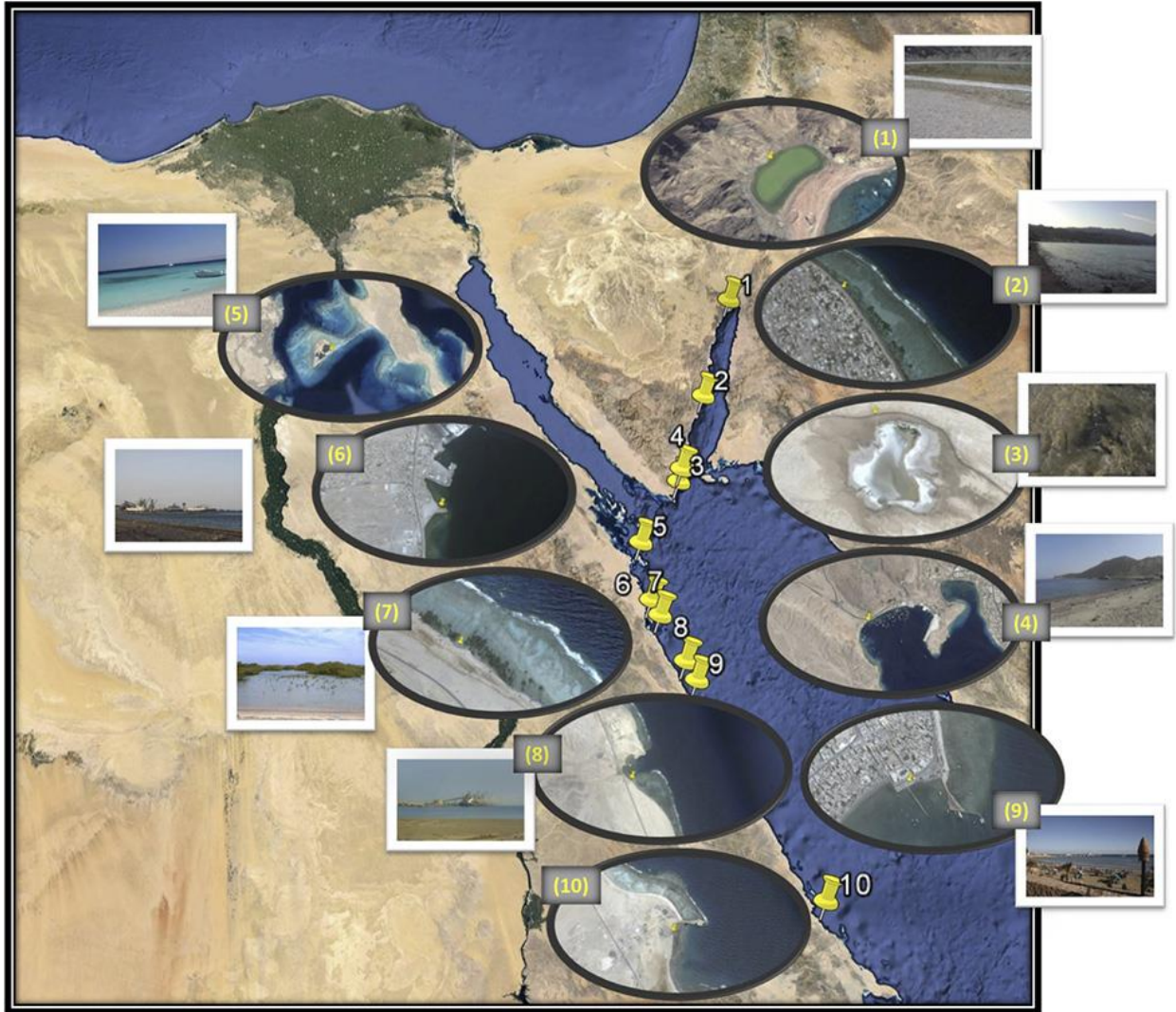


Figure 1: Map of the locations of the eight coastal sites (numbered 2, 4 and 5–10) and the two lakes (numbered 1 and 3). Image generated using Google, Image Landsat Data SIOA, NOAA, U.S. Navy, NGA, GEBCO. The adjacent zoomed image was generated by Image © 2014 DigitalGlobe (sample sites 1, 5 and 6), Image © 2014 CNES/Astrium (sample sites 2 and 4), Data SIOA, NOAA, U.S. Navy, NGA, GEBCO, Image © 2014 CNES/Astrium (sample site 3) and Image © 2014 CNES/Astrium, Data SIOA, NOAA, U.S. Navy, NGA, GEBCO, Image © 2014 TerraMetrics (sample sites 7, 8, 9 and 10). Sample sites: 1. Solar Lake-W, 2. Assala-Dahab, 3. Saline Lake-RM, 4. Sharm El-Maya, 5. Abu-Monkar Island, 6. Safaga Port (Aluminum), 7. Safaga Port-mangrove, 8. Hamrawein Port, 9. Qusseir port and 10. S-Abu Ghoson port.

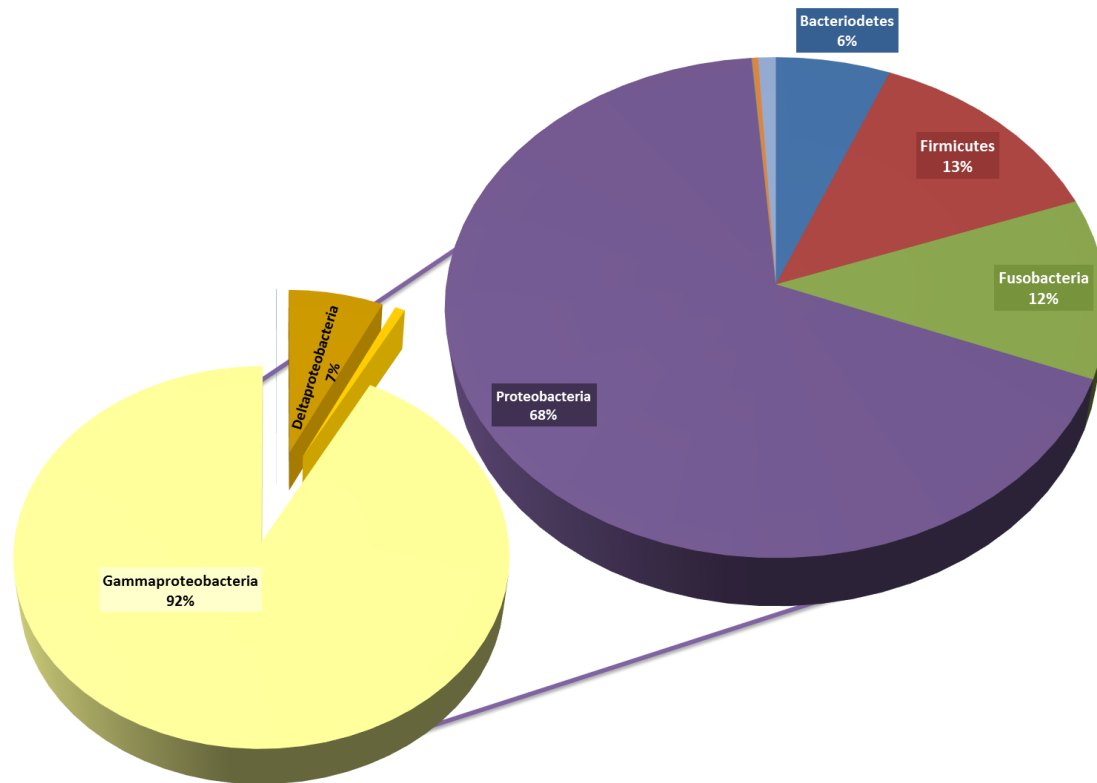


Figure 2: Pie chart representation of the total cultured phyla in all samples, showing 68% Proteobacteria, 13% Firmicutes, 12% Fusobacteria, 6% Bacteroidetes and unknown bacteria and organism phyla (percent abundance not shown). The composition was 92% γ -proteobacteria, 7% δ -proteobacteria and the remaining Proteobacteria were assigned as ϵ -proteobacteria.

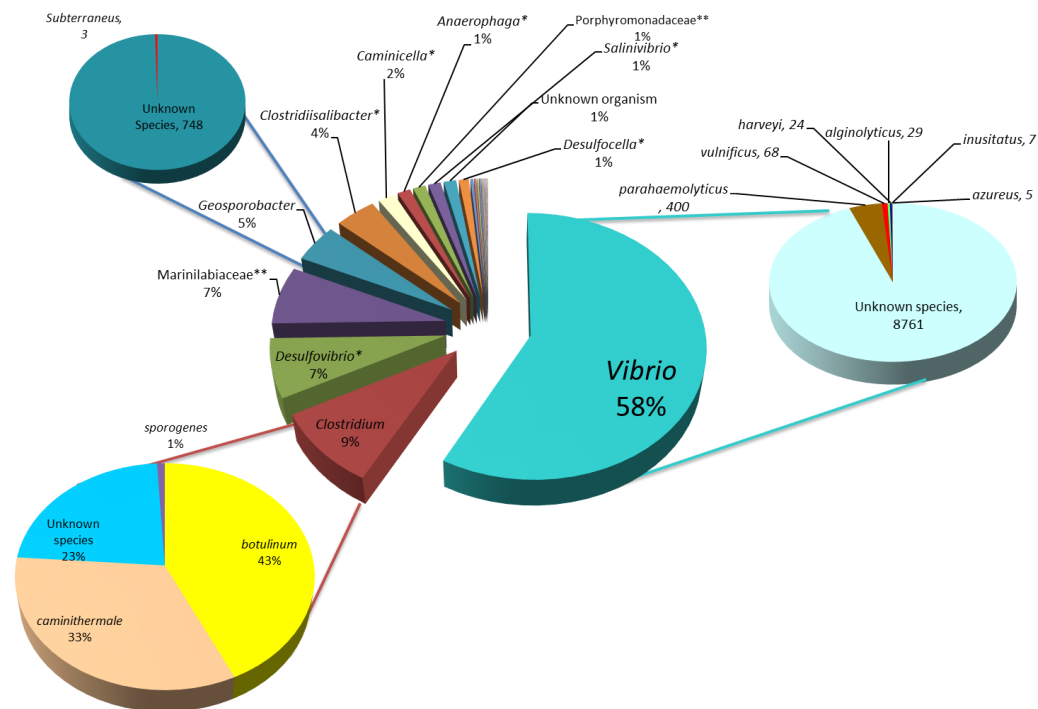


Figure 3: Solar Lake West genera are presented in the middle pie chart, predominated by *Vibrio* (58%), *Clostridium* (9%), unknown species of *Desulfovibrio* (7%) and *Geosporobacter* (5%). The *Vibrio*, *Desulfovibrio* and *Geosporobacter* are predominantly unknown species. The predominant *Clostridium* species is *C. botulinum* (43%). * unknown species; **unknown genus.

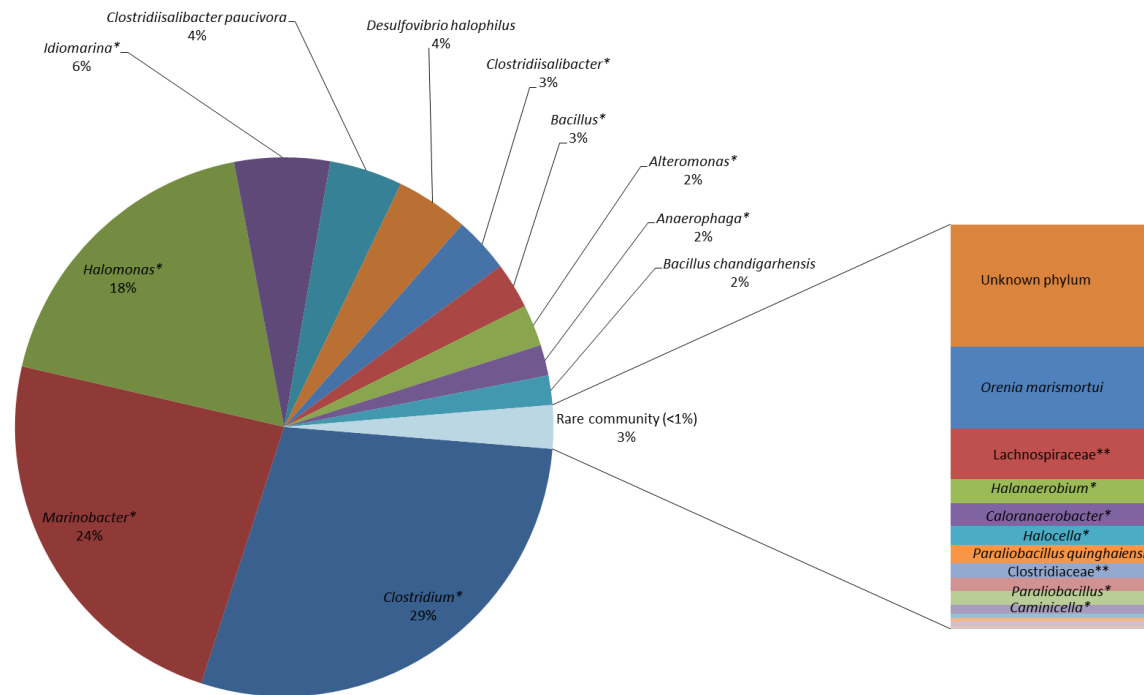


Figure 4: Saline lake bacterial consortia showing predominantly unknown species, including unknown species (*) of *Clostridium* (29%), *Marinobacter* (24%), *Halomonas* (18%), *Idiomarina* (6%), *Clostridiisalibacter* (3%), *Bacillus* (3%) and *Alteromonas* (2%). Known species identified include *Clostridiisalibacter paucivora* (4%), *Desulfovibrio halophiles* (4%) and *Bacillus chandigarhensis* (2%).

SUPPLEMENTARY MATERIAL

Table S1 | Bacterial communities comprising more than 0.1% in each site are represented.

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**CHAPTER 2: EGYPT'S RED SEA
COASTII: A MICROBIOME STUDY
REVEALING ECOSYSTEM SPECIFIC
HYDROCARBON DEGRADING
BACTERIAL CONSORTIA**

ABSTRACT

The Red Sea is considered one of the youngest oceanic systems with unique physical, geochemical and biological characteristics. The Red Sea is exposed to several different sources of pollution including tourism, industrialization, extensive fishing, oil processing and shipping. We analyzed the geochemical characteristics and microbial community of sediments along the Egyptian Red Sea coast. Our samples included 1- four ports for shipping aluminum, ilmenite and phosphate, 2-a site previously reported to have suffered extensive oil spills, 3-a reported tourism impacted site 4- two mangrove sites and 5-two lakes. We generated two major datasets in this study; i- measurements of Carbon, Hydrogen, Nitrogen and Sulfur, 29 metals and selected semi-volatile oil contents and ii- 16S rRNA Pyrotags, for each of the ten sediment Red Sea coastal sites. In this study we report an Egyptian Red Sea Coastal Microbiome, 30 different taxa, based on the taxonomic assignments of 16S rRNA Pyrotags to major bacterial groups. Hydrocarbon biodegrading bacteria predominated the majority of the Red Sea sites; particularly in two ports it reached up to 76% of the total identified genera. On the other hand, sulfate reducing and oxidizing bacteria dominated the two lakes, on the expense of other hydrocarbon metabolizers'. Despite the reported "Egyptian Red Sea Coastal Microbiome", individual sites showed unique evolution of their microbial communities based on minor intrinsic and imposed variation per sites.

1. INTRODUCTION

The Red Sea is exposed to different sources of pollution attributed to tourism, industrialization, extensive fishing, oil processing and shipping pollution. The Red Sea is prone to significant damage and serious environmental hazards because of the extensive oil exploration and numerous touristic attractions on the coast, in addition to being one of the highest oil traffic of marine water body (El-Sheshtawy, Khalil et al. 2014); 7% of the world seaborne trade is through the Red Sea and Gulf of Aden (Regional Organization for the Conservation of the Environment of the Red Sea and Gulf of Aden 2001).

Several bordering countries reported repetitive pollution outbreaks in the Red Sea (reviewed in (Mustafa, Abd-Elgawad et al. 2014)), including Egypt (Riegl and Velimirov 1991, El-Sorogy, Mohamed et al. 2012), Israel (Loya and Rinkevich 1980, Riegl and Velimirov 1991, Loya 2004, Abelson, Olinky et al. 2005), Yemen (Alkershi and Menon 2011), Saudi Arabia (Hanna and Muir 1990, Badr, El-Fiky et al. 2009, Ali, Elazein et al. 2011), Jordan (Al-Najjar, Rasheed et al. 2011), Sudan (Idris, Eltayeb et al. 2007) and Eritrea (Idris, Eltayeb et al. 2007). Several studies addressed “the anthropogenic impacts” on the Egyptian (Mansour, Nawar et al. 2011, Gladstone, Curley et al. 2013) and non-Egyptian Red Sea coast (PERSGA/GEF 2004, Gladstone, Curley et al. 2013, Furby, Apprill et al. 2014). The adverse consequences were evident in birds (Clark and Gorney 1987), fish (Ali, Ahmed et al. 2014), mussels (El Nemr, El-Sikaily et al. 2004, Khaled, El Nemr et al. 2004) and corals (El-Sikaily, Khaled et al. 2003, El Nemr, El-Sikaily et al. 2004).

The rich and diverse coral reefs, fish and mammals along the warm and clear water contributed to the extensive tourism in Red Sea Egypt; in 2010 43% of tourism in the Red Sea were in Egypt (Gladstone, Curley et al. 2013). The adverse environmental impacts of tourism was documented on the corals deteriorating health (Gladstone, Curley et al. 2013, Naumann, Bednarz et al. 2015). Metal deposition in the Red Sea was attributed to both crustal and anthropogenic sources from the gulf of Aqaba (Al-Taani, Rashdan et al. 2014). Additionally, natural factors contributed to the metal accumulation in the Red Sea; the grey mangrove *Avicennia marina* was reported to accumulate metals such as Cu, Pb and Zn (MacFarlane, Pulkownik et al. 2003). This mangrove species is the predominant species in the western Red Sea coast and the only species in the Egyptian Red Sea coast (Regional Organization for the Conservation of the Environment of

the Red Sea and Gulf of Aden 2001). Heavy metals pollution in the northern coast of the Egyptian Red Sea is mainly attributed to industrialization and population overgrowth in Suez. While in the southern part, ores and oil shipping and tourism are the main anthropogenic inputs for heavy metals pollution (El-Moselhy, Othman et al. 2014). Corals have been used as indicators for heavy metal pollution. Corals from polluted sites in Jeddah have shown to accumulate extensive heavy metals (Hanna and Muir 1990). An increase in metal concentration were reported in corals in El Qusseir and Hamrawein Ports and were attributed to urbanization, tourist activities, oil exploration in the gulf of Suez and the old shipping of phosphate at Hamrawein (near Qusseir area) (El-Sorogy, Nour et al. 2013). Higher heavy metals concentrations were detected in skeletons of corals from areas of intense human activities and developments in the Red Sea coast of Jordan, (Al-Rousan, Al-Shloul et al. 2012). Additionally, heavy metals (Cu, Cd, Mn, Pb, Fe and Zn) were concentrated in the liver, gills and muscles of fishes in a Red Sea heavy metal polluted areas (El-Moselhy, Othman et al. 2014). Accumulation of heavy metals from Red Sea water and sediments in clams (*Bivalve molluscs*) was also assessed to ensure safety levels on human (Mohammed, Mohamed et al. 2014).

Oil is considered a “chronic” source of pollution in the Red Sea and has been reported since the 70s (Loya 1975). A study from 1979-1981 confirmed the extensive oil pollution in the Red Sea area, especially in Ras-Gharib, Ras-Shukhair and the southern part of the Gulf of Suez. The study attributed this high level to the offshore oil fields exploration and processing; while it showed the absence of any dissolved hydrocarbons in Hurghada (Hanna 1983). Both diesel and motor oils contribute to the increase in polychromatic aromatic hydrocarbons (PAHs) content in the sediments. Motor oil are emitted from engines and has in addition to PAHs, high heavy metal contents such as Pb, Zn, Cu, Cr, Ni and Cd (Vazquez-Duhalt 1989). The hydrophobic nature of PAH allows the attachment to particulate materials in the sea. This makes sediments a very crucial component to assess the levels of hydrocarbons and other matters in any aquatic ecosystem. It also presents a very important tool in assessing the sources of oil pollution in coastal areas (Perra, Renzi et al. 2010). PAH in surface sediments along the Red Sea Egyptian coast were analyzed to determine the level of pollutants and its hazards to the environment (El Nemr, El-Sadaawy et al. 2014). At the microbial level, *Pseudomonas* species, known to contribute to oil and hydrocarbons bioremediation, was detected in Gemsa Bay-one of the highly polluted Red Sea sites (El-Sheshtawy, Khalil et al. 2014).

In this study, the microbial communities in ten different sites with touristic, industrialization, heavy metal shipping and oil shipping impacts were investigated. We assessed the effects of different anthropogenic sources on the bacterial community of the Red Sea coastal sediments. Our previous study assessed the effect of industrialization along the Red Sea coast using a culture dependent approach (Mustafa, Abd-Elgawad et al. 2014). The current study utilizes a cultured-independent approach, and therefore reports the entire microbial community composition in Egypt's Red Sea coast. We utilize an environmental genomic approach; through DNA extraction of sediment sampled across the coast followed by bacterial 16S rRNA pyrotagging. Additionally, we correlate the microbial community to geochemical and physical measurements to infer direct correlation between anthropogenic sources and evolution of microbes.

2. MATERIALS AND METHODS

2.1 Chemical Analyses

Chemical Analyses were performed on 0.5 grams of sediments from 0.5-1m depth, as previously described (Mustafa 2012). Carbon, Hydrogen, Nitrogen and Sulfur profiling was performed using the Thermo Electron Corporation FlashEA 1112 CHNS elemental analyzer as recommended by the manufacturer. Quantitative and qualitative metal and non-metal elements in sediments' samples were assessed using inductively coupled plasma optical emission spectroscopy ICP-OES. This technique was achieved using the Varian Vista MPX CCD Simultaneous axial ICP-OES. Total Petroleum Hydrocarbons were analyzed in our sediments using Northwest Total Petroleum Hydrocarbon Analytical Method (NWTPH-Dx) in which Diesel and Motor oils' percentages were measured. Physical parameters (pH, temperature, salinity, and dissolved oxygen) for the ten sites were previously reported in Chapter 1 (Mustafa, Abd-Elgawad et al. 2014).

2.2 Molecular Biology Analyses

The sediment sampling from the different Red Sea coastal sites and two lakes was previously reported (Mustafa, Abd-Elgawad et al. 2014) and presented in figure 1. DNA was extracted from 0.25 grams of sediments using the MoBio kits for all sites, with the exception to Sharm El-Maya were PowerMax® Soil DNA Isolation Kit (Cat. number 12888-100) was used (0.75 grams). This was because of Sharm El-Maya sediment nature. Triplicates isolations of three different samples per site were performed, the extracted DNA was then pooled in one tube then directed for PCR and sequencing. 16S rDNA V6 and V4 bacterial hyper-variable regions were amplified from the extracted DNA as previously described (Sogin ML, Morrison HG et al. 2006) using the primer sets previously described (Siam, Mustafa et al. 2012). The V6-V4 amplifications were followed by Pyrosequencing using 454 GS FLX Titanium technology.

2.3 Bioinformatics analysis

Taxonomic assignment of 16S rRNA were performed using the Visualization and Analysis of Microbial Population Structures, VAMPS, database; hosted by the Josephine Bay Paul Center, MBL, Woods Hole ([http://vamps.mbl.edu/resources/databases .php](http://vamps.mbl.edu/resources/databases.php)). Statistically significant assigned reads were subjected to Fisher Exact test, as previously described in (Siam, Mustafa et al. 2012, Mustafa, Abd-Elgawad et al. 2014).

3. RESULTS SECTION

3.1. Selected Geochemical Profiles in the Red Sea Coastal Sediments

The total organic Carbon, Hydrogen, Nitrogen and Sulfur (CHNS) and metals accumulated in the ten studied sites were measured (Table 1). The highest Carbon concentration was detected in Abu Monkar Island and Safaga Port (Mangrove); 12.04% and 11.88%, respectively. Our assay could only detect Nitrogen in the two lakes (0.13% in Saline Lake-RM and 0.2% in Solar Lake) and Abu Monkar Island (0.14%). Similarly, Sulfur was only detected in three sites; Abu Monkar Island (0.52%), Solar Lake-W (0.51%) and Safaga Aluminum Port (0.44%) (Table 1).

We classified the 29 elements detected in the Red Sea sampled sediments into two groups based on their total concentrations. Group I elements were measured in total percent this included Ca, Fe, Si, Sr, Mg, Al, K, Na and P (Table 1). Solar Lake-W showed the highest metal content and the highest concentration of Group I metals (Table 1) and half of group II elements (Table 1), this was followed by S-Abu Ghoson then Qusseir ports, in the total metal richness. Iron concentration, the highest element in all of the sites, greatly contributed to the metal richness in all sites, followed by Mn and Zn. Phosphorous concentrations in our samples was rather unique and can be represented in three categories; 1- Sharm El-Maya shows (61.6% equivalent to 616000 ppm) 2- the three ports: Hamrawein, Qusseir and S-Abu Ghoson (3300 ppm \pm 1652) and 3-the remaining sites (300 ppm \pm 284).

The highest relative concentration of Diesel oil was detected in Safaga Port (Mangrove) (67 mg/kg) and Safaga Aluminum Port (45 mg/kg), and the least concentration was detected in Abu Monkar Island (Table 1). The mangrove site of Safaga Port showed a strikingly higher Motor oil concentration (2690 mg/kg). The two lakes followed the Safaga sites in the Diesel oil content but not Motor oil, which showed its second highest content in Sharm El-Maya (74.7 mg/kg), followed by Qusseir Port (42.1 mg/kg)

3.2. Taxonomic Assignments of 16S rRNA Pyrotags to Major Bacterial Groups

A total of 140946 16S rRNA reads were generated by the amplification of 16S rRNA pyrotag sequencing (Table 2). Saline Lake-RM generated the highest number of reads (19628 \pm 775 reads). 139083 of the total reads (98.7%) were taxonomically assigned to 900 taxa, of which

381 were significantly detected (136314 reads, 96.7%). 1863 significant reads failed to be assigned to any known organism (Table 2).

Taxonomic assignment of reads to major bacterial groups significantly detected 32 taxa. 23 of the 32 significant phyla, represented the rare bacterial community (<1% of significant reads) (Figure 1). The major nine bacterial phyla ($\geq 1\%$ of significant reads) included Proteobacteria (55.2%), Actinobacteria (10.2%), Gemmatimonadetes (6.5%), Bacteroidetes (6.4%), Planctomycetes (4.4%), Firmicutes (3.3%), Chloroflexi (3.2%), Acidobacteria (2.2%), and an Unknown phylum (1.3%) (Figure 1). The 55% (76325 reads) assigned to Proteobacteria (Figure 1) included γ -proteobacteria (74/41% total proteobacteria /total significant reads %), α -proteobacteria (16/8.5%), δ -proteobacteria (9/5%), and β -proteobacteria (1/0.7%) (Figure 1).

3.3. Abundant and Rare Bacterial Genera in Red Sea Coastal Sites

The 360 genera significantly detected in the ten Red Sea analyzed sites showed the highest diversity in Qusseir Port (248 genera) and Safaga Port-mangrove (236 genera) and the least diversity in the two lakes; Saline Lake-RM (167 genera), Solar Lake-W (164 genera) and Abu Monkar Island (166 genera) (Table 2).

The genus *Vibrio* was detected in all of the sites with the exception to Saline Lake-RM and dominated both Abu Monkar Island (18%) and Hamrawein Port (14%). Other dominant genera included unknown genus of Enterobacteriaceae, Gemmatimonadetes, Pseudoalteromonas, Granulosicoccus, *Lamia*, *Marinobacter* and *Aestuariibacter*. It is worth noting that the bacterial genera in Safaga Port (Mangrove) were predominantly unknown members and only 1% of the reads were identified genera (Supplementary Table 1/Figure 2).

On the other hand, the rare bacterial taxa (<5%) were mostly detected in Safaga Port-mangrove (79%), followed by Qusseir Port (75%) and Safaga Aluminum Port (70%) (Figure 2 and Supplementary Table 1). Hamrawein Port showed the least representation of rare taxa (52%).

The shared Egyptian Red Sea coastal bacteria detected in all of the studied sites, referred to as “The Egyptian Red Sea Coastal Microbiome” revealed different taxa resolution, which included 1-nine genera, 2- seven unassigned genera of bacterial families, 3-unassigned families of five orders, 4-unassigned orders of five classes and 5-unassigned classes of Chloroflexi and Planctomycetes (Figure 3).

3.4. Unique Bacterial Taxa Detected in Each Red Sea Site

A few unique bacterial taxa were detected in individual sites (Figure 3). *Desulfobacterium vacuolatum*, *Thalassomonas loyana* and an unknown species of the genus *Neptuniibacter* were unique to Safaga Port (Mangrove), while *Psychroserpens mesophilus*, *Thermaerobacter subterraneus*, *Alteromonas genovensis*, *Congregibacter litoralis*, *Lentimonas marisflavi*, *Desulfofrigus sp.*, and *Tenacibaculum sp.*, were unique to Safaga Aluminum Port. In Assala Dahab five unique bacterial members were exclusively detected; *Marmoricola sp.*, *Haloferula sp.* including *Haloferula phyci*, *Luteolibacter sp.*, and *Delftia*. In Hamrawein Port, two pathogenic (*Dialister pneumosintes* and the genus *Aggregatibacter*) and two marine (*Marinomonas aquimarina* and *basaltis*) members were solely detected. Abu Monkar Island showed four unique members; *Alkaliflexus*, *Alkalibacterium*, *Pelagicoccus*; and *Sphingopyxis litoris*. Sharm El-Maya showed two unique genera; *Marinimicrobium* and *Altererythrobacter*. In Qusseir Port, *Actibacter sp.* and an unassigned genus of the family *Idiomarinaceae* were uniquely detected. *Pseudidiomarina sediminum*, and the methylotroph *Methylophaga marina* were detected only in S-Abu Ghoson Port. Interestingly, Saline Lake-RM showed the highest number of unique taxa, eight bacteria were detected uniquely in this lake; *Orenia marismortui*, *Caloranaerobacter sp.*, *Planifilum sp.*, *Methylohalobius crimeensis*, *Halobacteroides halobius*, *Halanaerobacter sp.*, *Halovibrio denitrificans* and an unassigned genus of Halobacteroidaceae (Figure 3). Solar Lake-W showed unique detection of *Halothiobacillus sp.*, and *Halocella cellulositytica*.

3.5. Inferred Metabolic Activities across Red Sea Coast

To infer general and specific metabolic activities of the different sites we assessed the known metabolic potential of the detected genera. The majority of the significant genera (genera > 1%) in Saline Lake-RM and Solar Lake-W were not identified at the genus level; only 24 and 28% of the 16rRNA reads were resolved at the genus level, respectively. On the other hand, Hamrawein port, Abu Monkar Island and Safaga Aluminum Port showed 74, 73 and 72% abundance of identified genera, respectively (Supplementary Figure 1).

In general genera known as oil degraders, sulfate reducers and oxidizers (SRB and SOB) predominated the sites (Figure 4), with the exception to Assala Dahab. Other identified but less predominant genera included known pathogens and nitrogen metabolizers (Figure 4). The genera

detected and their general relevant metabolic profile is summarized in Supplementary table 2. The highest abundance of oil/hydrocarbon degrading genera was detected in Hamrawein port (45 genera representing 76% of the identified genera) (Figure 4), followed by the Safaga Aluminum Port (51 genera/74% of the identified genera). On the other hand, the mangrove site of the port showed less oil degrading genera (40%), yet maintained diversity of oil degraders (49 genera). Qusseir Port showed the highest taxonomic diversity (246 genera) including oil degraders (54 genera) (Figure 4).

The two lakes, Saline Lake-RM and Solar Lake-W, showed the highest abundance for SOB and SRB (24.03% and 18.81%), respectively. Hamrawein port that showed the highest abundance for hydrocarbon degrading bacteria (76.2%,) and the least abundance for the sulfur reducing and oxidizing bacteria (1.84%).

Nitrogen metabolizing genera were mostly detected in Sharm El-Maya and Saline Lake, 18.8% and 15.2%, respectively. Pathogenic genera were detected in all the sites with the exception to Safaga Port (Mangrove) and Solar Lake-W; and in a negligible amount in Saline Lake-RM (0.02%). 7.3% of the identified genera in Abu Monkar Island were assigned as pathogenic (Figure 4 and Supplementary Table 2). Ubiquitous marine bacteria, reached maximum abundance at Assala Dahab on the expense of the abundance of Hydrocarbon degraders, SRB and SOB (Figure 4 & Supplementary Table 2).

4. DISCUSSION:

4.1. Establishing a geochemical and microbial community datasets to evaluate anthropogenic impacts on the Red Sea coast

In this study we analyzed geochemical characteristics and microbial community of sediments along the Egyptian Red Sea coast; eight coastal sites and two lakes. The selection was based on i-feasibility and accessibility of sampling and ii-reported/documented site-specific anthropogenic impact. The two lakes studied were the Solar Lake at the Gulf of Aqaba (Solar Lake-W) and Saline Lake inside the Ras Muhammed National Park protected area (Saline Lake-RM). The coastal sites included 1- four ports for shipping aluminum (Safaga Aluminum Port), ilmenite (S-Abu Ghoson Port) and phosphate (Qusseir Port and Hamrawein Port), 2-a site previously reported to have suffered several oil spills (Sharm El-Maya) and 3-a tourism impacted site (Assala Dahab) (Siam, Mustafa et al. 2012).

In order to correlate microbial community profiles with geochemical data we generated two major datasets in this study; 1- measurements of Carbon, Hydrogen, Nitrogen and Sulfur (CHNS), metals and hydrocarbon and 2- 16S rRNA Pyrotags, for each of the ten sediment Red Sea coastal sites. Nitrogen was only detected in the two lakes (0.13% in Saline Lake-RM and 0.2% in Solar Lake-W) and Abu Monkar Island (0.14%) and Sulfur in three sites; Abu Monkar Island (0.52%), Solar Lake-W (0.51%) and Safaga Aluminum Port (0.44%) (Table 1). Solar Lake-W showed the highest metal content (1880.9 ppm/31.3%), followed by S-Abu Ghoson (1043.2ppm/29.7%) and Qusseir Port (1105.4 ppm/29.2%). The iron richness in these sites greatly contributed to the metal richness in such sites.

Selected heavy metals can be used as an indication of terrigenous contamination, including Fe, Mn, Ni, Zn, Pb, V, Cu (Mansour, Nawar et al. 2000, Chen and Kandasamy 2008, Salem, Khaled et al. 2014). Based on the standardized toxicity of heavy metals in aquatic sediments (Persaud, Jaagumagi et al. 1993) the Fe in Solar Lake-W (1.99%) is close to the Lowest Effect Level (2%). Similarly, the phosphorus levels in four different Red Sea coastal sites exceeded the standardized Severe Effect Level (2000 ppm). Such pronounced pollution requires an effective management plan (Persaud, Jaagumagi et al. 1993). Additionally, the five rare earth elements, Tm, Ce, Y, Sc and Yb, show almost the same abundance pattern across the ten sites.

To determine the effect of oil pollution on the microbiome, the semi-volatile petroleum hydrocarbon contents (Diesel and Motor oils) were measured in the Red Sea sites. The highest relative concentration of diesel oil was detected in Safaga port sites, Safaga Port (Mangrove) (67 mg/kg) and Safaga Aluminum Port (45 mg/kg), and the least concentration was detected in Abu Monkar Island (Table 1). On the other hand, the mangrove site of Safaga Port showed a substantially higher Motor oil concentration (2690 mg/kg).

A total of 140946 16S rRNA reads were generated by amplification of V6-V4 16S rRNA tags and pyrosequencing (Table 2). More than 98.7% of reads were taxonomically assigned to 900 taxa, and 96.7% were significantly detected (Table 2).

4.2. The Egyptian Red Sea Coastal Microbiome

Based on the assigned 16S rRNA reads in our samples we detected a total of 30 different bacterial taxa in the Red Sea coastal sites and we refer to as the “Egyptian Red Sea Microbiome”. Unassigned taxa (genera, families and orders) were highly abundant in the “Red Sea Microbiome”, however, nine genera, seven families, five orders and five bacterial classes were detected (Figure 3 and Supplementary Table 1). The most abundant phyla included Proteobacteria (50.4%), as expected, followed by Actinobacteria (15.8%) and Planctomycetes (15.1%).

Roseovarius was the only assigned genera of α -proteobacteria detected in our “Red Sea Microbiome”. Previous studies reported the involvement of *Roseovarius* in the degradation of Poly Aromatic Hydrocarbons (PAHs) (Vila, Nieto et al. 2010) and alkane (Vila, Nieto et al. 2010). Moreover, several reports documented its’ role in crude oil and PAH biodegradation in marine environments (Brakstad and Lødeng 2005, McKew, Coulon et al. 2007, Wang, Lai et al. 2008, Vila, Nieto et al. 2010, Chronopoulou, Sanni et al. 2015). *Ralstonia* was the only assigned genera of β -proteobacteria in the “Red Sea Microbiome”. Maximum abundance was detected at Assala Dahab (249 reads +/- 68.75). *Ralstonia eutropha* was reported to degrade chlorinated aromatic compounds (Priyadarshi, Shukla et al. 2014). Yet, other species of *Ralstonia* are known pathogens for humans (Ryan and Adley 2014) and plants (Genin and Denny 2012). *Nitrosococcus* and *Coxiella* were the two assigned genera of γ -proteobacteria in the “Red Sea Microbiome”. *Nitrosococcus* members are aerobic obligate ammonia-oxidizing bacteria (AOM) (Campbell, Chain et al. 2011, Stein, Campbell et al. 2013) (Campbell, Chain et al. 2011). Surprisingly, the

three sites where nitrogen was detected in our study, the two lakes and Abu Monkar Island, shows the least abundance of *Nitrosococcus*.

Actinobacteria are presented in the “Red Sea Microbiome” by two members; *Conexibacter* and an unclassified family of Acidimicrobiales. *Conexibacter* was detected in all sites, however, it was strikingly more abundant in the Saline Lake-RM (74.4% of the total detected *Conexibacter*). *Conexibacter* plays a major role in the nitrification process and dominates nitrogen-rich soil (Deng, Gu et al. 2015). Note that Saline Lake-RM is one of three sites where nitrogen was detected. *Conexibacter* saccharolytic potential may contribute to the carbon cycling in these sites (Monciardini, Cavaletti et al. 2003, Pukall, Lapidus et al. 2010, Seki, Matsumoto et al. 2012).

Eight members belong to the phyla Planctomycetes; the genera *Rhodopirellula*, *Blastopirellula*, *Pirellula* and *Planctomyces* in addition to an unassigned genera of Planctomycetaceae, unassigned order of Phycisphaerae, and an unassigned class of Planctomycetes were detected. This phyla is abundant in different marine ecosystems and can convert organic materials to carbon dioxide (Richter, Richter-Heitmann et al. 2014). N-acetylglucosamine is the main carbon and nitrogen source in *Rhodopirellula* and it encounters 110 genes for sulfatases. *Rhodopirellula baltica* is a marine bacterium known for being a model organism for aerobic carbohydrate degradation (Wecker 2009). All of the above *Rhodopirellula* characteristics along with the fact that sulfated polysaccharides are found largely in marine ecosystems (Senni, Pereira et al. 2011), suggests its’ contribution to marine global carbon and sulfur cycling (Kertesz 2000).

Based on the identified genera in the Red Sea microbiome and their known metabolic and biochemical potential, the Red Sea Microbiome are chronic constituents of this marine ecosystem.

4.3. Quantitative and qualitative unique oil degrading microbial community in the Red Sea ports

In the ten Red Sea Egyptian coastal sites 21 oil degrading genera were detected. Note that 61 shared oil-degrading genera were not significantly detected in all the sites (<0.5%) (Supplementary Table 2). A study on the dynamics of marine microbial communities following an oil spill showed shifting towards an increase of *Alcanivorax*, *Roseovarius*, *Marinobacter*, and *Methylophaga*, leading to complete removal of all linear and branched alkanes (Vila, Nieto et al.

2010). These genera were detected in our sites suggesting a similar environmental response to the oil pollution.

Despite the presence of hydrocarbon biodegrading bacteria in all the Red Sea sites, two ports analyzed revealed quantitative and qualitative uniqueness in these taxa. Hamrawein Port is one of the oldest harbors for phosphate. This port did not show comparably high levels of oil, despite the high, in fact highest, abundance for hydrocarbon utilizing/degrading bacteria (76.2% of the assigned genera/45 genera). Two of the four Hamrawein Port unique bacteria (Figure 3), *Marinomonas aquimarina* and *Marinomonas basaltis*, are known hydrocarbon degraders (Kang, Jang et al. 2012). Safaga Aluminum Port (73.8%/51 genera) was similarly dominated by known hydrocarbon degraders, this included *Marinobacter*, *Pseudoalteromonas*, *Alcanivorax*, *Arcobacter*, *Cycloclasticus*, *Pseudomonas* and *Sphingomonas*. The coastal marine sulfide-oxidizing bacterium *Arcobacter*, was only detected in the three sulfur-detected sites (Abu Monkar Island, Safaga Aluminum Port and Solar Lake-W), and was previously shown to produce sulfur as a metabolic end product (Wirsén, Sievert et al. 2002).

Safaga Aluminum Port and Safaga Port (Mangrove) showed similar diversity of hydrocarbon degrading bacteria, however the abundance of these degraders in the port was almost twice (74%) the abundance in the mangrove part of the port (40%). On the other hand, the SRB/SOB diversity and abundance weren't significantly different in the two sites. Safaga Aluminum port showed 16 different SRB/SOB (11.18%), while Safaga Port-Mangrove showed 13 SRB/SOB (18.75%); 12 are shared with the 16 Safaga Aluminum port SRB/SOB, and the remaining one is one of the Safaga Port (Mangrove) unique taxa.

The highly detected abundance and the unique diversity of the genera in these two ports may suggest an efficient oil degrading microbial community in Red Sea ports to ensure the effective elimination of pollutants.

4.4. Abundance of Sulfur oxidizers and reducers, and reduced abundance of hydrocarbon degraders in the two Red Sea lakes

SRB/SOB are known to play major roles in biodegradation of hydrocarbon and their derivatives (Fukui, Harms et al. 1999, Kleindienst 2012). Saline Lake-RM and Solar Lake-W exhibited higher abundance for SRB/SOB communities versus the rest of the sites. Saline Lake-

RM showed the highest contents for SRB/SOB (24.03%), followed by Solar Lake-W (18.81%). Solar Lake-W showed specific high activity for sulfur because of 1- abundance of SRB/SOB microbial members, 2- is one of the three only sulfur-sites (Table 1) and 3- contained only two unique taxa, both sulfur-metabolizing and halophilic. *Halocella cellulositytica*, is a halophilic cellulose degrader isolated previously from a hypersaline lagoon (Simankova, Chernych et al. 1993). The second Solar Lake-W unique taxon is the sulfur metabolizer *Halothiobacillus* sp.. Saline Lake-RM showed unique microbial community and patterns, and the highest number of unique bacteria. This included four halophiles; *Halovibrio denitrificans*, *Halanaerobacter* sp. (anaerobic), *Halobacteroides halobius* (anaerobic), *Methylohalobius crimeensis* (high methylotrophic bacteria isolated from hypersaline lakes) (Heyer, Berger et al. 2005), and an unassigned genus of *Halobacteroidaceae*. In addition to, two anaerobic unique taxa, *Orenia marismortui* and *Caloranaerobacter* sp.

On the other hand the hydrocarbon degraders constituted 18.87% and 52.13% of the microbial genera in Saline Lake-RM and Solar Lake-W, respectively. The higher abundance for SRB/SOB communities on the expense of the diversity of hydrocarbon metabolizers' in the two lakes is observed (Supplementary table 2). This suggests different reaction to oil pollution than coastal sites.

Individual sample sites seem to be affected by both the intrinsic geochemical and physical characteristic and the extrinsic factors. This should be considered when assessing industrialization and anthropogenic impact on the microbial communities. The ten sites along the Egyptian coast of the Red Sea investigated in this study are predominantly oil-polluted sites. Despite the reported "Egyptian Red Sea Coast Microbiome", individual sites showed unique evolution of their microbial communities that were particularly evident in the hydrocarbon degrading bacterial genera. These hydrocarbon consortia could be simply subcategorized into three categories that have selectively evolved in port, lakes and mangrove forest. Therefore, both the original environment and the imposed environment have dictated the microbial community.

TABLES

Table 1: Chemical analyses performed for individual sediment sites in this study:

1- Carbon, Hydrogen, Nitrogen and Sulfur measurements in percent (%), 2- Metal and Non-metal analysis (nine elements concentrations presented in percent (%), and twenty elements in Part Per Million (PPM)) and 3-Selected hydrocarbon analysis of percentages of diesel and motor oils.

Element	Solar Lake-W	Assala Dahab	Saline Lake-RM	Sharm El-Maya	Abu Monkar Island	Safaga Aluminum Port	Safaga Port (Mangrove)	Hamrawein Port	Qusseir Port	S-Abu Ghoson Port
CHNS analysis measured in %										
C	2.39	1.59	2.46	0.34	12.04	2.82	11.88	3.94	2.63	5.51
H	0.93	0	0.73	0	0.72	0.67	0.69	0.68	0.72	0.61
N	0.2	0	0.13	0	0.14	0	0	0	0	0
S	0.51	0	0	0	0.52	0.44	0	0	0	0
Percent Elements										
Ca	4.7	2.24	9.2	1.09	26.43	13.31	11.08	10.52	10.91	16.15
Fe	1.99	0.23	0.6	0.25	0.018	0.6	0.13	0.87	1.46	1.23
Si	19.83	21.29	15.62	19.83	0.22	8.51	10.13	10.87	13.7	10.09
Sr	0.13	0.0131	0.16	0.0000178	0.52	0.27	0.13	0.12	0.12	0.000029
Mg	2.63	0.34	2.39	0.19	1.63	0.68	0.98	1.78	1.45	0.97
Al	1.4	0.0936	0.54	0.1	0.0181	0.54	0.11	0.62	1.08	0.55
K	0.44	0.0227	0.0978	0.0175	0.016	0.12	0.0321	0.0826	0.15	0.0578
Na	0.0796	0.0229	0.0697	0.0118	0.24	0.16	0.0733	0.0969	0.0975	0.16
P	0.083	0.00888	0.0242	61.6	0.0102	0.0315	0.0105	0.25	0.22	0.52
PPM Elements										
B	143	3.8	110	4.4	36.7	18.5	78.6	20.3	25.6	33.5
Ba	142	2.8	35	3.6	10.8	133	3.6	29.9	113	107
Ce	25	6.3	17	20.8	2	11.8	3.4	8.3	12.4	6.5
Cd	0.4	0	0.2	0	0	0.2	0	0.4	0.5	0.4
Co	3	0	1.2	0	0.4	0.4	0.3	1.4	2.5	5
Cr	30.4	0.8	8.6	1	2.3	4.5	3	24	41.8	20.1
Cu	10.5	0.2	2.7	1	0.4	7.1	1.1	2.9	11.8	23.6
Li	20.9	2.3	7.6	0.9	0.9	4	1	3.9	8.1	1.6
Mn	267	33.8	167	122	4.1	78.1	20.8	153	179	288
Nb	155	10.9	46.9	6.8	3.7	55.3	11.9	36.1	78.6	60.3
Ni	13.7	0.4	3	0	0	1.8	0.7	9.7	22.8	16.2
Pb	4.9	1.2	3.2	1.6	0.6	3.5	0.6	1.1	11.7	6
Sc	5.4	0.3	1.2	0.3	0	0	0.4	2.3	3.5	2.5
Ti	867	62.9	266	41.1	20.2	302	66.5	207	440	346
Tm	102	7.4	31	4.8	3.8	36.8	8.2	24.4	52.6	41
V	35.9	2.3	14.3	2.5	0.5	12.2	6.4	25	32.6	47.3
Y	9.6	2.5	5	5.7	0.9	5.5	1.3	6.5	7.8	7.6
Yb	1.1	0.3	0.6	0.6	0.1	0.6	0.1	0.7	0.9	0.9
Zn	44.1	7.1	16.9	10.7	1.2	17.9	3.1	23.8	60.2	27.6
Zr	0	2.2	9.6	2.7	0.8	3.3	1	0	0	2.1
Hydrocarbon Analysis										
Diesel (mg/kg)	9.7	<2.6	10.1	7.8	<5.8	45.3	67	3.7	7	2.8
Motor Oil (mg/kg)	<4.9	6.4	<10.2	74.7	<11.7	143	2690	12.4	42.1	5.9

Table 2: 16S rRNA Pyrotag dataset

	Solar Lake-W	Assala Dahab	Saline Lake-RM	Sharm Maya	El- Abu Monkar Island	Safaga Aluminum Port	Safaga Port (Mangrove)	Hamrawein port	Qusseir Port	S-Abu Ghoson Port	Totals
Total number of reads	12780	15270	19628	16406	13822	11513	12312	14651	12560	12004	140946
Number of total Taxa	225	239	275	297	212	263	335	263	375	285	900
Significant assigned reads	12218	14866	19057	15836	13453	11155	11833	14205	12081	11610	136314
Significant unassigned reads	317	145	245	223	192	109	166	174	133	159	1863
Number of significant taxa	158	175	164	195	167	202	234	197	246	198	381

FIGURES

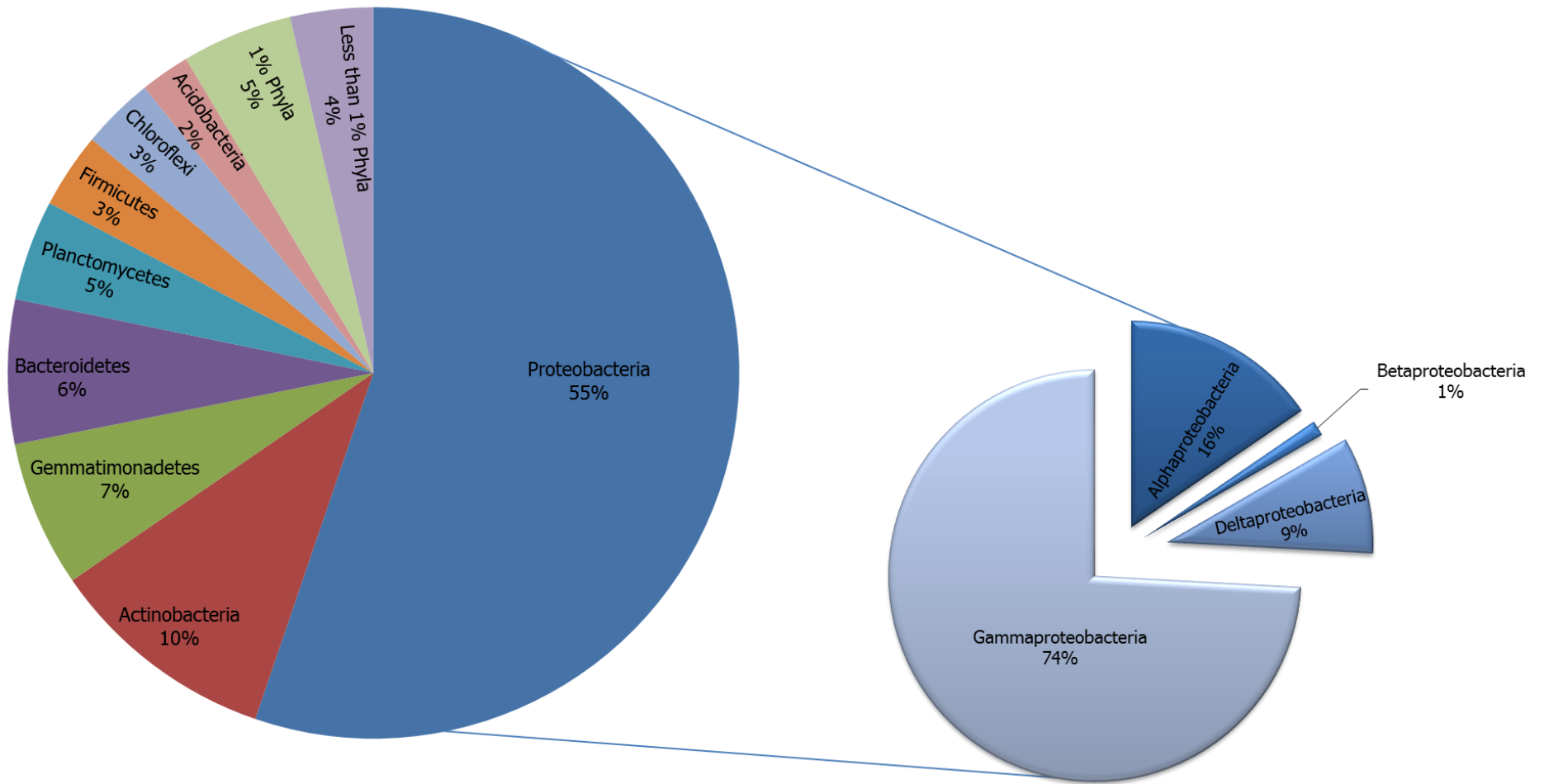


Figure 1: Total Significant taxa assignment of 16S rDNA reads at the phyla level

Figure 2: Map of the ten studied sites and taxa assignment of 16S rDNA reads at the phyla level of individual site

Pie chart presenting the bacterial communities of individual sites across the Red Sea. The map was generated using two images using Google , Image Landsat Data SIO, NOAA, U.S. Navy, GEBCO, using the coordinates (17° 20' 52.45" N, 28° 15' 51.14" E, elev 1605ft and eye alt 1226.72 mi) and (28° 32' 06" N, 35° 53' 07.54" E, elev 3706 ft and eye alt 345.52 mi)

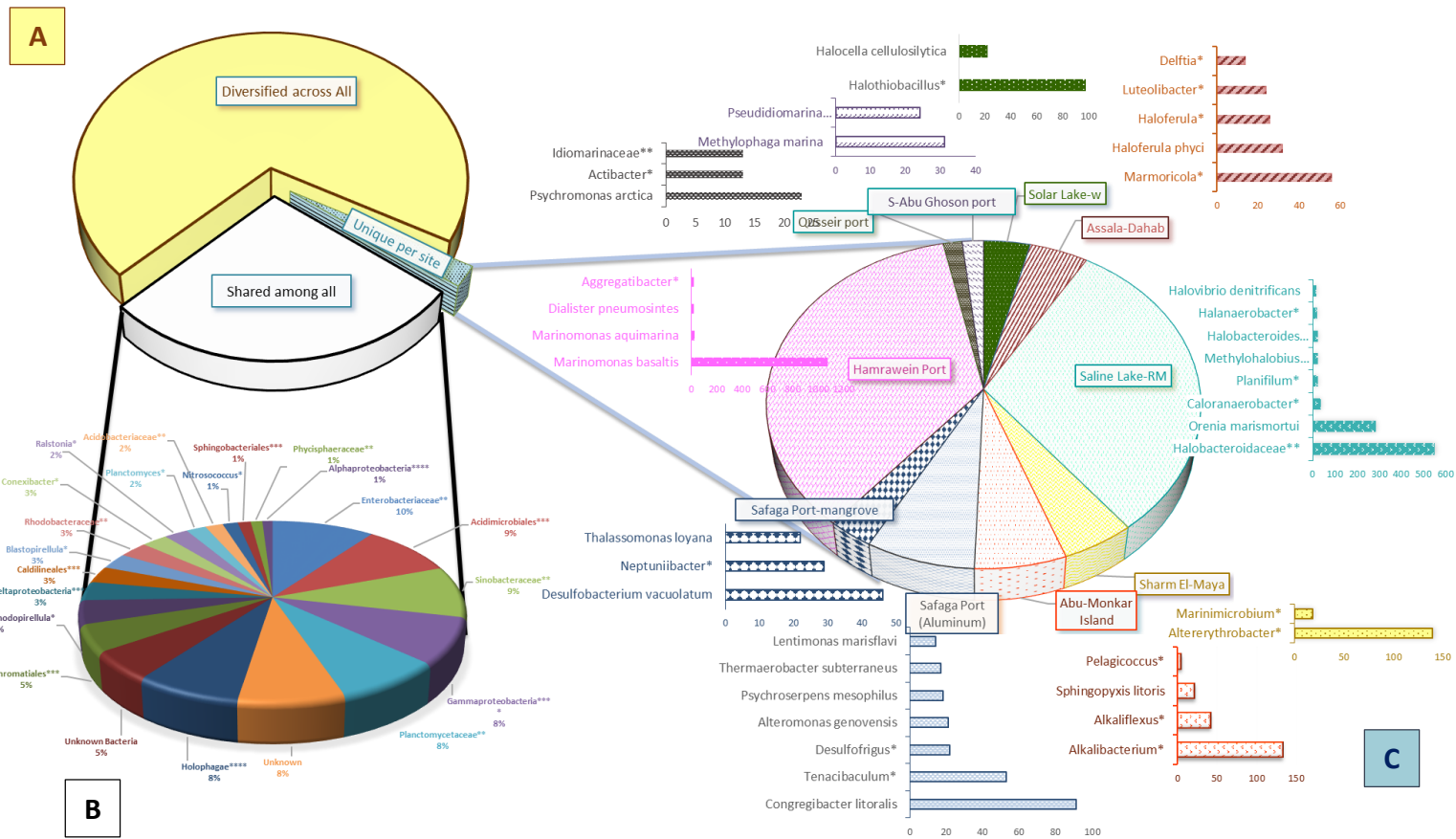


Figure 3: Total generated taxa, showing the “Egyptian Red Sea Coastal Microbiome” and the “Unique taxa per site” A- Total generated taxa constituting shared (B) and unique (C) taxa. B-The shared microbiome (>40% of total taxa) presented as the “Egyptian Red Sea Coastal Microbiome” and include 30 different taxa. C-Magnification of the unique taxa for individual sites are presented as bar graphs.

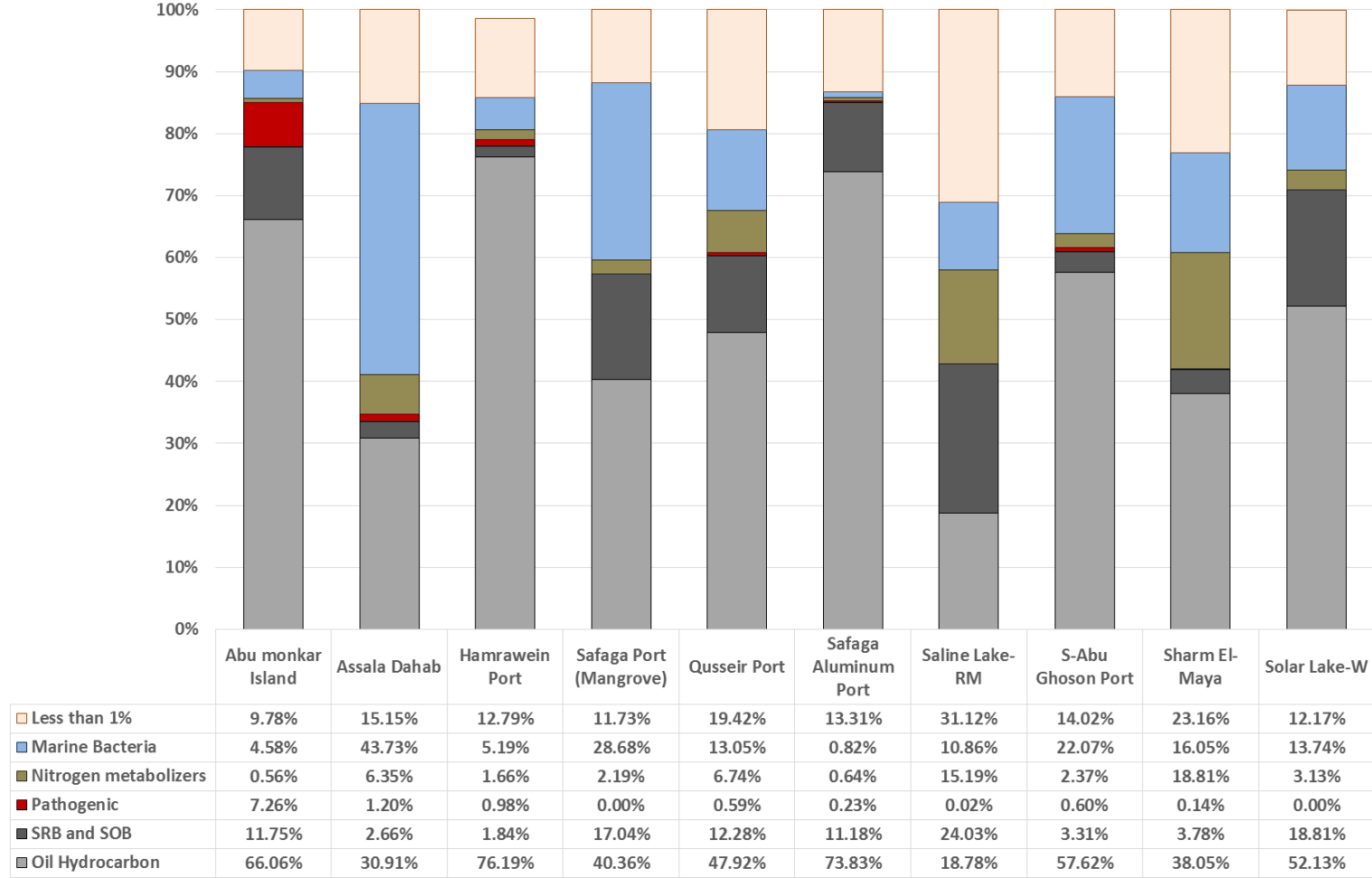
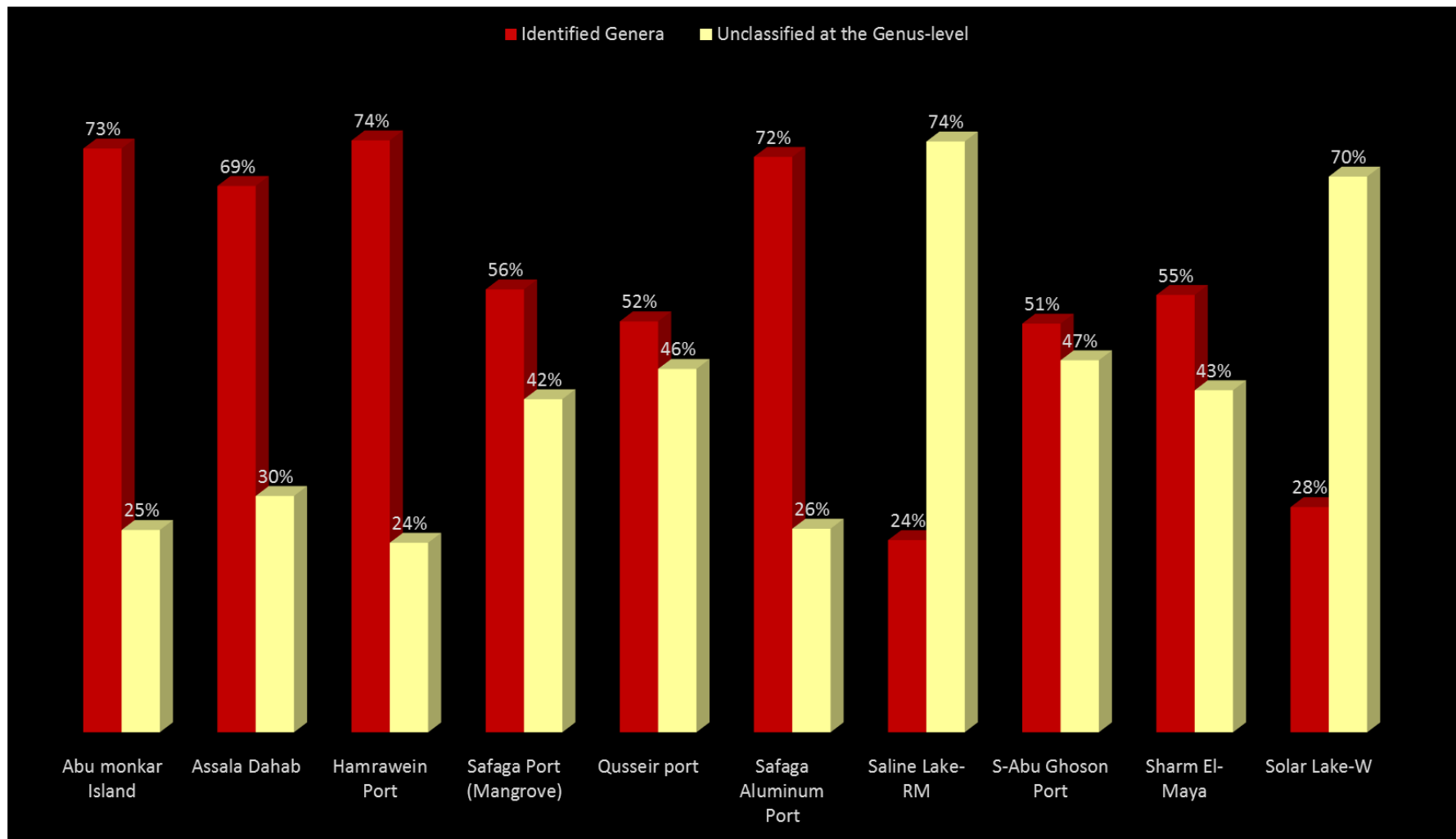


Figure 4: Inferred metabolic profiles across the Egyptian Red Sea coast. Based on the known metabolic potential of identified genera, the bar graph presents the quantitative and qualitative metabolic potential of the sites summarized in six main inferred categories including; hydrocarbon degraders, Sulfur- Oxidizing and Reducing Bacteria (SRB&SOB), Nitrogen Metabolizers, pathogenic, ubiquitous marine bacteria and insignificant genera (< 1%)

SUPPLEMENTARY MATERIAL

Supplementary table 1: Raw data (taxonomy and abundance) for all taxa per site, used in generating figures 2 &3, highlighted for the three main categories: “Egyptian Red Sea Microbiome”, Unique bacteria per site, rest of bacteria diversified across the ten sites.

Supplementary table 2: Raw data (taxonomy and abundance) for genera used in generating the six metabolic categories.



Supplementary figure 1: Identified versus unidentified genera across the ten studied sites. Note the opposite pattern (an increase in unidentified genera) in two lakes for the rest of the sites.

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**CHAPTER 3: EGYPT'S RED SEA TWO
HYPERHALINE ECOSYSTEMS'
BACTERIAL COMMUNITIES**

ABSTRACT

Two hypersaline aquatic ecosystems, Solar Lake (near Taba) and Saline Lake (in Ras Muhammed protected area), on the coast of the Red Sea showed intriguing characteristics. Both of them have connection to the Red Sea water, yet, they originated differently. Solar Lake is a basin on the rift of the Red Sea which has been thoroughly studied before, while Saline Lake is considered as a sort of saltern where the Red Sea waters covers it frequently, however, it is not studied before. Both of these ecosystems, show similar characteristics, of high salinity, high temperature and low oxygen. Sediments samples were collected from the coast of those two lakes on two different years 2011 and 2013. DNA isolation from 2011 samples, was performed using two approaches, Culture-Dependent and Culture-Independent approaches. For 2013 samples, Culture-Independent approach was used to isolate DNA. Bacterial taxonomy revealed three major patterns, where halophilic, anaerobic and sulfur metabolizing bacteria were dominating the two datasets. Interestingly, more than 50% similarity between the microbiota of the two datasets were detected. Similarly interesting, members of the Solar Lake bacteria in our samples, have been reported to dominate the lake more than 40 years ago. Our results revealing the fact that those two lakes are typical hypersaline ecosystems where the oxygen dissilience is scarce and the sulfur metabolism is the dominant metabolic pathway for its microbiota; and how our bacterial datasets for these lakes are known to be inhabitants and adaptable to these harsh conditions.

1. INTRODUCTION

Saline and hypersaline environments are potential habitats for great diversity of prokaryotes, mainly bacteria and archaea. They are known to act as biotopes for salt-tolerating and salt-loving microbes. More than 20 different metabolic processes are being ran by microbial activities at hypersaline environments. Of these, oxygenic photosynthesis, anoxygenic photosynthesis, chemolithotrophic oxidation of sulfur compounds and dissimilatory sulfate reduction, are among those very well studied pathways in different saline and hypersaline ecosystems. It is known that oxygen is poorly dissolved in those environments so it is expected to find a lot of anaerobic microbiota at such environments (Oren 2006); therefore, anoxygenic photosynthesis is used to be reported from these environments. Another important criterion about the salt lakes is that, often, their bottoms are black sulfide-rich sediments, which makes the dissimilatory sulfate reduction rate is quite high in these environments; and this, in turns favors the inhabitation of different Sulfate-Reducing Bacteria (SRB). The fate of this anaerobic sulfate reduction is the production of the toxic smelled hydrogen sulfide.

Solar Lake was discovered in 1968 (Por 1968), has an intense smell of hydrogen sulfide. Solar Lake has been characterized since its discovery by several extreme conditions including high evaporation rate which led to salinity reaching maximum of 180‰ and seasonal extreme temperatures' fluctuations of 16° C in winter and 60° C in summer (Jørgensen and Cohen 1977). This seasonal cycles are a result of the stratification criteria of this lake, which is generated due to a seepage from the sea water to the surface of the lake creating a pycnocline. This stratification (from September to July), caused a zonation in the microbiota of the lake, where the bottom of the lake is known to be floored by cyanobacterial mats in addition to the creation of benthic cyanobacterial mats and other diversified microbial plates (Cohen, Krumbein et al. 1977). These cyanobacteria preserves a record of 4,600 years of the lake. The temperature of the lake reaches 27° C only during the short period of holomixis which lasts from 4 to 13 weeks. Solar Lake used to be considered as a model example for studying oxygen-sulfide microbial metabolism (Jørgensen, Revsbech et al. 1979). The studies on Solar Lake' cyanobacterial mats and their microbial communities, even those that degrade cyanobacteria have been well studied since the discovery of the lake (Cohen, Padan et al. 1975, Jørgensen and Cohen 1977, Krumbein, Cohen et al. 1977, Jørgensen, Revsbech et al. 1979, Walsby, Van Rijn et al. 1983). The cyanobacterial mats

of this lake reached 1m thickness in which the distributions of different metabolic compounds including sulfur compounds, organic carbon and carbonates; and microbial communities of sulfate-reducing bacteria, anaerobic and heterotrophic bacteria along with the rate of the different metabolic activities have been studied and reported (Jørgensen and Cohen 1977).

The dominant microbes reported in the Solar Lake include the unicellular cyanobacteria *Aphanocapsa littoralis*, *Aphanothece hazophytica* and the filamentous *Microcoleus sp.*, *Oscillatoria spp.*, the diatoms *Amphora coffeaeformis*, and *Nitzschia sp.*, photosynthetic and colorless sulfur bacteria, in addition to several heterotrophic bacteria (Jørgensen and Cohen 1977). These bacteria were described to be diversified in forms of plates during the stratification period, where the upper most layer of the lake. Epilimnion (0-1m), showed general low concentration of microbes, mainly dominated by budding bacteria (*Caulobacter sp.*), in addition to few diatoms and cyanobacteria. In the Metalimnion (1-2.5m), the spirochaetes and the filamentous bacteria were the dominant bacteria and were restricted to this layer in addition to the dominance of several spore forming bacteria and rod heterotrophic bacteria. Interestingly, the major photosynthetic organism in this layer is the phototrophic sulfur bacteria *Chromatium violescens*. Deeper than this layer is the Hypolimnion, it is characterized by the high rate of sulfur metabolism where extreme high numbers of the green sulfur bacteria *Prosthecochloris sp.* were detected, resembling the second plate of photosynthetic bacteria after *Chromatium violescens*. The lower Hypolimnion is being dominated by the filamentous cyanobacteria *Oscillatoria limnetica* and *Oscillatoria salina*, where the highest rate of primary production is measured. So, collectively, during the stratification period, a strata of photosynthetic microbiota alternate with strata of chemoorganotrophic and/or chemolithotrophic bacteria; are predominating the lake microbiota.

Interestingly, although information about Saline Lake is almost depleted, as no one till now tried to study this ecosystem thoroughly, still it shows similar basic chemical and physical structures to Solar Lake reported recently (Chapter 2 and (Mustafa, Abd-Elgawad et al. 2014). In these reports, authors described the high salinity of the lake reaching 150 ppt, in addition to the limited oxygen dissolved 1.94 mg/L, with moderate thermophilic (31.7 °C) and slight alkaline conditions (PH 8.24). These anaerobic conditions favored the activities of sulfur metabolizing bacteria, generally presented in (chapter 2). The personal observation during the sampling process showed intense oil contents in the coastal sediments of the lake. On the other hand, another group

managed to isolate two novel halophilic bacteria from this hypersaline Lake (Romano, Lama et al. 2007, Romano, Orlando et al. 2011).

Here in this paper, we are describing the microbial communities harboring the coastal sediments of these two hypersaline lakes, using 16S rDNA taxonomic analysis. These data were generated from samples collected in 2011 and 2013. Datasets from 2011 samples have been generated using two main approaches described in the previous chapters, the Culture-Dependent and the Culture-Independent approaches. In addition to generating a Culture-Independent dataset from samples collected in 2013. The three datasets for each lake are described.

2. MATERIALS AND METHODS

2.1. Site description and sample collection:

The general characteristics of the two lakes and the sampling procedure from them were described in Chapters 1 &2. The sampling has done for the same sites in May 2011 and in April 2013. The samples have been taken through using sediments core of 0.5 m length from coastal sediments of the two lakes.

2.2. Chemical and Physical analyses:

Chemical analyses for the two lakes, performed on 0.5 grams of sediments from 0.5-1m depth, included Carbon, Hydrogen, Nitrogen and Sulfur profiling using oxidation of the samples by flash combustion using a Thermo FlashEA 1112 elemental analyzer. The combusting gases were then separated by passage through a chromatographic column using He as a carrier gas, and were detected with thermal conductivity detector, reported in Chapter 1. Quantitative and qualitative metal and non-metal elements in the sediments were assessed using inductively coupled plasma optical emission spectroscopy ICP-OES. This technique was achieved using the Varian Vista MPX CCD Simultaneous axial ICP-OES. Total Petroleum Hydrocarbons were analyzed using Northwest Total Petroleum Hydrocarbon Analytical Method (NWTPH-Dx); previously described in Chapter 2.

2.4 Molecular Biology Analyses

The sediment sampling from the different Red Sea coastal sites and two lakes was previously reported (Mustafa, Abd-Elgawad et al. 2014) and presented in figure 1. DNA was extracted from 0.25 grams of sediments using the MoBio kits for all sites, with the exception to Sharm El-Maya were PowerMax® Soil DNA Isolation Kit (Cat. number 12888-100) was used (0.75 grams). This was because of Sharm El-Maya sediment nature. Triplicates isolations of three different samples per site were performed, the extracted DNA was then pooled in one tube then directed for PCR and sequencing. 16S rDNA V6 and V4 bacterial hyper-variable regions were amplified from the extracted DNA as previously described (Sogin ML, Morrison HG et al. 2006) using the primer sets previously described (Siam, Mustafa et al. 2012). The V6-V4 amplifications were followed by Pyrosequencing using 454 GS FLX Titanium technology.

2.5 Bioinformatics analysis

Taxonomic assignment of 16S rRNA were performed using the Visualization and Analysis of Microbial Population Structures, VAMPS, database; hosted by the Josephine Bay Paul Center, MBL, Woods Hole ([http://vamps.mbl.edu/resources/databases .php](http://vamps.mbl.edu/resources/databases.php)). Statistically significant assigned reads were subjected to Fisher Exact test, as previously described in Chapter 1 (Siam, Mustafa et al. 2012, Mustafa, Abd-Elgawad et al. 2014).

2.3. Molecular Biology Analyses

For the samples taken in 2011, two approaches for DNA isolation were used. The Culture-Dependent approach, in which the DNA was extracted from bacterial cultures prepared as previously described in Chapter 1. While the other approach is the Culture-Independent approach, the DNA was extracted from 0.25 grams of sediments using the MoBio PowerSoil® kit (Cat no. 12830-50). Triplicates isolations of three different samples per site were performed, the extracted DNA was then pooled in one tube then directed for PCR and sequencing. (reported in Chapter 2).

For the DNA extracted through the two approaches; 16S rDNA V6 and V4 bacterial hyper-variable regions were amplified from the extracted DNA as previously described (Sogin ML, Morrison HG et al. 2006) using the primer sets The primers utilized in this study were V6-V4 primer pairs (bacterial: 1046R CGACRRCCATGCANCACT and 518F CCAGCAGCYGCGGTAAN; archaeal: 1048R CGrCrGCCATGyACCwC, arc517F1 GCCTAAAGCATCCGTAGC, arc517F2 GCCTAAArCGTyCGTAGC, arc517F3 GTCTAAAGGGTCyGTAGC, arc517F4 GCTTAAAGnGTyCGTAGC, and arc517F5 GTCTAAArCGyyCGTAGC; previously described (Siam, Mustafa et al. 2012). The V6-V4 amplifications were followed by Pyrosequencing using 454 GS FLX Titanium technology, in case of the 2011 Culture-Dependent and 454 Culture-Independent DNA.

On the other hand, samples taken in 2013; the DNA was extracted directly from 0.25 grams of the sediments' particles using the MoBio PowerSoil® kit. The extracted DNA have been used to amplify the same V6V4 hypervariable regions using the primers reported with the 2011 samples however, the 16S rDNA V6 V4 hypervariable regions were sequenced using the Illumina technology.

2.4. Bioinformatics analysis

For the 2011 Culture-Dependent and Culture-Independent approaches; Taxonomic assignment of 16S rRNA were performed using the Visualization and Analysis of Microbial Population Structures, VAMPS, database; hosted by the Josephine Bay Paul Center, MBL, Woods Hole (http://vamps.mbl.edu/resources/databases_.php). Statistically significant assigned reads were subjected to Fisher Exact test, as previously described in (Siam, Mustafa et al. 2012, Mustafa, Abd-Elgawad et al. 2014). For the 2013 datasets; QIIME taxonomic classification of 16S rRNA

tags was done using USEARCH 6 that performed de-replication and 97% identity clustering with reference sequences. Unknown reads are disregarded and they are not considered in % calculation with this approach.

3. RESULTS

3.1. Physical and Chemical Analyses:

The general physical and chemical parameters for those two lakes have been assessed and described previously in Chapters 1&2. The main criteria for those lakes are the high salinity which reached 149.8 ppt in Saline Lake-RM and 107.9 ppt in Solar Lake-W. Temperatures reached, in May, 34.7° C in Solar Lake-W and 31.5° C in Saline Lake-RM, with DO (Dissolved Oxygen) concentrations 1.94 mg/L and 2.77 mg/L in Saline Lake-RM and Solar Lake-W, respectively.

Chemically, through running CHNS analysis, Solar Lake-W was one of the only three sites, analyzed at that time, where sulfur were detected and it showed almost the highest concentration at this lake (0.51%), while there was not any sulfur detected at Saline-Lake-RM. The two lakes were of the only three sites where nitrogen was detected, as it showed concentrations 0.2% and 0.13% in Solar Lake-W and Saline Lake-RM, respectively. Interestingly, with regards to the metal and non-metal analysis reported in Chapter 2, Solar Lake-W, showed the highest concentrations in almost all of the 39 measured elements, in regards to the other 10 sites tested. Also, Saline Lake-RM, considered to have significant high concentrations of these elements after the severely polluted ports.

3.2. Molecular analyses:

3.2.1. Saline Lake:

Three different datasets, 2011 Culture-Dependent, 2011 Culture-Independent and 2013 datasets, have been generated for Saline Lake-RM sediments samples. 2011 Culture-dependent dataset revealed 18,577 total number of reads (Mustafa, Abd-Elgawad et al. 2014), (Chapter 1), represented in 8 different phyla constituting 54 taxa. After performing the significant test; this dataset retrieved only 28 significant taxa, 23 of them are above 0.05% (Supplementary Table 1a). The phyla of the Culture-Dependent dataset are presented in (Figure 1a), and dominated by Proteobacteria (55%), Firmicutes (43%) and Bacterioidetes (2%). Interestingly, these three phyla have been displayed as dominant phyla in the three datasets.

2011 Culture-independent dataset have generated 19,628 total reads represented by 31 different bacterial phyla involving 275 different taxa. Significantly, the dataset retrieved 164 significant taxa, of which 108 has an abundance more than 0.05% (Supplementary Table 1b). The major phyla

detected in this dataset are Gemmatimonadetes (38.18%), Proteobacteria (18.19%), Firmicutes (9.7%), Chloroflexi (4.78%), Planctomycetes (4.43%), Bacteroidetes (4.40%) and Actinobacteria (4.21%); all the phyla detected in this dataset are presented in (Figure 1b).

Similarly, 2013 Culture-Independent dataset generated 30,436 total number of reads represented by 34 bacterial phyla. The 2013 datasets generated 69 different taxa with abundance more than 0.05%. However, the rare taxa <0.05% counted for 116 taxa (Supplementary Table 1c). The dominant phyla revealed in this dataset are Proteobacteria (42.65%), Bacteroidetes (32.32%), Firmicutes (10.24%), OP1 (2.43%), Caldithrix (2.56%), Gemmatimonadetes (1.91%), SAR406 (1.50%) and Spirochetes (1.49%), (Figure 1c). Interestingly, Cyanobacteria has been detected in both 2011 and 2013 Culture-Independent Saline Lake-RM datasets in a comparable abundance (0.17%) and (0.14%), respectively.

Interestingly, 5 different taxa have been detected in the three generated datasets; the genera *Bacillus sp.*, *Clostridiisalibacter sp.*, *Halanaerobium sp.*, and *Desulfovibrio sp.* and an unassigned family of Clostridiales (Table 1). While 23 taxa have been found in the both Culture-Independent generated datasets (2011 and 2013). Also, 9 taxa has been detected in the two 2011 datasets (Culture-Dependent and Culture-Independent), and only 3 taxa, *Halomonas sp.*, *Marinobacter sp.*, and Clostridiaceae have been detected in both 2011 Culture-Dependent dataset and 2013 Culture-Independent dataset.

2013 dataset showed the dominance for Desulfobacteraceae, one of the 23 repetitively detected bacteria, constituting more than 20% of the generated reads (Figure 2a& Supplementary table 1). This was followed by an un-assigned class of the phylum Bacteroidetes (19.6%), the genus *Acinetobacter* (9.6%), Desulfohalobiaceae (4.8%), and *Salinibacter* (3.8%), Halanaerobiaceae (3.5%), Flammeovirgaceae (2.7%), Bacteroidales (2.6%), KB1 (2.4%), Halobacteroidaceae (2.4%), BA059 (2.1%), and SB-5 (2%). From another point of view, 2013 dataset showed 36 different halophilic and sulfur metabolizing bacteria, constituting more than 39% of the generated dataset, and including the most dominant bacteria of the site (Supplementary table 2a). Of the total reads, these 36 bacteria were dominated by Desulfobacteraceae (20.4%), Desulfohalobiaceae (4.8%), *Salinibacter sp.* (3.8%), Halanaerobiaceae (3.5%), Halobacteroidaceae (2.4%), *Desulfovermiculus sp.* (1.5%), *Halanaerobacter sp.* (0.7%), Halanaerobiales (0.3%), *Halorhodospira sp.* (0.3%), Halobacteriaceae (0.3%), *Halogeometricum*

sp. (0.2%), *Marinobacter sp.* (0.2%), *Salisaeta sp.* (0.2%), Desulfarculaceae (0.1%), Ectothiorhodospiraceae (0.07%), *Haloferax sp.* (0.07%), Dehalococcoidetes (GIF9) (0.06%), Piscirickettsiaceae (0.06%), *Acetohalobium sp.* (0.06%); *Desulfovibrio sp.* (0.06%), *Desulfitobacter sp.* (0.05%), *Thioalkalivibrio sp.* (0.05%) and the rare bacteria (<0.05%) include *Halorhabdus sp.*, Desulfuromonadales, Halobacteriaceae, Dehalococcoidaceae, *Thiohalorhabdus sp.*, *Desulfarculus sp.*, *Haloplasma sp.*, *Haloarcula sp.*, *Desulfomonile sp.*, *Methanohalobium sp.*, *Halothiobacillus sp.*, *Halomonas sp.*, Halomonadaceae, and Marinicellaceae (Supplementary Table 2a).

2011 Culture-Independent data showed dominance for an unassigned family of Gemmatimonadetes constituting more than 38.2%, followed by an unassigned genus of Ectothiorhodospiraceae (4%). Interestingly, this taxon has been also detected, in 2013 dataset three times, the unassigned genus (0.1%), along with additional two identified genera of this family; *Halorhodospira* (0.3%) and *Thioalkalivibrio* (0.05%) (Figure 2b and Supplementary Table 1b). This was also the case with Gemmatimonadetes, as three assigned classes of this phylum has been detected in the 2013 dataset; Gemm-4 (1.8%), Gemm-2 (0.13%), and JL-ETNP-Z39 (0.01%). The third most abundant bacteria in 2011 Culture-Independent dataset is *Conexibacter* which resembles (3.5%) of Saline Lake reads. Two members of the order Anaerolineales, have been detected among the most dominant bacteria of this lake; an unassigned family of Anaerolineales (2.8%) and the family Anaerolinaceae (1.8%). Also, the family Halobacteroidaceae was among the most dominant bacteria in Saline Lake-RM representing (2.8%). The halophilic sulfur metabolizing genera *Thiohalorhabdus sp.*, *Desulfovermiculus sp.*, *Thiohalomonas sp.*, and *Thiohalophilus sp.*, represented 2.6%, 1.9%, 0.1% and 0.02% respectively. Moreover, other than those previously mentioned taxa, several substantial halophilic bacteria have been detected in significant abundance ranging from 2.01% to 0.01% (Supplementary Table 2b). This includes; but not restricted to, in order, *Orenia sp.*, *Clostridiisalibacter sp.*, *Salinibacter sp.*, *Haloplasma sp.*, Oceanospirillales, *Halobacteroides*, *Salisaeta sp.*, *Halanaerobium sp.*, *Marinilabiaceae*, *Halocella sp.*, *Methylohalobius sp.*, *Halobius sp.*, *Haliscomenobacter sp.*, *Halanaerobacter sp.*, *Halovibrio sp.*, *Anaerophaga sp.*, *Haliangium sp.*, *Marinoscillum sp.*, *Dehalogenimonas sp.*, *Salinisphaera sp.*, *Alcanivorax sp.*, and *Maritimibacter sp.*. Similarly, several known sulfur metabolizers (including obligatory lithoautotrophs) have been detected, also, as both dominant and rare bacteria ranging in concentration from 1.9% to 0.01%. These include, but not restricted to,

after the aforementioned two halophilic sulfur metabolizers; *Desulfovermiculus sp.*, Desulfuromonadales, Acidithiobacillales, Desulfobacteraceae, *Desulfurivibrio sp.*, *Pelobacter sp.*, *Thioalkalispira sp.*, *Desulfobulbus sp.*, Desulfarculaceae, Desulfobacterales, *Desulfobacterium sp.*, *Nitrospina sp.*, *Desulfopila sp.*, *Desulfovibrio sp.*, *Desulfosarcina sp.*, *Desulfonema sp.*, *Geobacter sp.*, *Sulfurimonas sp.*, and *Acidithiobacillus sp.* (Supplementary Table 2b). After the dominance of the class Gemmatimonadetes which constituted 38.2% of the total datasets, the previously mentioned halophilic and sulfur metabolizing bacteria constituted, collectively, 20% of the total reads generated from the lake, represented by 40 different taxa.

On the other hand, 2011 Culture-Dependent DNA generated 24 taxa. Several identified genera have dominated this dataset of Saline Lake-RM (Figure 2c and Supplementary Table 1c). The most dominant genera are *Clostridium sp.* (28.5%), *Marinobacter sp.* (23.5%), *Halomonas sp.* (18.3%), *Clostridiisalibacter sp.* (7.7%), *Idiomarina sp.* (5.7%), *Bacillus sp.* (4.5%), *Desulfovibrio sp.* (4.5%), *Alteromonas sp.* (2.5%), *Anaerophaga sp.* (1.8%), Unknown (0.7%) and *Orenia sp.* (0.5%). Although the whole dataset consists of only 24 significant taxa, those 24 taxa showed also a similar picture of halophilicity and sulfur cycling; where more than 62.5% of this Culture-Dependent dataset were represented by several halophilic and sulfur metabolizing bacteria; most of them were already detected in 2011 Culture-Independent dataset. These halophilic and sulfur metabolizing bacteria were represented by *Marinobacter sp.*, *Halomonas sp.*, *Clostridiisalibacter sp.*, *Idiomarina sp.*, *Desulfovibrio*, *Anaerophaga sp.*, *Orenia sp.*, *Halanaerobium sp.*, *Caloranaerobacter sp.* (Thermophilic), and *Halocella sp.* (Supplementary Table 2c).

3.2.2. Solar Lake-W:

Similar to Saline Lake-RM, three datasets have been generated for Solar Lake-W sediments' samples. 2013 Culture-Independent dataset showed 7967 total number of reads represented in 41 phyla compiled 212 taxa (Supplementary Table 3a). These phyla were dominated by Proteobacteria (32.44%), *Caldithrix* (10.14%), *Spirochaetes* (10.1%), *Planctomyetes* (9.2%), *Bacterioidetes* (8.77%), *Chloroflexi* (7.04%), *Firmicutes* (5.34%), OP8 (2.49%), *Cyanobacteria* (2.26%), *Fibrobacteres* (1.39%), *Gemmatimonadetes* (1.28%) and OD1(1.27%) (Figure 3a).

2011 Culture-Independent dataset, retrieved total number of reads 12,780 represented in 33 phyla and 225 taxa, filtered significantly to 164 taxa (Supplementary Table 3b). The dominant

phyla of this dataset are Proteobacteria (59.05%), Chloroflexi (7.67%), Gemmatimonadetes (6.64%), Bacteroidetes (5.81%), OP1 (4.15%), Unknown (2.48%), Deferribacteres (2.07%), Planctomycetes (1.74%), Nitrospirae (1.72%), Spirochaetes (1.49%), Firmicutes (1.33%), and OP3 (1.18%) (Figure 3b).

The 2011 Culture-Dependent dataset retrieved 16,053 total reads, represented in 9 phyla comprising 56 different taxa, significantly, these taxa have been filtered into 27 taxa only (Supplementary Table 3c). The phyla retrieved by this dataset are Proteobacteria (68.02%), Firmicutes (20.59%), Bacteroidetes (9.72%), Unknown (1.26%), Spirochaetes (0.3%), Thermotogae (0.07%), Planctomycetes (0.02%), Nitrospirae (0.01%), and OP1 (0.01%).

On the contrast to the case with Saline Lake-RM; 2013 Culture-Independent dataset for Solar Lake-W showed the highest number of taxa than both the 2011 Culture-Independent and Culture-Dependent datasets. 2013 dataset retrieved 212 taxa, while 2011 Culture-Independent approach retrieved 164 taxa, and 27 taxa for the Culture-Dependent approach. Interestingly, 38 of 2011 Culture-Independent taxa are shared with 2013 dataset, while only 3 taxa of the Culture-Dependent dataset, Clostridiaceae, *Clostridiisalibacter sp.*, and *Salinivibrio sp.*, are shared with the 2013 dataset (Table 2). The order Clostridiales and the genera *Desulfovibrio sp.*, *Halomonas sp.* and *Vibrio sp.* have been detected in the three datasets. The bacteria shared among the three datasets are presented in (Table 2).

2013 dataset showed the dominance to the unclassified bacteria *Caldithrix* candidate divisions (KSB-1[GW-22]) representing more than 10% of the whole dataset (Figure 4a and Supplementary Table 3a). This was followed by *Spirochaeta sp.*, which is one the bacteria that are recurrently detected in both of the datasets, 2013 Culture-Independent (7.3%) and 2011 Culture-Independent (1.2%) datasets. This was followed by the candidate divisions OPB11 of the class Anaerolineae (4.9%), AKAU3564 of (2.8%), and OP8(HMMVPog-54)(2.5%), (TG3;TG3-1;MAT-CR-H6-H10) of Fibrobacteres (1.3%), OD1 (1%), Gemm-2 of Gemmatimonadetes (1%), an unassigned order of Deltaproteobacteria (4.8%), an unassigned genus of the following: Phycisphaeraceae (4.3%), Ruminococcaceae (3.7%), Desulfarculaceae (2.8%), Rhodobacteraceae (2.4%), Saprospiraceae (2.3%), Spirochaetaceae (2.1%), and the genera *Desulfovermiculus sp.* (2.9%), *Desulfomonile sp.* (2.6%), *Microcoleus sp.* (2.1%), *Salinivibrio sp.* (1.5%), *Pseudoalteromonas sp.* (1.3%), and *Pseudomonas sp.* (1.1%) From another point of view;

analyzing this dataset revealed more than 33 different halophilic and sulfur metabolizers have been detected in the 2013 dataset constituting 16.3% of the whole dataset, presented in (Supplementary Table 4a). This was dominated by unassigned genera of Desulfobacteraceae (3.7%) and Desulfarculaceae (2.8%), and the genera *Desulfovermiculus sp.* (2.9%) and *Desulfomonile sp.* (2.6%).

On the other hand, 2011 Culture-Independent dataset was mostly dominated by an unassigned genus of Enterobacteriaceae, constituted more than 37% of the whole dataset (Figure 4b and Supplementary Table 3b). This is one of the taxa that are repetitively detected in both the 2011 and 2013 Culture-Independent datasets. Moreover, the genus *Enterobacter sp.*, a member of Enterobacteriaceae, has been significantly detected in 2013 Culture-Independent dataset. The second most abundant taxa in the 2011 Culture-Independent dataset is an unassigned order of Gemmatimonadetes (6.6%), followed by the candidate division OP1 (4.1%), *Psychroflexus sp.* (3.2%), *Dehalogenimonas sp.* (2.7%), *Idiomarina sp.* (2.6%), an unassigned family of Anaerolineales (2.5%), Unknown (2.5%), an unassigned genus of Anaerolinaceae (2.1%), followed by the halophilic genera *Halomonas sp.* and *Marinobacter sp.* (1.8%/each) (Figure 4b and Supplementary Table 3b). Interestingly, 2011 Culture-Independent dataset revealed almost exactly the same pattern of the 2013 dataset of encountering several halophilic and sulfur metabolizing bacteria, where 36 different halophilic and sulfur metabolizers have been detected constituting 18.3% of the whole dataset (Supplementary Table 4b). This was dominated by *Dehalogenimonas sp.* (2.7%), *Idiomarina sp.* (2.6%), *Halomonas sp.* (1.8%), *Marinobacter sp.* (1.8%), Ectothiorhodospiraceae (1.2%), and *Desulfovermiculus sp.* (0.96%).

2011 Culture-Dependent dataset revealed 27 different taxa that showed also similar pattern to other previously mentioned datasets. *Vibrio sp.* dominated this dataset by representing more than half of it (58%). This was followed by *Clostridium sp.* (9.4%), *Desulfovibrio sp.* (7.2%), Marinilabiaceae (7.1%), *Geosporobacter sp.* (4.7%), *Clostridiisalibacter sp.* (4.2%), *Caminicella sp.* (1.7%), *Anaerophaga sp.* (1.3%), Porphyromonadaceae (1.3%), Unknown (1.3%), *Desulfocella sp.* (1%), and *Salinivibrio sp.* (1%) (Figure 4c and Supplementary Table 3c). Out of the 27 detected taxa, 8 different significant halophilic and sulfur metabolizers have been detected representing 22.14% of the whole dataset (Supplementary Table 4c). This was dominated by *Desulfovibrio sp.* (7.2%), Marinilabiaceae (7.1%), *Clostridiisalibacter sp.* (4.2%), *Anaerophaga*

sp. (1.3%), *Salinivibrio sp.* (1.3%), *Desulfocella sp.* (1%), *Orenia* (0.1%), and *Halomonas sp.* (0.01%).

3.2.3. Saline Lake-RM versus Solar Lake-W:

Interestingly, more than 50% of the data detected at the lakes, were shared between the two lakes. The three datasets of Saline Lake-RM have been compiled together, the duplicates have been removed and the total number of the taxa retrieved reached 332 taxa. Similarly, the three datasets of Solar Lake-W have been compiled together and after the removal of duplicates the total number of taxa retrieved was 355. Of these taxa, 221 different taxa are shared between the two lakes (Supplementary Table 5)

4. DISCUSSION

Most of the planet is saline (Oren 2006). The environment is considered to be saline when its salinity ranges between 3 to 15%, equivalent to 30 ppt to 150 ppt; while it is considered hypersaline when its salinity ranges between 15 to 30%, i.e. from 150 ppt to 300 ppt; (Rafael, Sánchez-Porro et al. 2011). Based on that, Saline Lake-RM (salinity: 149.8 ppt), is considered to be a hypersaline environment; while Solar Lake-W (salinity: 107.9 ppt), considered as a high saline environment; however, it used to be considered hypersaline lake since its discovery because the salinity there were recording from 150 to 180 ppt (Jørgensen and Cohen 1977, Jørgensen, Revsbech et al. 1979). Saline and Hypersaline environments are characterized by other several harsh conditions, such as low dissolved oxygen, alkaline PH, high or low temperatures (in our case, the two lakes show high temperatures), and high concentrations of metals and/or toxic compounds (Oren 2002, Rafael, Sánchez-Porro et al. 2011). All of these characteristics are displayed by the two lakes analyzed in this study. Interestingly, environments that show higher salt concentrations than seas and oceans are the nearshore small ecosystems where the rate of evaporation is high. Although the two lakes are near-shore ecosystems, they did not originate the same way; also, both of them their waters mixes with the Red Sea but with different mechanisms. Saline Lake-RM is a saltern which is being formed due to the Red Sea tide and the evaporation of the Red Sea water that covers it frequently; which is not the case in Solar Lake-W. Solar Lake is a basin at the edge of the sea that is filled with brine and only connected to the sea water, through a seepage (Cohen, Krumbein et al. 1977), and the evaporation there happens only to the lake's water itself. However, both lakes provide extreme conditions for their inhabitants, consequently, microbial inhabitants of such environments should be designated to survive these stressful conditions. The two lakes show extreme saline conditions which in turns would favor the halophilic, moderate and extreme halophilic inhabitants. It has been reported previously that Solar Lake-W does not harbor any sort of animal life and dominated by microbial communities (Jørgensen, Revsbech et al. 1979), while not enough research has been done on Saline-Lake-RM; only one group published two articles about isolating two novel halophilic bacteria from there (Romano, Lama et al. 2007, Romano, Orlando et al. 2011). Other stressful conditions characterizing the two lakes, include the low dissolved oxygen, especially in Saline Lake-RM, 1.94 mg/L; and also they both possess a slight alkaline PH condition, 8.24 in Saline Lake-RM and 8.17 in Solar Lake-W, in addition to the high temperatures and high metal contents of the two lakes, reported in chapter 1&2 (Mustafa, Abd-

Elgawad et al. 2014). So they mainly should be halophiles; but this could be supplemented with being resistant to other extreme conditions, for example, being thermophilic, alkaliphilic or anaerobic halophiles. Most of the halophilic bacteria are members of Gammaproteobacteria, high G+C Firmicutes, Bacteroidetes, Cyanobacteria and Spirochaetes (Oren 2006). This makes Gammaproteobacteria dominant class in most of the marine ecosystems. In our data, the Gammaproteobacteria was the most dominant class in the following datasets: 2011 Culture-Dependent Solar Lake-W dataset, 2011 Culture-Independent Solar Lake-W dataset, and 2011 Culture-Dependent Saline Lake-RM dataset; and the second most dominant in 2011 Culture-Independent Saline Lake-RM dataset. While in 2013 datasets for the two lakes, it showed the third and the fifth most dominant class in Saline Lake-RM and Solar Lake-W, respectively. Also, Firmicutes and Bacteroidetes showed dominance in all of the datasets generated for the two lakes.

In regards to the low oxygen content in those two saline ecosystems. The anaerobic halophiles were detected in the datasets of these two lakes, such as the order Halanaerobiales. Members of this order have been detected in all of the datasets including, interestingly, the Culture-Dependent datasets, although these cultures were not grown on anaerobic conditions, (this could be interpreted as we isolated DNA from spores or cells of these bacteria were in the sediments particles added to the media). Three different genera of this order have been detected in Saline Lake-RM Culture-Dependent dataset; *Orenia marismortui*, *Halanaerobium sp.*, and the cellulolytic halophile *Halocella sp.*. More interestingly, the same three genera just mentioned, were also detected in the Culture-Independent dataset for the same site along with five more members of the same anaerobic halophilic order; an unassigned genus of Halobacteroidaceae, *Orenia sp.*, *Halobacteroides sp.*, *Halobacteroides halobius*, *Halanaerobacter sp.* Interestingly, some of these members require a temperature range for growth from 30° C to 47° C with an optimal temperature from 37° C to 47° C, such as *Halobacteroides halobius* (De Vos, Garrity et al. 2009), which explains its adaptation to three stressful conditions of the lake, the high temperature, salinity and low dissolved oxygen. Also the anaerobic order Bacteroidales was one of the dominant taxa in the Solar Lake-W 2013 dataset.

Interestingly, through all of the generated datasets, Proteobacteria, Firmicutes and Bacteroidetes showed dominance display. Firmicutes are characterized by having a rigid cell wall,

and most of the members of this phyla are known to produce endospores that makes them resist desiccation and survive other harsh conditions (De Vos, Garrity et al. 2009).

Solar Lake has been extensively studied since 1970s. It is characterized by high temperature, high salinity, low dissolved oxygen and high sulfur content, noticed even by the intense smell of sulfur during the sampling process. It has, also, been characterized by the spatial distribution of its microbiota, mainly cyanobacterial mats and bacteria, this zonation was mainly due to the annual cycle of the lake (Krumbein, Cohen et al. 1977). Early documentations were emphasizing and dissecting, mainly, the cyanobacterial and microbial sulfur metabolism in the Lake as the dominant biochemical pathway ran by the lake' microbiota (Cohen, Padan et al. 1975, Cohen, Krumbein et al. 1977, Cohen, Krumbein et al. 1977, Jørgensen and Cohen 1977, Jørgensen, Kuenen et al. 1979, Jørgensen, Revsbech et al. 1979, Walsby, Van Rijn et al. 1983). It has been known that the bottom of the lake has a carpet of cyanobacterial mats along with their bacterial communities harboring the nearby sediments. This was not the only place where cyanobacterial mats described, it has been known that these cyanobacterial mats exist in four different areas of the lake with their own different types. The four types of cyanobacteria recorded at Solar Lake, at that time, were 1- Flat shallow-water mat, 2- Pinnacle mat on the upper slope of the lake, 3- Flocculose mat at the bottom of the lake (anaerobic cyanobacteria), and 4- cyanobacterial and other bacterial films on the slower slope. The cyanobacterial there on the coast of the lake reached up to 1m thick, with a sulfate reduction rate 10,000 times more than the bottom of the lake (Jørgensen and Cohen 1977). Interestingly, our samples have been taken from the coastal sediments of the lake. Based on that several sulfate reducers have been detected extensively in our datasets for this lake, some of them were reported since 1970s to be the main players of sulfur metabolism in the lake, such as *Desulfovibrio sp.* which has been reported since that time to play a major role with the cyanobacterial biomass assimilation. Not only this, but also, more importantly, a novel species of this genus has been isolated from the anoxic layer of cyanobacterial mats of Solar Lake, *Desulfovibrio halophilus* (Caumette, Cohen et al. 1991), while another species has been isolated from the oxic mat layer of the same lake, *Desulfovibrio oxyclinae* (Krekeler, Sigalevich et al. 1997). This genus has been detected in the three datasets of the lake, representing 0.3% in the Culture-Independent dataset, 0.4% in the 2013 Culture-Independent dataset, and 7.2% in the Culture-Dependent dataset. This does not necessarily mean that *Desulfovibrio sp.*, based on the cultured dataset, has a dominant display in the site, because the other larger pictures, 2013 and

2011 Culture-Independent datasets, are not showing that. It could be simply because the media favored the growth of certain bacteria over the others, but it does not have to reflect the real picture of bacterial abundance. However, other sulfate-reducers with a mesophilic to moderately thermophilic anaerobic halophiles, which agrees with Solar Lake-W conditions, have also been detected, such as members of Desulfohalobiaceae. These represented by *Desulfovermiculus sp.* (2.9%) and (0.1%) in 2013 and 2011 Culture-Independent datasets, respectively; and *Desulfohalobium sp.* (0.07%) in the 2013 dataset, which has been isolated from a hypersaline lake (Kuever, Rainey et al. 2005). Interestingly, several anaerobic sulfate reducers of the order Desulfobacterales have also detected in the three datasets of the lake, with a better coverage displayed by 2011 Culture-Independent dataset where it showed 8 members of this order. In this dataset, the taxa of this order detected are: an unassigned genus of Desulfobacteraceae (0.7%), *Desulfobacterium sp.* (0.3%), *Desulfurivibrio sp.* (0.3%), *Desulfobulbus sp.* (0.1%), an unassigned class of Desulfobacterales (0.1%), *Desulfonema sp.* (0.05%), *Desulfococcus sp.* (0.02%), *Desulfopila sp.* (0.01%). While in the 2013 dataset this order was represented by *Desulfococcus sp.* (4.7%), an unassigned genus of Desulfobacteraceae (3.7%), and an unassigned genus of Desulfobulbaceae (0.14%); and only one genus was detected in the 2011 Culture-Dependent dataset, *Desulfocella sp.* (1%) (Supplementary Tables 2a, 2b, 2c). Consequently, some of these sulfate reducers have been detected repeatedly in the three datasets of Solar Lake-W and presented in (Table 2). Interestingly, these members have also detected in Saline Lake-RM 2013 and 2011 Culture-Independent datasets. An unassigned genus of Desulfobacteraceae is the only taxa of this order, Desulfobacterales, detected in the Saline Lake-RM 2011 dataset. While in 2011 Culture-Independent dataset, more interestingly, almost the same genera of this order detected in the Solar Lake-W 2011 Culture-Independent dataset, has also been detected in the Saline Lake-RM Culture-Independent dataset. These are the following 8 taxa: *Desulfobacterium sp.*, *Desulfonema sp.*, *Desulfosarcina sp.*, *Desulfobulbus sp.*, *Desulfopila sp.*, *Desulfurivibrio sp.*, unassigned genus of Desulfobacteraceae, and unassigned family of Desulfobacterales.

Importantly, although Cyanobacteria was the dominant microbiota in the lake since its discovery in 1970s, in our data, Cyanobacteria were detected in minute amounts in the analyzed datasets of the Solar Lake-W. It was detected only in 2011 and 2013 Culture-Independent datasets, representing only 0.26% and 2.26% respectively. However, one of the cyanobacteria that were known to be a dominant cyanobacteria at the lake during its discovery, *Microcoleus sp.*, has been

detected in our 2013 dataset representing 2.1% of the dataset (most of the total Cyanobacteria in this dataset, 2.26%). Other than *Microcoleus sp.*, the candidate division 4C0d-2;YS2, Stramenopiles, and *Cyanothece sp.*, have been detected in the 2013 dataset (Supplementary Table 3a), while unassigned Cyanobacteria was the representative Cyanobacteria of the Solar Lake-W 2011 Culture-Independent dataset (Supplementary Table 3b). Also, more interestingly, Cyanobacteria has been detected in Saline Lake-RM but in smaller concentrations; however with almost the same taxa and pattern detected in the Solar Lake-W datasets where the candidate division 4C0d-2;YS2, Chlorophyta, Stramenopiles, *Cyanothece sp.*, and *Microcoleus sp.* has been detected in the 2013 dataset, while it was represented by unassigned Cyanobacteria having almost the same concentrations of the above mentioned members (0.14%). Similarly interesting, the desiccation-resistant bacteria, Myxococcales, have been detected with several members in both the Saline Lake-RM two datasets (2013 and 2011 Culture-Independent) and Solar Lake-W two datasets (2013 and 2011 Culture-Independent), (Supplementary Tables 1a,b & 3a,b). The interesting fact about the detection of these bacteria is that it is known to degrade Cyanobacteria (Kuever, Rainey et al. 2005), and also, this degradation in the Solar Lake-W by this bacteria has been reported very early (Krumbein, Cohen et al. 1977). The members of Myxococcales detected in the Solar Lake-W 2013 dataset are an unassigned family of Myxococcales (1.9%) and Cystobacterineae (0.14%), while in the Solar Lake-W 2011 Culture-Independent dataset only the unassigned family of Myxococcales (0.19%) was detected. Similarly, in Saline Lake-RM 2013 dataset an unassigned family of Myxococcales (0.13%), Cystobacterineae (0.01%), and the genus *Plesiocystis sp.* (0.03%) were detected, while in the other dataset, 2011 Culture-Independent, the detected members were an unassigned family of Myxococcales (0.4%), Polyangiaceae (0.05%), and *Haliangium sp.* (0.03%) (Supplementary Tables 1 a,b & 3 a,b). Similarly interesting, *Flexibacter sp.*, has been detected in Solar Lake-W dataset and this bacteria is known to lyse cyanobacterial filaments and grow on their fragments (Abed, Kohls et al. 2010).

Adapting to different stressful conditions together has been also revealed through the dominant bacteria of Solar Lake-W. The fact that it is dominated, in the 2013 dataset, by candidate division of the unclassified bacteria *Caldithrix* reflects the environmental characteristics of the Solar Lake-W, as these bacteria are known to be frequently inhabitant to environments with thermophilic, halophilic, and sulfide-rich conditions (Alauzet and Jumas-Bilak 2014). This was more confirmed by the second most abundant bacteria; the genus *Spirochaeta sp.*, which has been

also detected in the 2011 culture-independent dataset; this genus is known to be obligatory or facultative anaerobic that is commonly occur in H₂S containing environments (Krieg, Whitman et al. 2011). Also, Phycisphaeraceae, one of the dominant bacteria detected in the 2013 dataset, which has been also detected, twice, in 2011 Culture-Independent dataset, is known to be facultative anaerobic bacteria which its members have been, firstly, isolated from marine algae (Fukunaga, Kurahashi et al. 2009). Interestingly, 3.7% of the 2013 dataset was assigned to the clostridia family: Ruminococcaceae, which is known to be strictly anaerobic and usually isolated from rumen of animals and humans but not from environmental samples, also, ammonia is required for their growth; and the lake showed significant nitrogen concentration 0.2% (De Vos, Garrity et al. 2009). Also, three taxa of the class Dehalococcoidetes (0.8%), have been detected in this dataset; this class comprise newly discovered lineage of Chloroflexi, its identified members till now are known to be marine strictly anaerobic, mesophilic and chemotrophic bacteria, with significant ecological importance, as they are able to reductively dehalogenate chlorinated alkanes (Moe, Yan et al. 2009).

Adapting to the high salinity condition of the Solar Lake-W, several halophilic bacteria have been detected in the three datasets, including, the following sulfur metabolizers' halophiles; *Halanaerobium sp.*, *Marinobacter sp.*, *Halothiobacillus sp.*, *Desulfobacteraceae*, *Desulfococcus sp.*, *Desulfovermiculus sp.*, *Orenia sp.*, and Ectothiorhodospiraceae, which have been detected in both 2013 and 2011 Culture-Independent datasets (Table 2). In addition to several others halophiles detected in each data separately, such as, in 2013 dataset, six members of the strictly anaerobic moderate halophiles Halanaerobiales (0.2%), and Marinicellaceae (0.2%).

Several bacteria have been shown to be repetitively detected in the three datasets, confirming their significance to the lake. Some of these, were of the sulfate reducers mentioned earlier. Others were also reflecting the general environmental conditions of the lake including the high salinity, high temperature and the high rate of other types of sulfur metabolism; these such as, the three genera *Desulfovibrio sp.*, *Halomonas sp.* and *Vibrio sp.*

Although the two lakes are different in their origin and in their physical descriptions and also in their environmental impacts and they are not in close proximity to each other, they are showing great similarities starting from the physical and chemical conditions till the microbial inhabitants. The two lakes are sharing around 70% of their microbiota. Both lakes are showing

extreme salinity conditions, low oxygen, high temperature and significant metal concentrations. In regards, several anaerobic and halophilic bacteria were detected in both lakes and were dominating the taxa of each site. Although, there were no sulfur detected in Saline Lake-RM, a lot of sulfur metabolizing bacteria were detected in dominant concentrations. Solar Lake-W has both sulfur and sulfur metabolizers were detected. Our data confirming the old reported data about the high sulfur metabolism at the lake, although it did not show the same dominance for the main player, Cyanobacteria. Abundance of Cyanobacteria in our data does not reflect at all having meters-thick mats, however, Cyanobacterial degradation due to sulfate reduction and other microbial degradation, mentioned above, at the lake has been reported since old ages (Krumbein, Cohen et al. 1977), it could now being almost degraded, because, during our samples, we did not notice any Cyanobacteria or Stromatolites on the shores of the lake as was reported. Interestingly, Saline Lake-RM has shown Cyanobacteria but in a very minute abundance, even less than those detected at Solar Lake-W. A lot of different investigations need to be ran on Saline Lake-RM to start identifying it and its ecological significance.

As expected, 2011 Culture-Dependent dataset showed to be closer to 2011 Culture-Independent dataset than to 2013 Culture-Independent dataset in the two lakes. This is simply because of 1- the same date of sampling and 2- using the same sequencing approach and database. Also, a good characteristic about the 2011 Culture-Dependent approach over the other two Culture-Independent datasets, is that it helped us to magnify ecologically significant bacteria that would have been neglected if only the uncultured approaches were used; such as Clostridiaceae, *Halomonas sp.* and *Marinobacter sp.* in Saline Lake-RM (table 1), and *Desulfovibrio sp.*, *Vibrio sp.*, Marinilabiaceae, *Clostridium sp.*, *Caminicella sp.*, *Clostridiisalibacter sp.* in Solar Lake-W (Table 2), which were magnified, sometimes, even to 100 times. However, generally, through looking at the two uncultured datasets, 2013 and 2011 datasets, the later shows a clearer and sometimes bigger image about the site' microbiota.

TABLES

Table 1: Taxa shared among the three Saline Lake-RM datasets, and their abundance per dataset

Saline Lake-RM			
Taxon	2013 Culture-Independent (%)	2011 Culture-Independent (%)	2011 Culture-Dependent (%)
Bacillus	0.03	0.03	2.77
Clostridiales**	0.11	0.08	0.02
Clostridiisalibacter	0.13	1.54	3.33
Halanaerobium	0.01	0.32	0.16
Desulfovibrio	0.06	0.02	0.06
Bact	0.02	2.53	0
Halanaerobacter	0.70	0.10	0
OD1****	0.00	0.39	0
Planctomycetes****	0.01	0.17	0
Phycisphaerales**	0.04	0.10	0
Phycisphaeraceae*	0.03	0.31	0
Rhodobacteraceae*	1.87	0.34	0
Roseovarius	0.003	0.01	0
Rhodospirillaceae*	0.04	0.07	0
Rhodovibrio	0.11	0.25	0
Bdellovibrionaceae*	0.003	0.02	0
Desulfobacteraceae*	20.40	0.38	0
Desulfovermiculus	1.45	1.90	0
Desulfuromonadales**	0.03	0.81	0
Myxococcales**	0.14	0.37	0
Gammaproteobacteria***	0.03	0.57	0
Chromatiales**	0.01	0.67	0
Ectothiorhodospiraceae*	0.07	3.99	0
Enterobacteriaceae*	0.01	0.02	0
Pseudomonas	0.02	0.01	0
Spirochaetaceae*	0.23	0.02	0
Spirochaeta	1.22	0.93	0
TM6	0.01	1.20	0
Clostridiaceae*	0.01	0	0.1
Halomonas	0.01	0	18.33
Marinobacter	0.23	0	23.54
Anaerophaga	0	0.08	1.84
Sphingobacteriales**	0	0.76	0.03
Caloranaerobacter	0	0.18	0.15
Caminicella	0	0.51	0.09
Clostridium	0	0.38	28.58
Lachnospiraceae*	0	0.04	0.32
Halocella	0	0.17	0.12
Orenia marismortui	0	1.45	0.52
Unknown	0	1.25	0.79

Table 1: Taxa shared among the three Saline Lake-RM datasets, and their abundance per dataset

Solar Lake-W			
Taxon	2013 Culture-Independent (%)	2011 Culture-Independent (%)	2011 Culture-Dependent (%)
Clostridiales**	0.16	0.44	0.025
Desulfovibrio	0.36	0.20	7.18
Halomonas	0.05	1.79	0.01
Vibrio	0.07	0.05	54.58
Bact	0.68	0.52	0
Propionibacterium	0.04	0.06	0
BRC1	0.01	0.05	0
Anaerolinaceae*	0.01	2.05	0
Streptococcus	0.02	0.04	0
Halanaerobium	0.24	0.02	0
Orenia	0.02	1.59	0
Nitrospiraceae*	0.08	0.07	0
Nitrospira	0.01	0.35	0
OD1	1.06	0.12	0
Planctomycetes	0.01	0.06	0
Phycisphaerales**	0.08	0.07	0
Phycisphaeraceae*	4.30	0.11	0
Alphaproteobacteria***	0.04	0.05	0
Rhodobacteraceae*	2.37	0.07	0
Rhodospirillales**	0.60	0.23	0
Rhodospirillaceae*	0.23	0.02	0
Inquilineus	0.05	0.05	0
Rhodovibrio	0.04	0.34	0
Deltaproteobacteria**	4.80	0.66	0
Desulfobacteraceae*	3.69	0.02	0
Desulfococcus	0.47	0.96	0
Desulfovermiculus	2.92	0.30	0
Myxococcales**	1.90	0.30	0
Gammaproteobacteria***	0.01	1.78	0
Marinobacter	0.69	0.02	0
Ectothiorhodospiraceae*	0.02	1.21	0
Halothiobacillus	0.05	0.77	0
Enterobacteriaceae*	0.43	37.18	0
Acinetobacter	0.35	0.02	0
Spirochaetaceae*	2.13	0.20	0
Spirochaeta	7.34	1.21	0
Clostridiaceae*	0.05	0	0.18
Clostridiisalibacter	0.44	0	4.17
Salinivibrio	1.48	0	1.00
Marinilabiaceae*	0	0.2347	7.10
Clostridium	0	0.0235	2.16
Camincella	0	7.80E-05	1.73
Unknown	0	2.4804	1.26
Pseudoalteromonas	0	0.0391	0.04
Fusibacter	0	0.2426	0.03
Sphingobacteriales**	0	0.0939	0.02

FIGURES

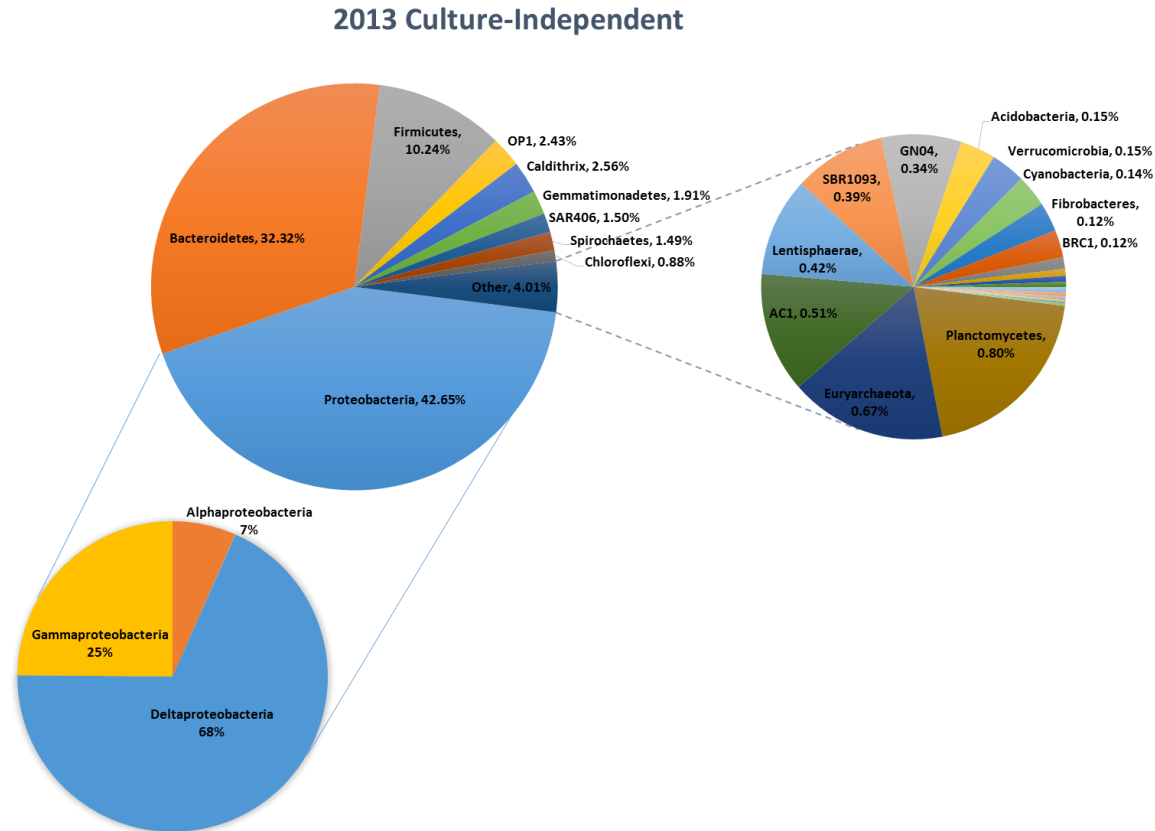


Figure 1a: Pie chart presentation of the bacterial phyla (>0.1%) retrieved from 2013 Culture-Independent dataset of Saline Lake-RM, showing the dominance of Proteobacteria (43%); which presented by the composition of Deltaproteobacteria (68%), Gammaproteobacteria (25%), and Alphaproteobacteria (7%).

2011 Culture-Independent

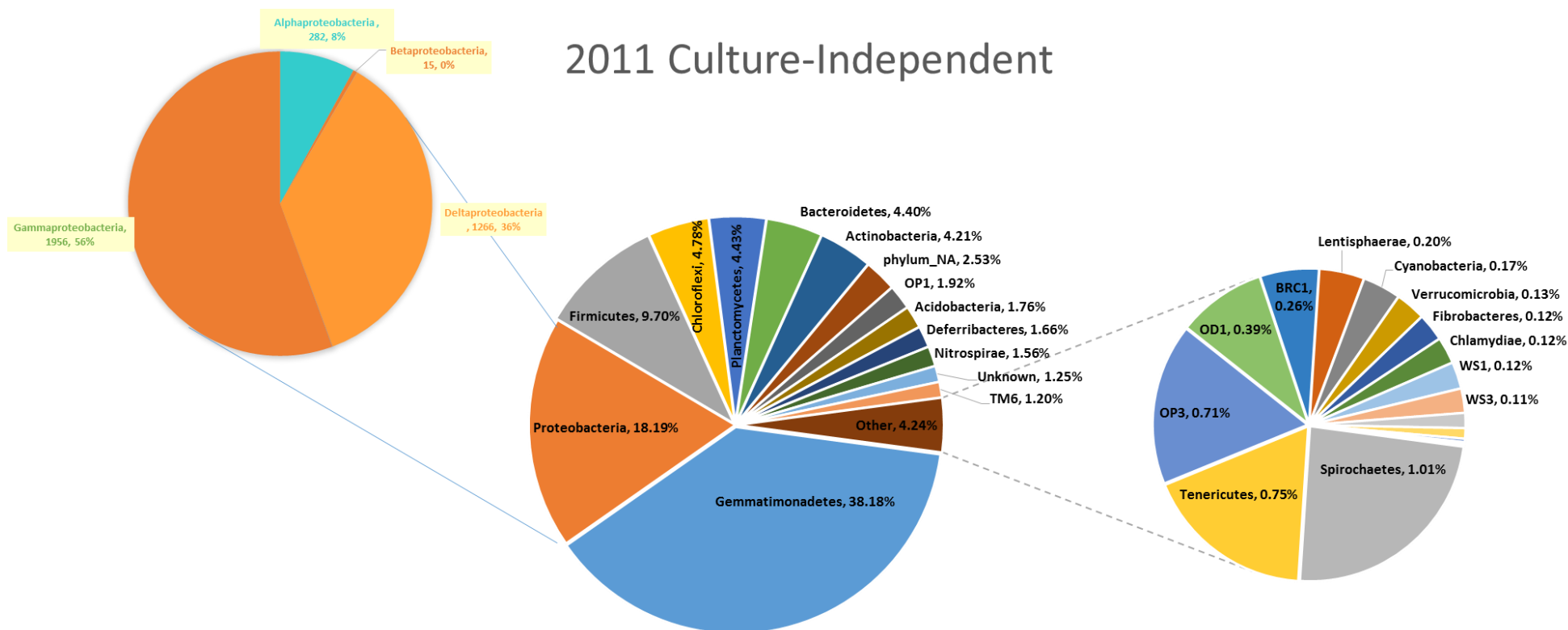


Figure 1b: Pie chart presentation of the bacterial phyla (>0.1%) retrieved from 2011 Culture-Independent dataset of Saline Lake-RM, showing the dominance of Gemmatimonadetes (38.18%), while Proteobacteria was the second most dominant phyla which dominated by the composition of Gammaproteobacteria (56%), Deltaproteobacteria (36%) and Alphaproteobacteria (8%).

2011 Culture-Dependent

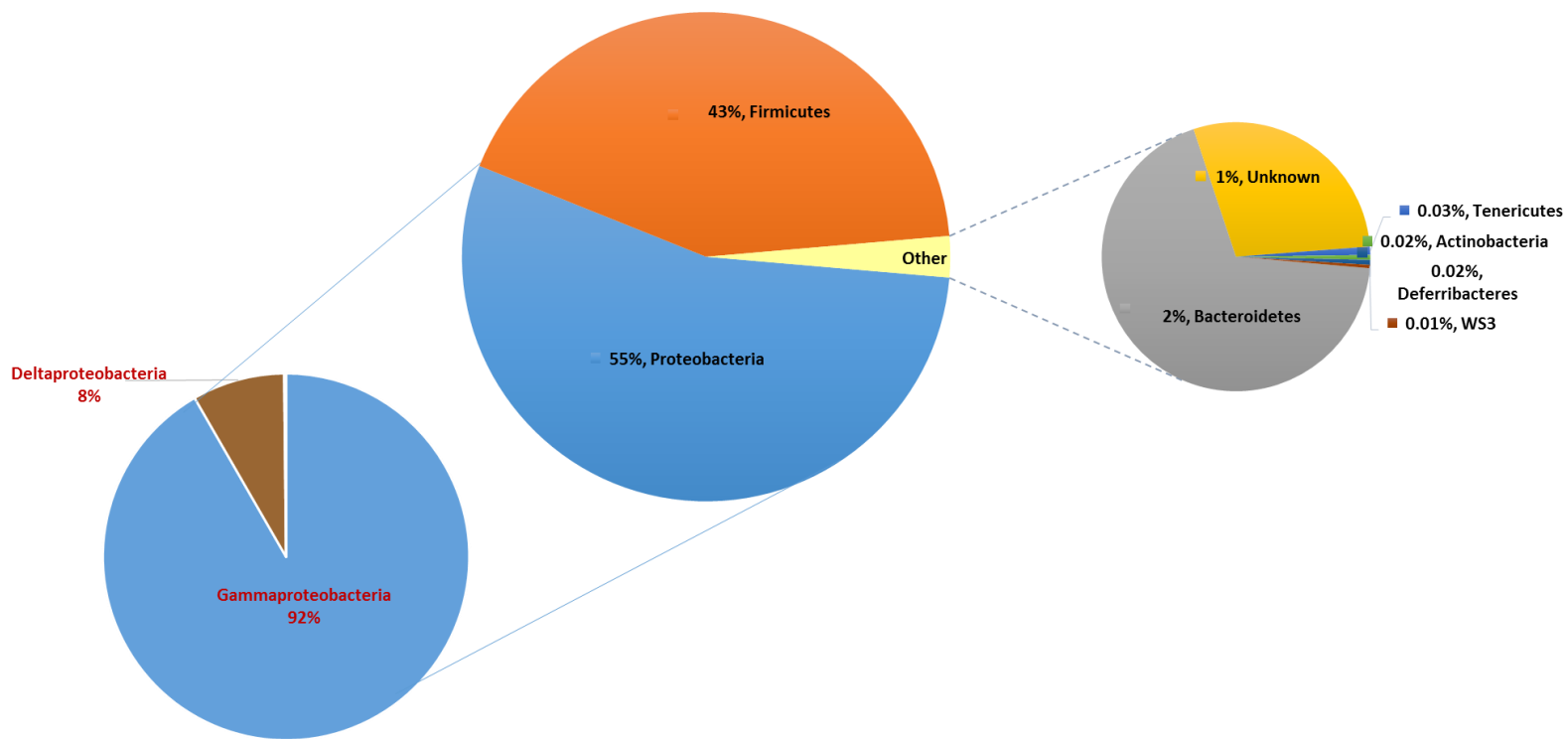


Figure 1c: Pie chart presentation of the total bacterial phyla retrieved from 2011 Culture-Dependent dataset of Saline Lake-RM, showing the dominance of Proteobacteria (55%), which represented mainly by Gammaproteobacteria (92%).

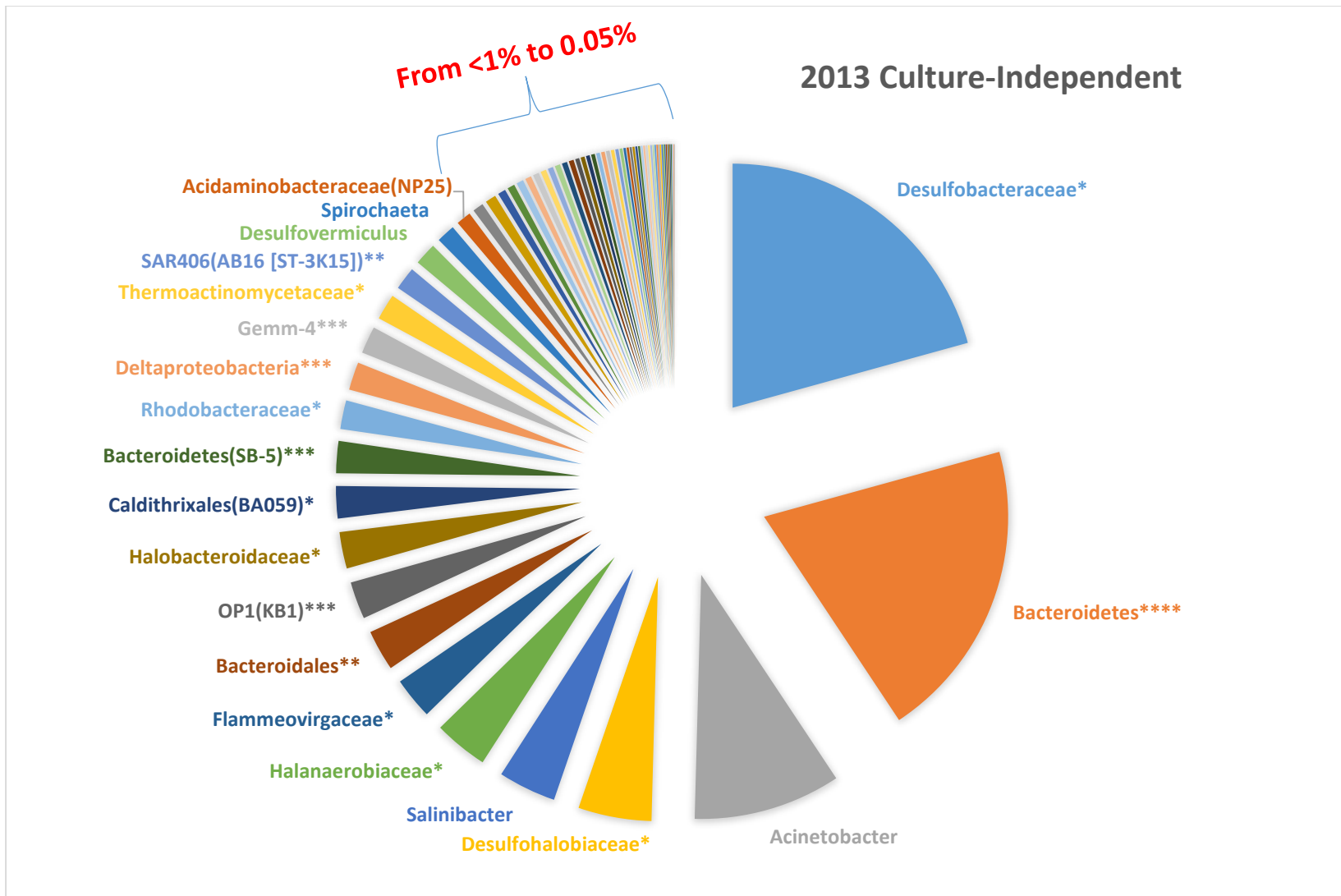


Figure 2a: Pie chart representation of the total taxa (>0.05%) of Saline Lake-RM generated through 2013 Culture-Independent dataset, showing the dominance of an unassigned genus of Desulfobacteraceae (20.4%) and the phyla Bacteroidetes (19.6%). * unassigned genus, ** unassigned family, * unassigned order, **** unassigned class.**

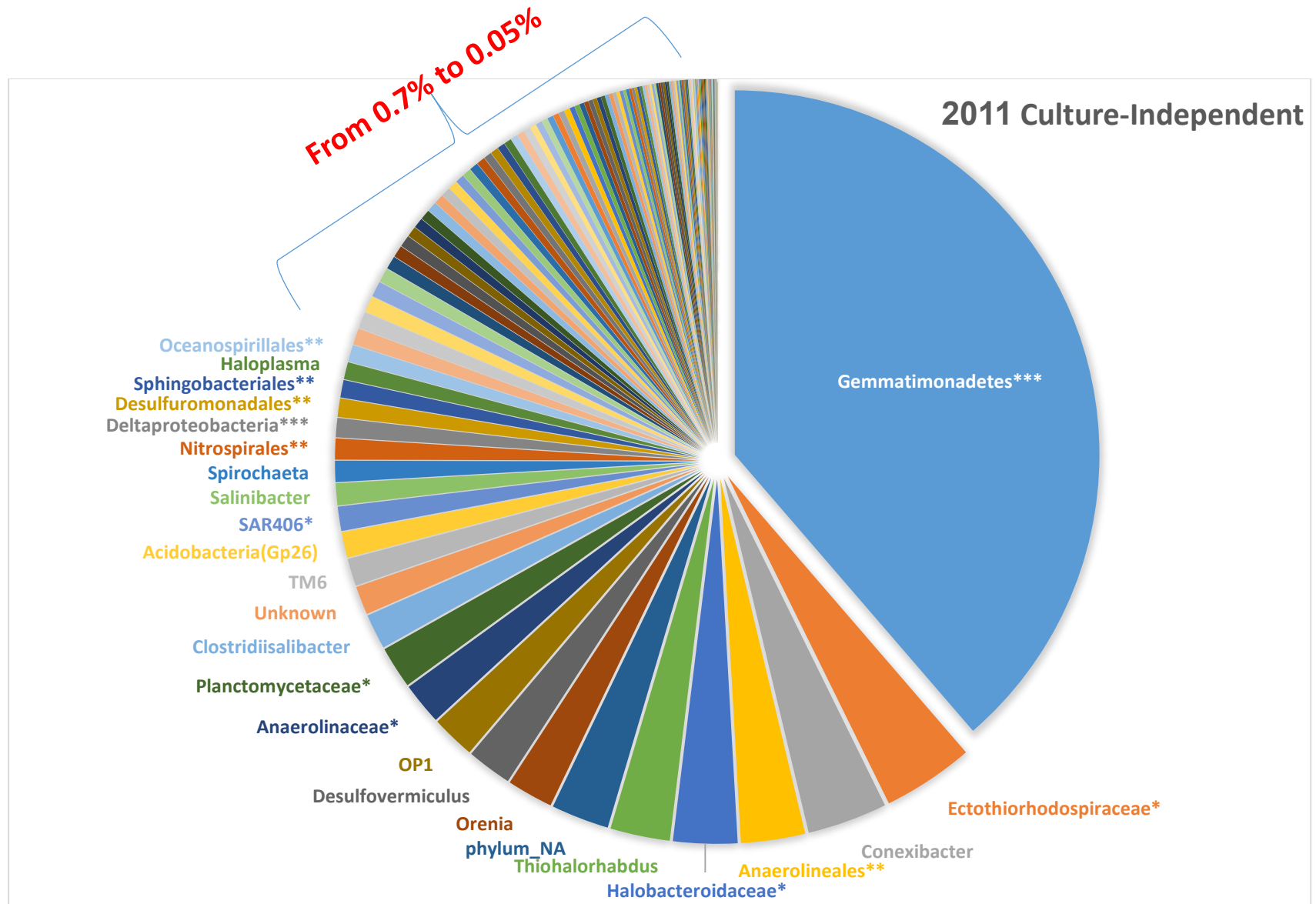


Figure 2b: Pie chart representation of the total taxa (>0.05%) of Saline Lake-RM generated through using the 2011 Culture-Independent approach, showing the dominance of an unassigned order of Gemmatimonadetes (38.2%). * unassigned genus, ** unassigned family, * unassigned order, **** unassigned class.**

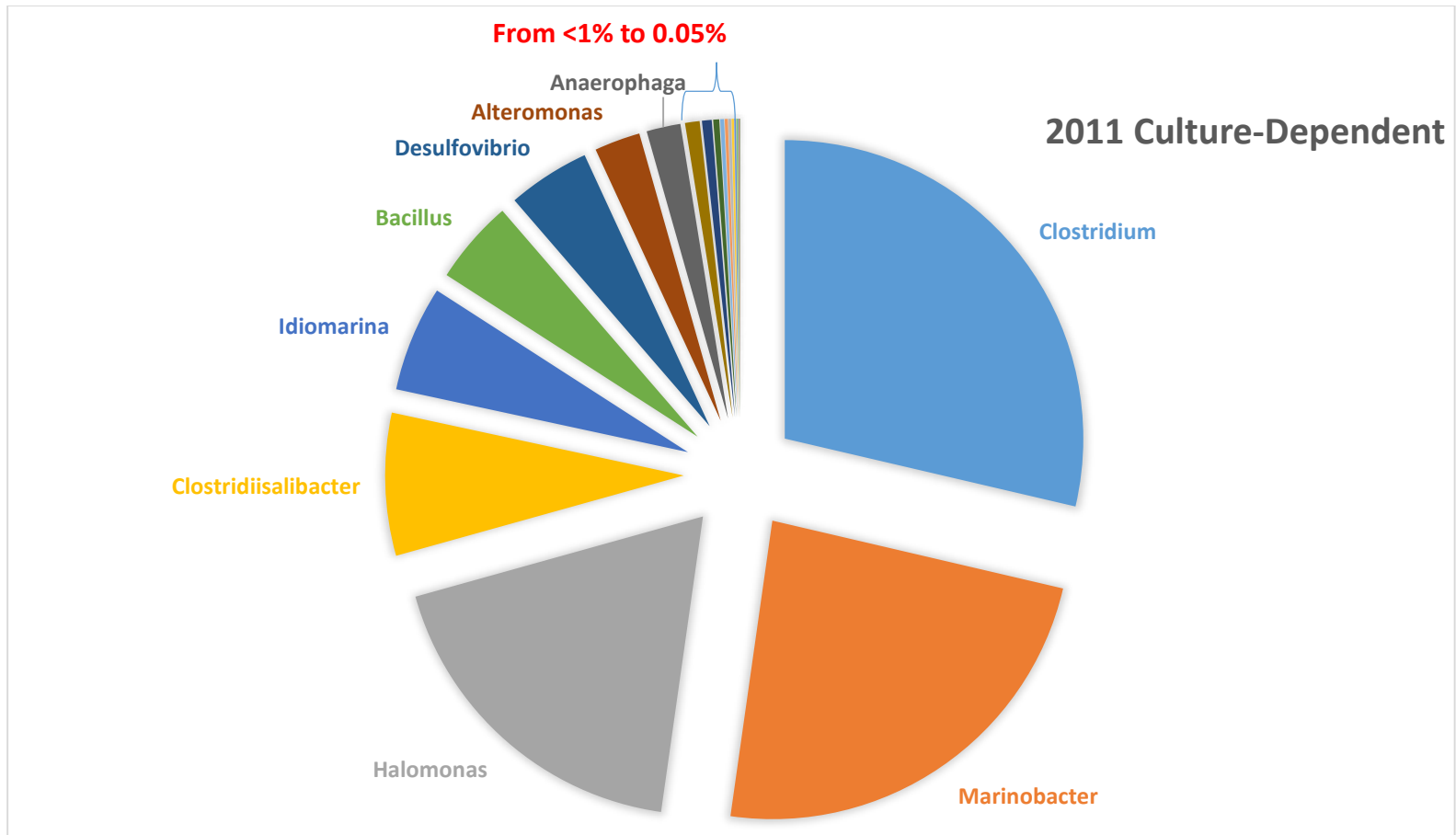


Figure 2c: Pie chart representation of the total taxa (>0.05%) of Saline Lake-RM generated through using the 2011 Culture-Dependent approach, showing the dominance of the genera *Clostridium* sp. (28.6%), *Marinobacter* sp. (23.5%), and *Halomonas* sp. (18.3%).

2013 Culture-Independent

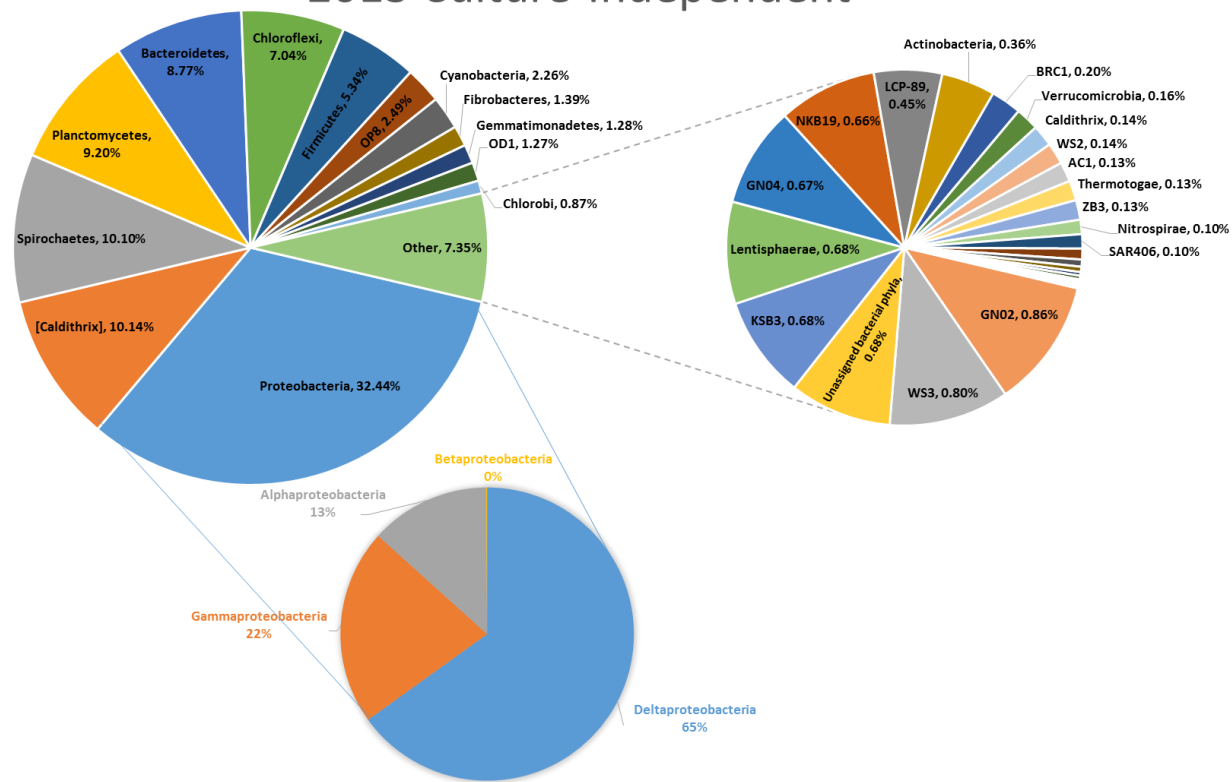


Figure 3a: Pie chart presentation of the bacterial phyla (>0.1%) retrieved from 2013 dataset of Solar Lake-W, showing the dominance of Proteobacteria (32%); which presented by the composition of Deltaproteobacteria (65%), Gammaproteobacteria (22%), and Alphaproteobacteria (13%).

2011 Culture-Independent

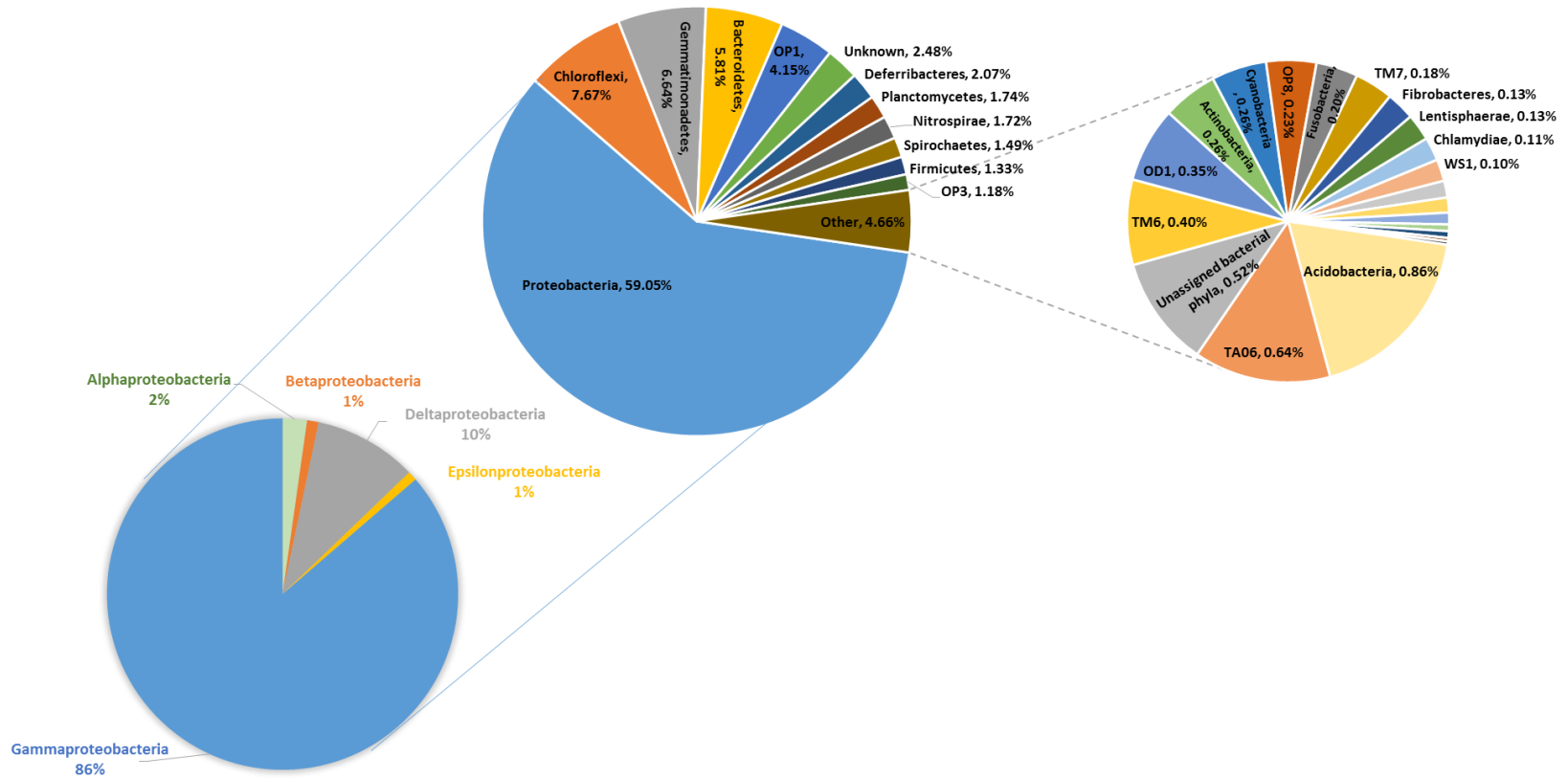


Figure 3b: Pie chart presentation of the bacterial phyla (>0.1%) retrieved from 2011 Culture-Independent dataset of Solar Lake-W, showing the dominance of Proteobacteria (59%) which dominated by the composition of Gammaproteobacteria (86%), Deltaproteobacteria (10%).

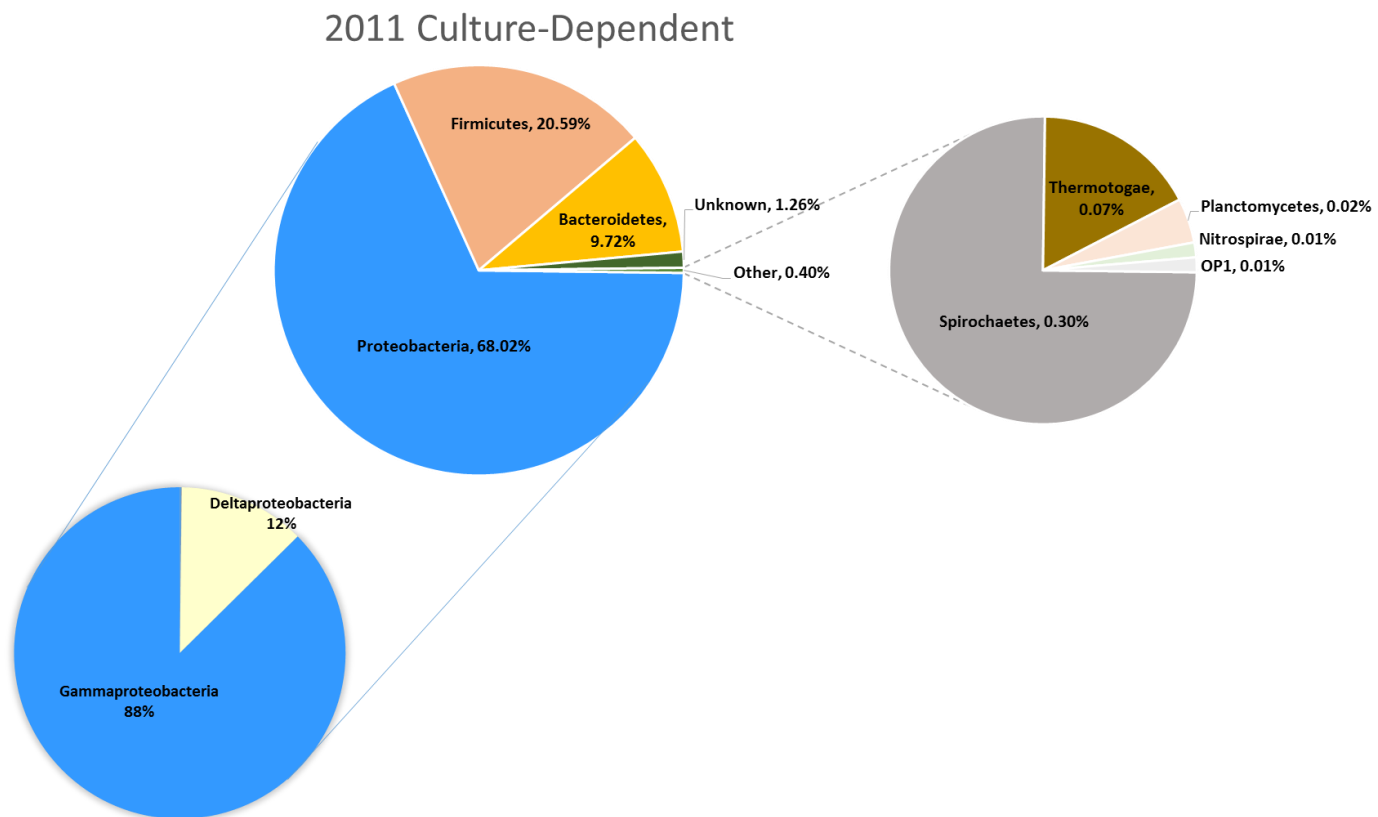


Figure 3c: Pie chart presentation of the total bacterial phyla retrieved from 2011 Culture-Dependent dataset of Solar Lake-W, showing the dominance of Proteobacteria (68%), which represented only by Gammaproteobacteria (88%) and Deltaproteobacteria (12%).

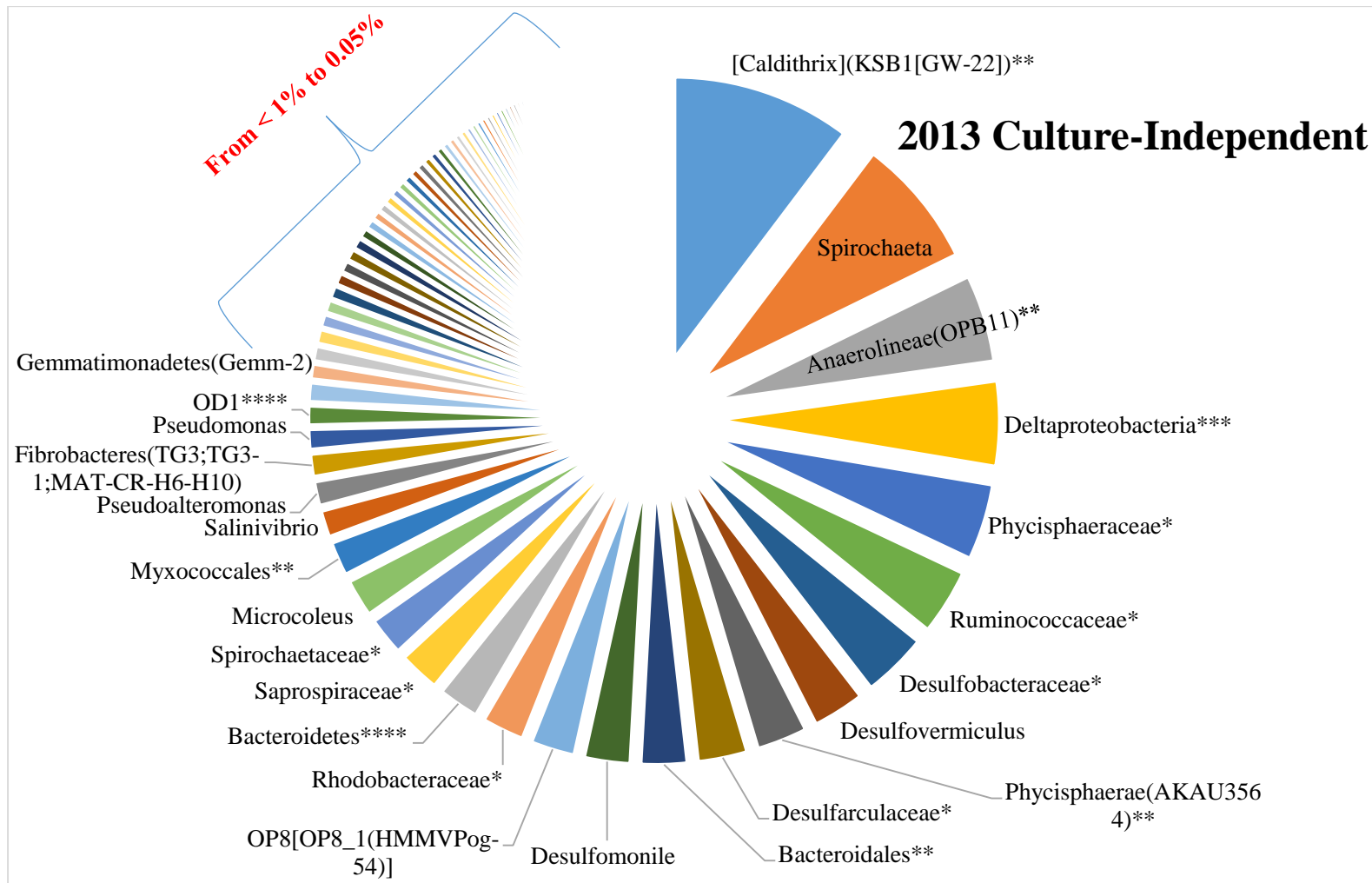


Figure 4a: Pie chart representation of the total taxa (>0.05%) of Solar Lake-W generated through 2013 Culture-Independent dataset, showing the dominance of Caldithrix (10%) and Spirochaeta (7.3%). * unassigned genus, ** unassigned family, * unassigned order, **** unassigned class.**

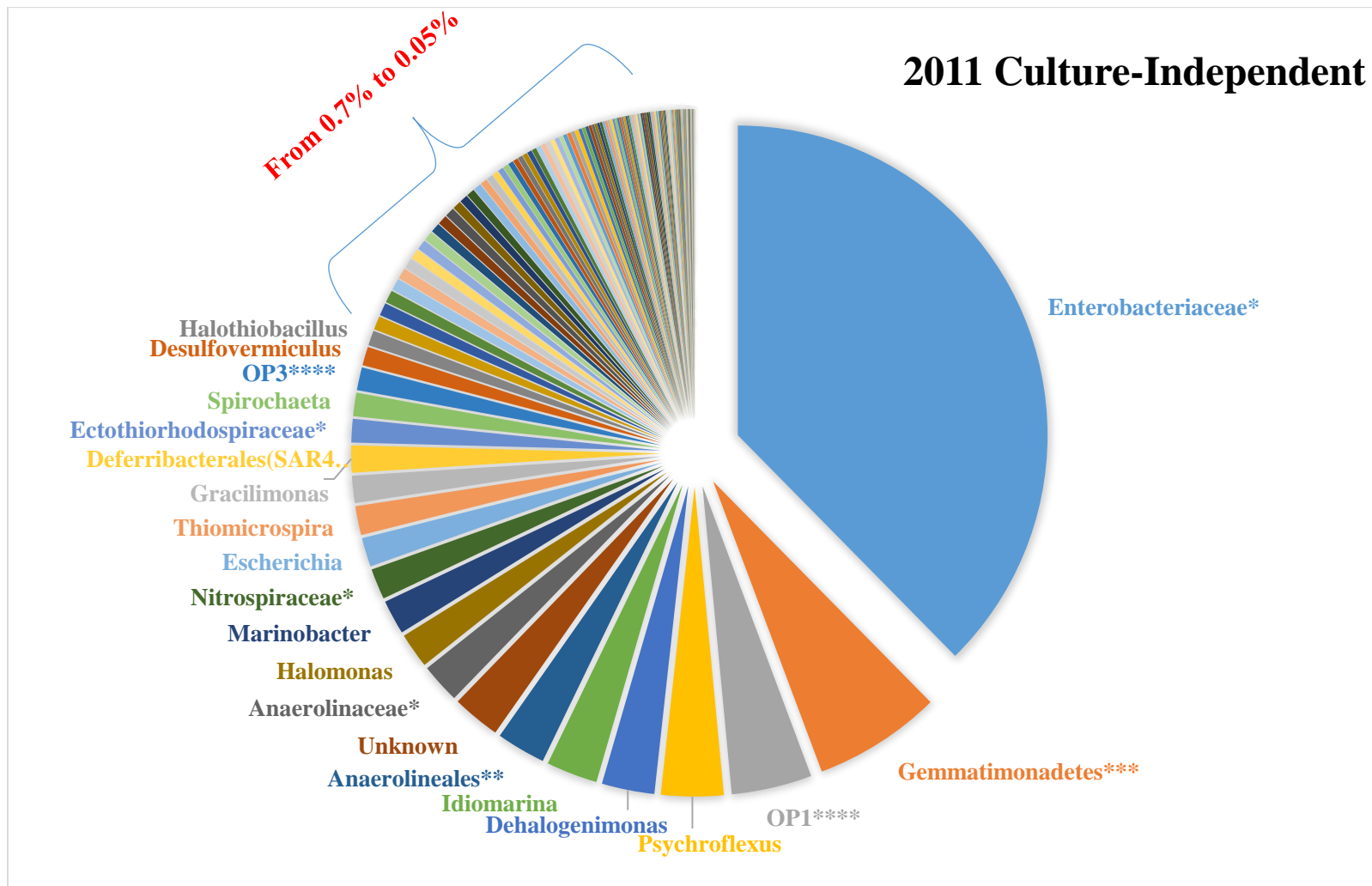


Figure 4b: Pie chart representation of the total taxa (>0.05%) of Solar Lake-W generated through using the 2011 Culture-Independent approach, showing the dominance of an unassigned genus of Enterobacteriaceae (37.2%) and unassigned order of Gemmatimonadetes (6.6%) * unassigned genus, ** unassigned family, * unassigned order, **** unassigned class.**

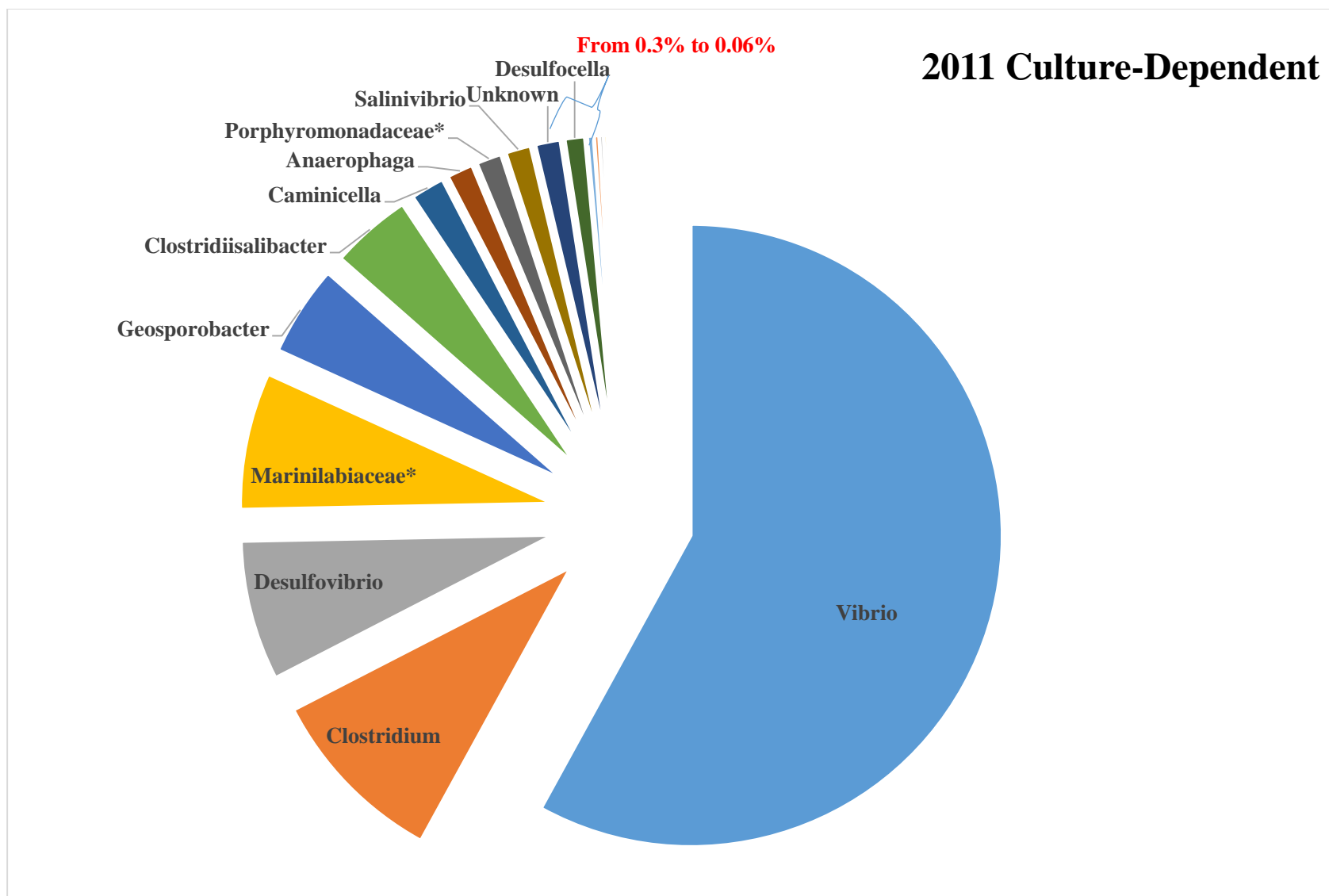


Figure 4c: Pie chart representation of the total taxa (>0.05%) of Solar Lake-W generated through using the 2011 Culture-Dependent approach, showing the dominance of the genus *Vibrio* sp. (54.8%).

SUPPLEMENTARY MATERIAL

Supplementary Table 1a: Saline Lake-RM' 2013 dataset

Supplementary Table 1b: Saline Lake-RM' 2011 Culture-Independent dataset

Supplementary Table 1c: Saline Lake-RM' 2011 Culture-Dependent dataset

**Supplementary Table 2a: Halophilic and Sulfur metabolizing taxa in Saline Lake-RM'
2013 Culture-Independent dataset**

**Supplementary Table 2b: Halophilic and Sulfur metabolizing taxa in Saline Lake-RM'
2011 Culture-Independent dataset**

**Supplementary Table 2c: Halophilic and Sulfur metabolizing taxa in Saline Lake-RM'
2011 Culture-Dependent dataset**

Supplementary Table 3a: Solar Lake-W' 2013 Culture-Independent dataset

Supplementary Table 3b: Solar Lake-W' 2011 Culture-Independent dataset

Supplementary Table 3c: Solar Lake-W' 2011 Culture-Dependent dataset

Supplementary Table 4a: Halophilic and Sulfur metabolizing taxa in Solar Lake-W' 2013 dataset

**Supplementary Table 4b: Halophilic and Sulfur metabolizing taxa in Solar Lake-W'
2011 Culture-Independent dataset**

**Supplementary Table 4c: Halophilic and Sulfur metabolizing taxa in Solar Lake-W' 2011
Culture-Dependent dataset**

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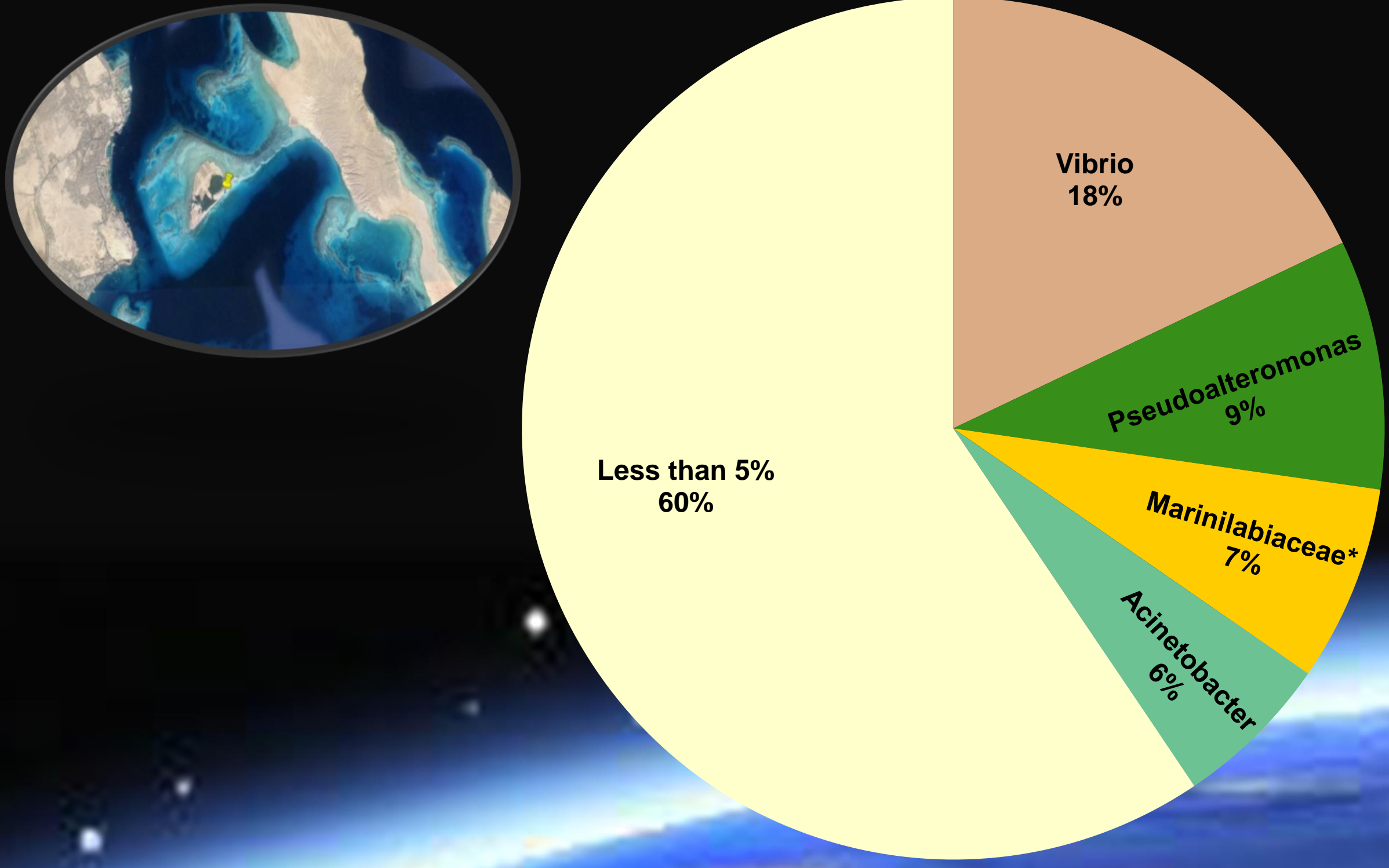
Conclusion

Red Sea is a complex environment where several rich, complex and fruitful ecosystems are engulfed within its environment. Dense mangrove forests, hard and soft corals, sponges, fisheries, invertebrates, and Red Sea waters and sediments are all considered to be complex ecosystems that act as a shelter for other organisms. The common organisms to be symbiont for all of these ecosystems are the microbial communities. These microbial communities support the nutrients for their host in addition to also act as an immune system for their host against other microbial communities. Also, the microbial-host' tissues relationship results in the production of several secondary metabolites that show pharmacological significance. On the other hand, Red Sea environment is being attacked chronically by several types of pollution, by which Red Sea different ecosystems are being dramatically affected. It is a matter of fact that almost all of the Red Sea ecosystems are being underestimated in research studies in regards to similar unique biodiverse ecosystems. More importantly hazardous effects of diverse sources of on the different living communities of the Red Sea are also being dramatically underestimated. However, the most studied ecosystems in terms of effects of pollution are the mangroves, corals and fishes, especially within the Egyptian borders. Up to our knowledge, this is the first study to describe bacterial communities harboring the coastal sediments of the Egyptian Red Sea proper and Gulf of Aqaba; in addition to shedding the light upon the relation between the communities structures and abundances and the source of pollution impacting the site. Ten different sites have been visited in 2011, 8 of them are coastal sites in addition to two lakes. Of these 8 coastal sites, four of them shown to be shipping ports for phosphate, Ilmenite and Aluminum, in addition to a mangrove forest near the aluminum port. That was in addition to a touristic-impacted site and a chronically oil polluted sites. For the two lakes, one of them is discovered and studied thoroughly since 1968 till 1998, while the other lake have never been studied before. Sediments samples collected from the coasts of these ten sites at a 0.5m depth using core sampler. Physical parameters including PH, temperature and dissolved oxygen have been measure for each site. Few grams of the sample have been inoculated on site in a marine broth. DNA was extracted from the ten cultured and subjected for high-throughput sequencing technology; and this approach was referred to as the 2011 Culture-Dependent approach. On the other hand, DNA was also extracted directly from the sediments' particles and subjected for high-throughput sequencing; and this approach has been referred to as

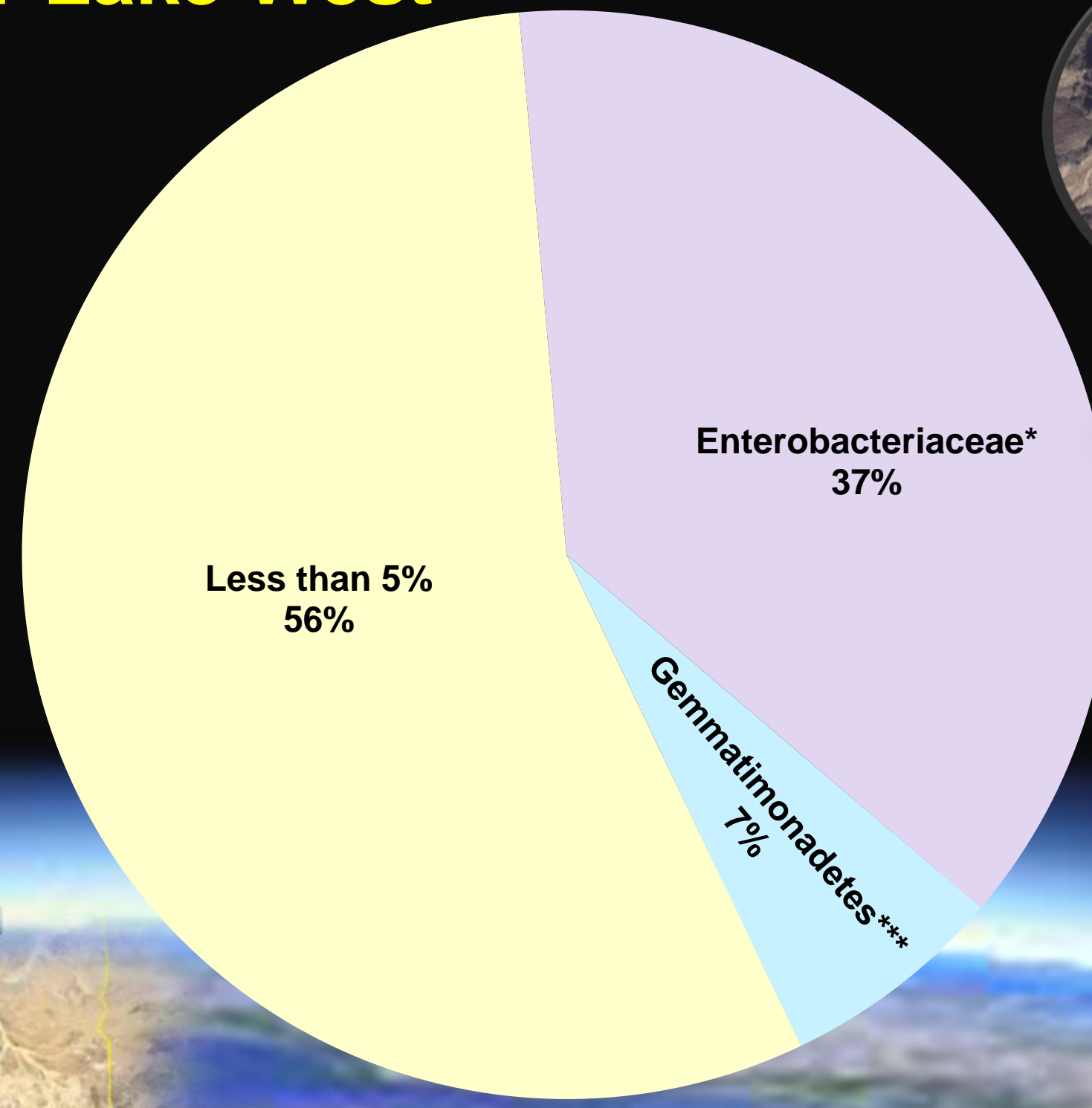
2011 culture-independent approach. The two lakes, due to their founded unique characteristics, have been visited another time in 2013. DNA has been extracted directly from the sediments particles of these samples and subjected again to high throughput sequencing; this approach has been referred to as 2013 Culture-Independent approach. Data generated through the three approaches 2011 Culture-Dependent, 2011 Culture-Independent and 2013 Culture-Independent have been subjected to deep descriptive analysis. Carbon, Hydrogen, Nitrogen and Sulfur profiling have been assessed for each site. In addition to metal and non-metal analysis where 29 elements have been measured. Petroleum hydrocarbon contents in each site has been evaluated through measuring motor and diesel oil concentrations in each site. Data retrieved from all types of analyses were used to thoroughly describe each site scheme and microbiota; and to try to correlate between the sites' impacts and the living microbiota. The first approach, 2011 Culture-Dependent have revealed three main findings. The first was the detection of potential pathogenic bacterial taxa in the analyzed sites. In addition to the detection of 28 different species of *Vibrio* across the coast; and this has been referred as "Egyptian marine *Vibriosis* phenomenon". Finally, through this approach, a unique pattern of oil degrading bacteria diversity were detected, as the types of degrading bacteria detected in the coastal sites were different from those detected in the lakes, which showed unique incidences to the lake. On the other hand, the 2011 Culture-Independent approach revealed the repetitive detection of 30 different bacterial taxa in the ten sites; and this pattern have been referred to in this study as 'Egyptian Red Sea Coastal Microbiome'. Despite the reported "Egyptian Red Sea Coastal Microbiome", individual sites showed unique evolution of their microbial communities based on minor intrinsic and imposed variation per sites. A second finding of this approach is the fact that hydrocarbon biodegrading bacteria predominated the majority of the Red Sea sites; particularly in two ports it reached up to 76% of the total identified genera. On the other hand, sulfate reducing and oxidizing bacteria dominated the two lakes, on the expense of other hydrocarbon metabolizers'. Almost all of the findings and readings of the two previous approaches revealed unique patterns for the two lakes, which also have been more clarified through comparing the two previous approaches along with the 2013 Culture-Independent dataset. Interestingly, the three datasets showed that most of the bacteria that harbor the two lakes are halophilic, anaerobia, anaerobic halophiles and sulfate reducing bacteria. Generally, our data reflects the environmental impacts of each site. This drives the attention to the dramatic status of the Egyptian Red Sea coast in the shipping ports and the chronically oil polluted sites, while it

droves the attention of the ecological significance of the two lakes for their pattern in sulfur metabolism under extreme conditions. Instant conservational actions are needed to help improving or at least stoping the deteriorating status of the Egyptian Red Sea coast.

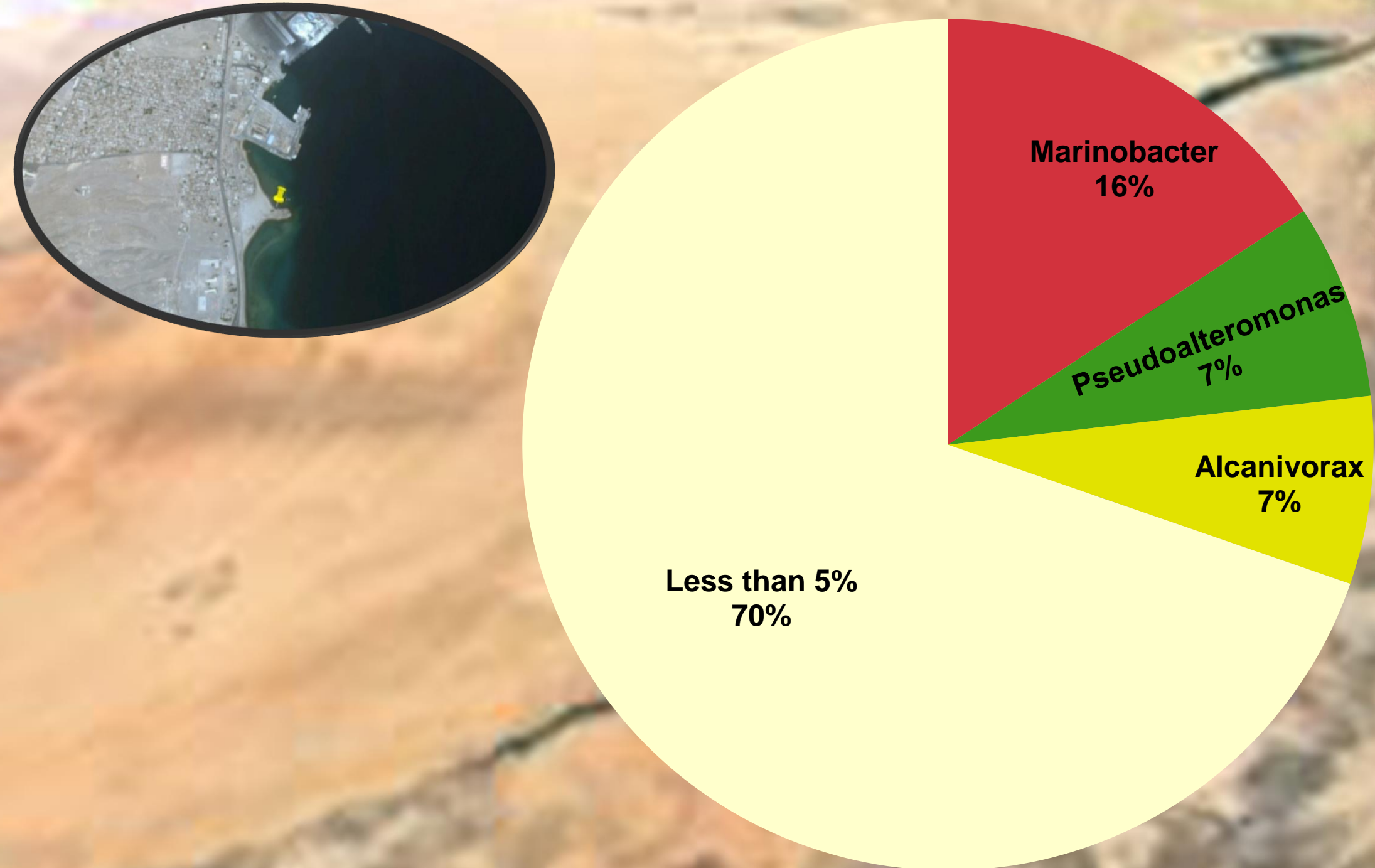
5- Abu Monkar Island



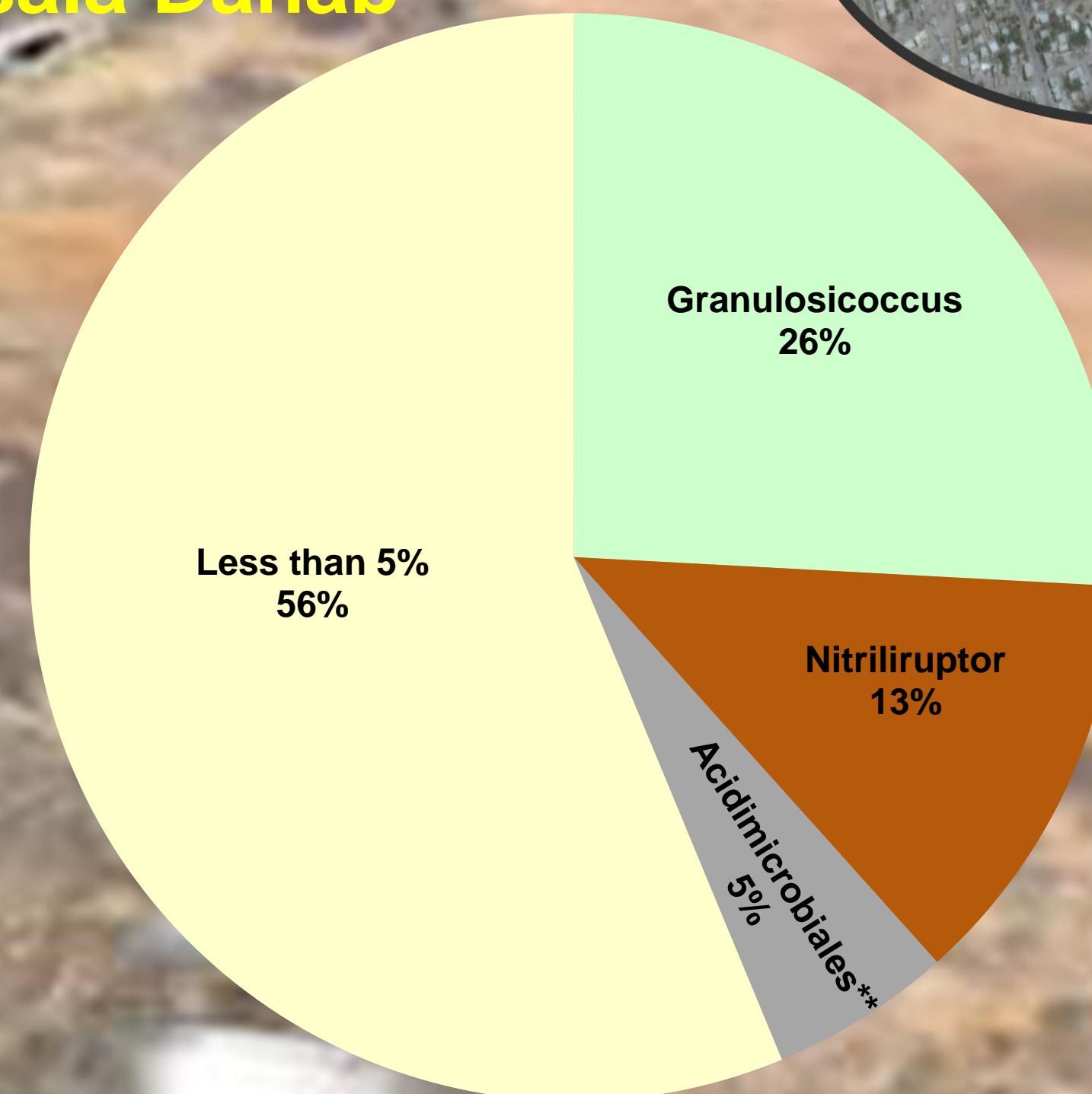
1- Solar Lake West



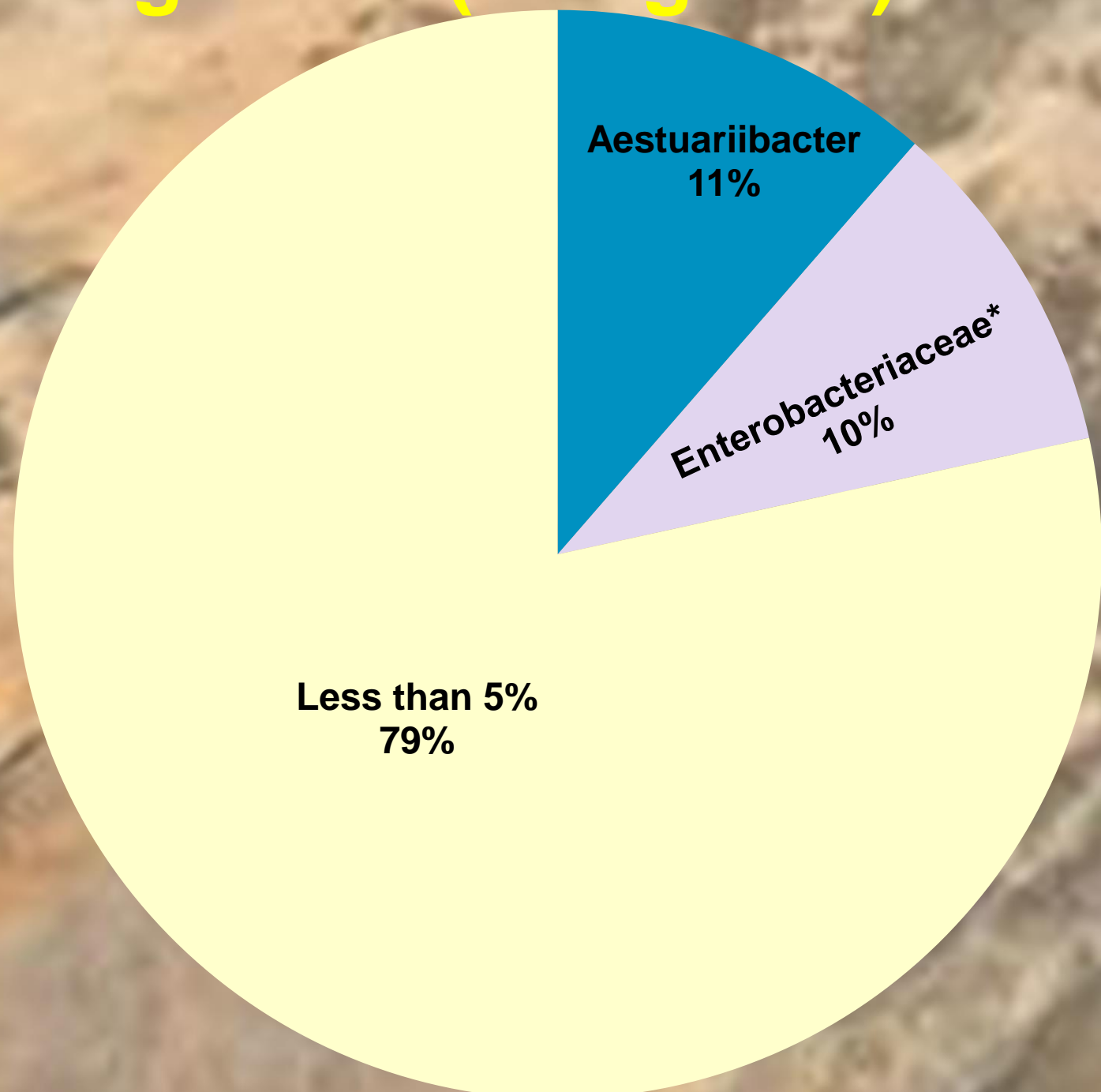
6- Safaga Aluminum Port



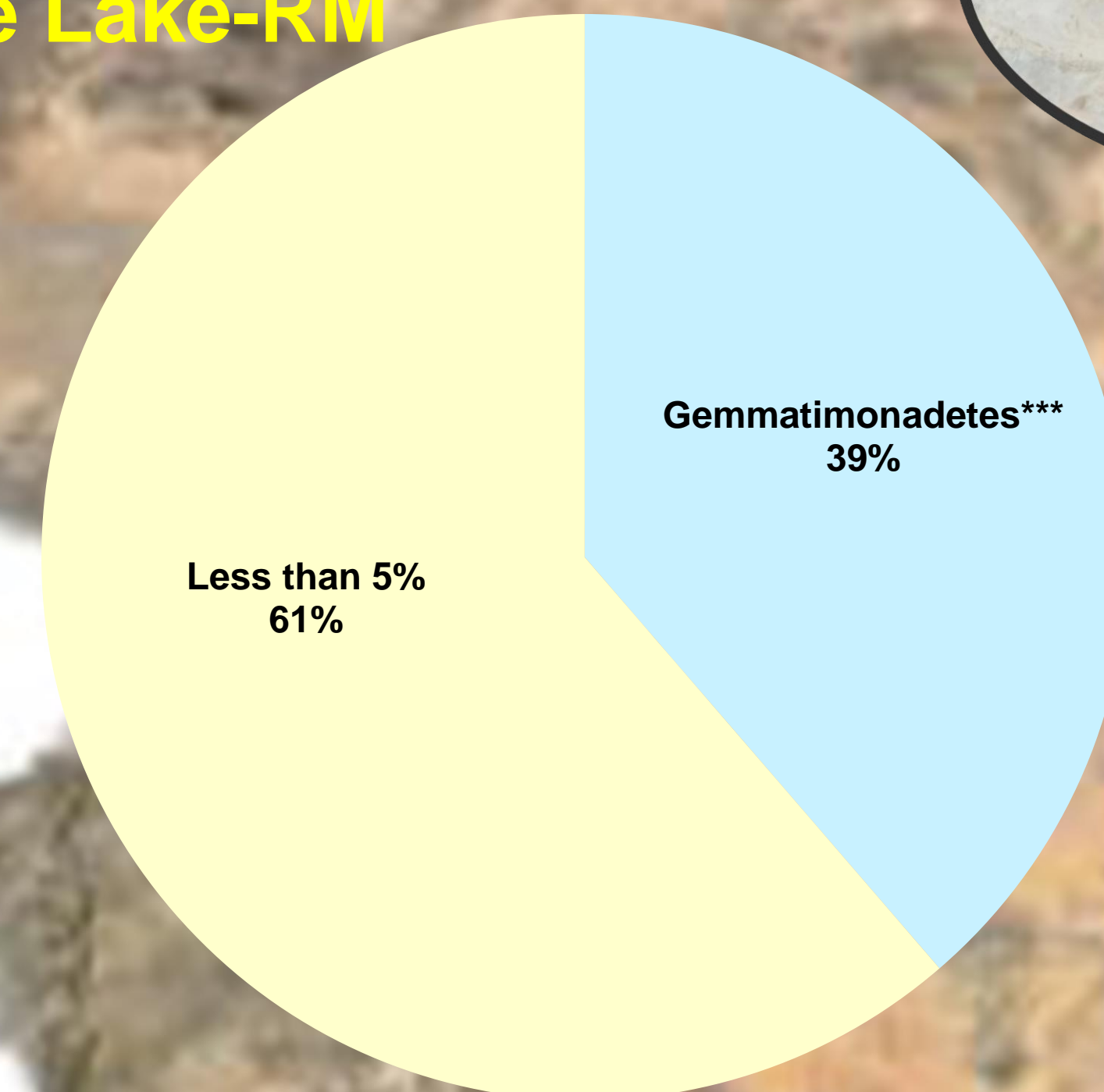
2- Assala Dahab



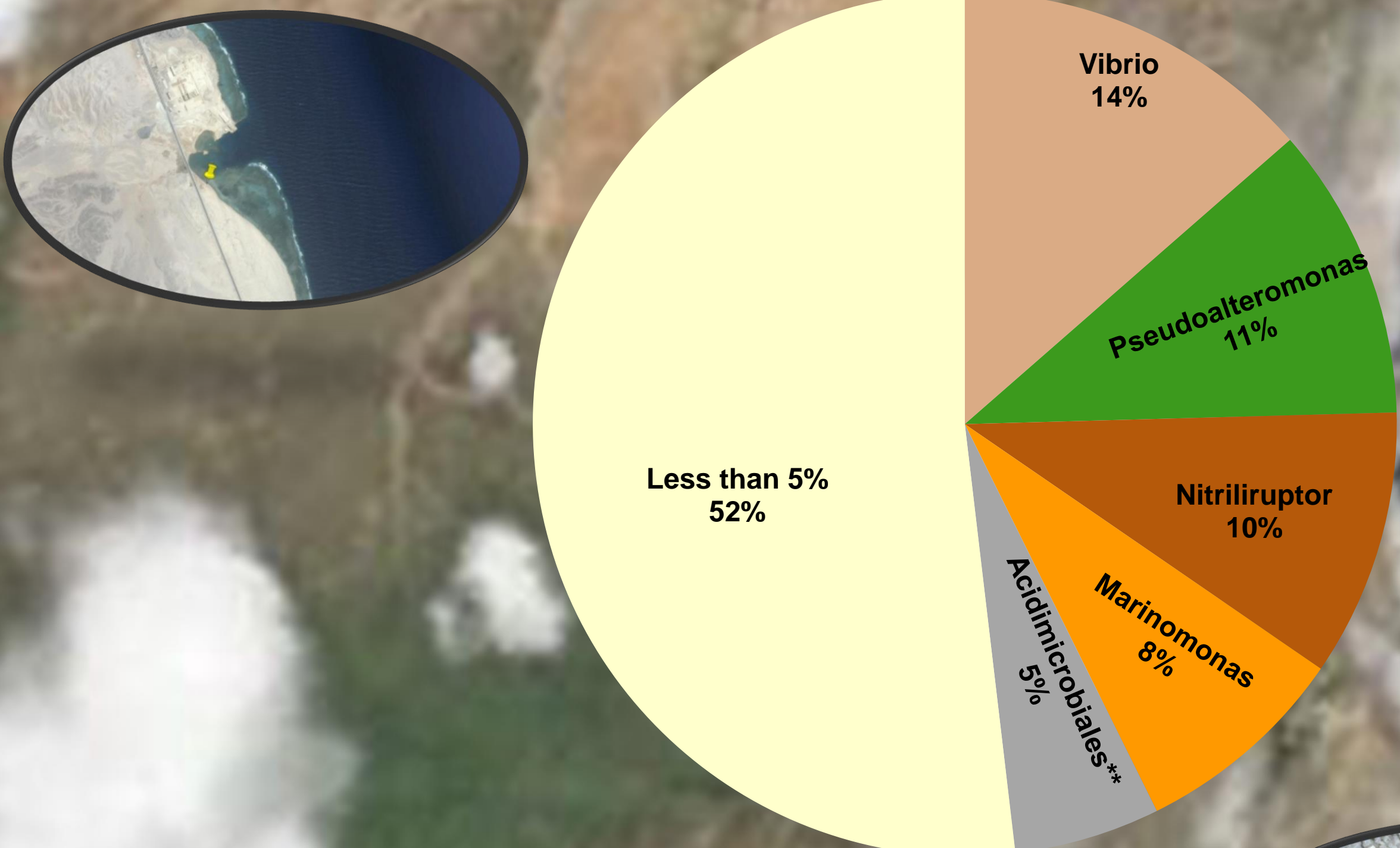
7- Safaga Port (Mangrove)



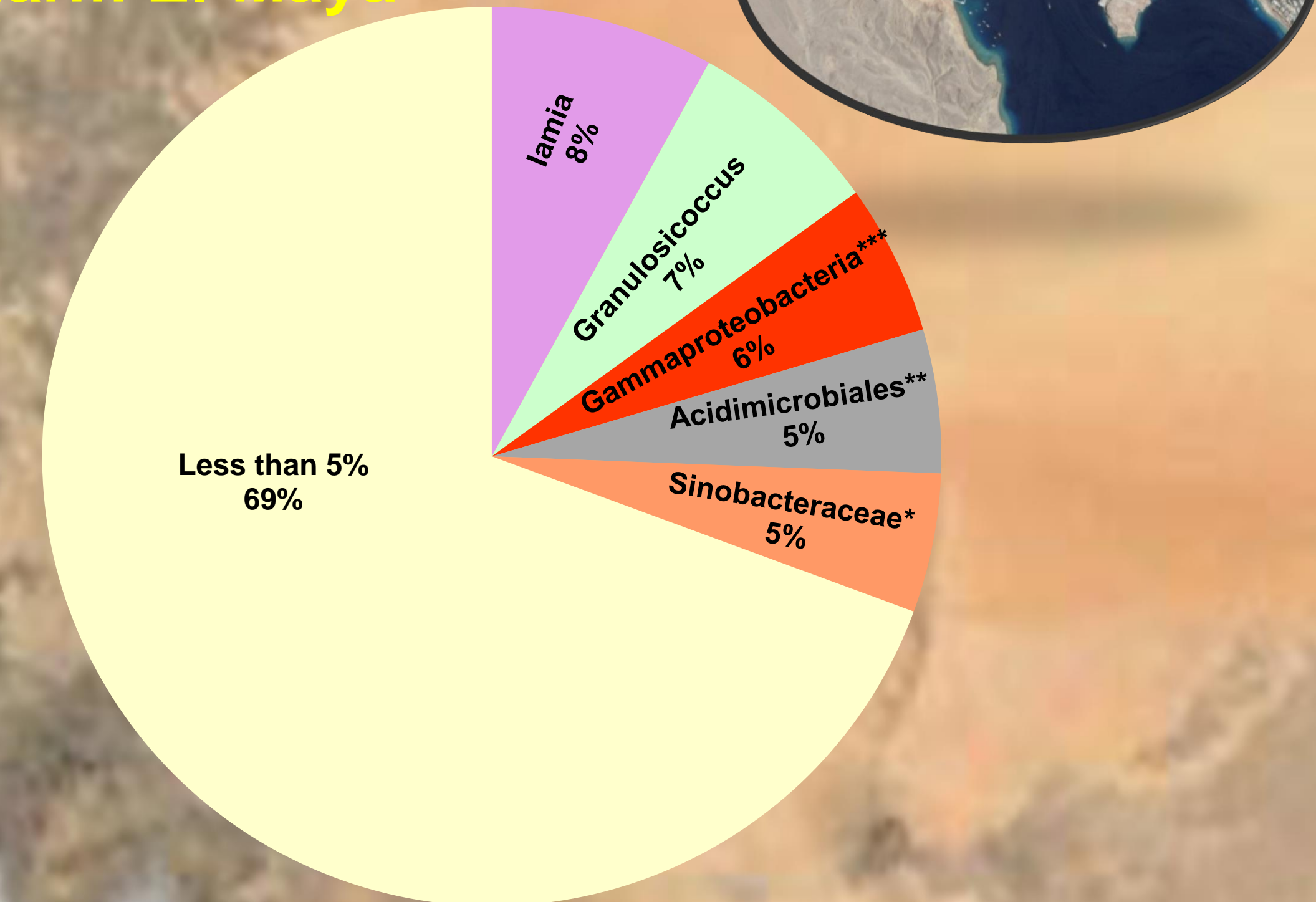
3- Saline Lake-RM



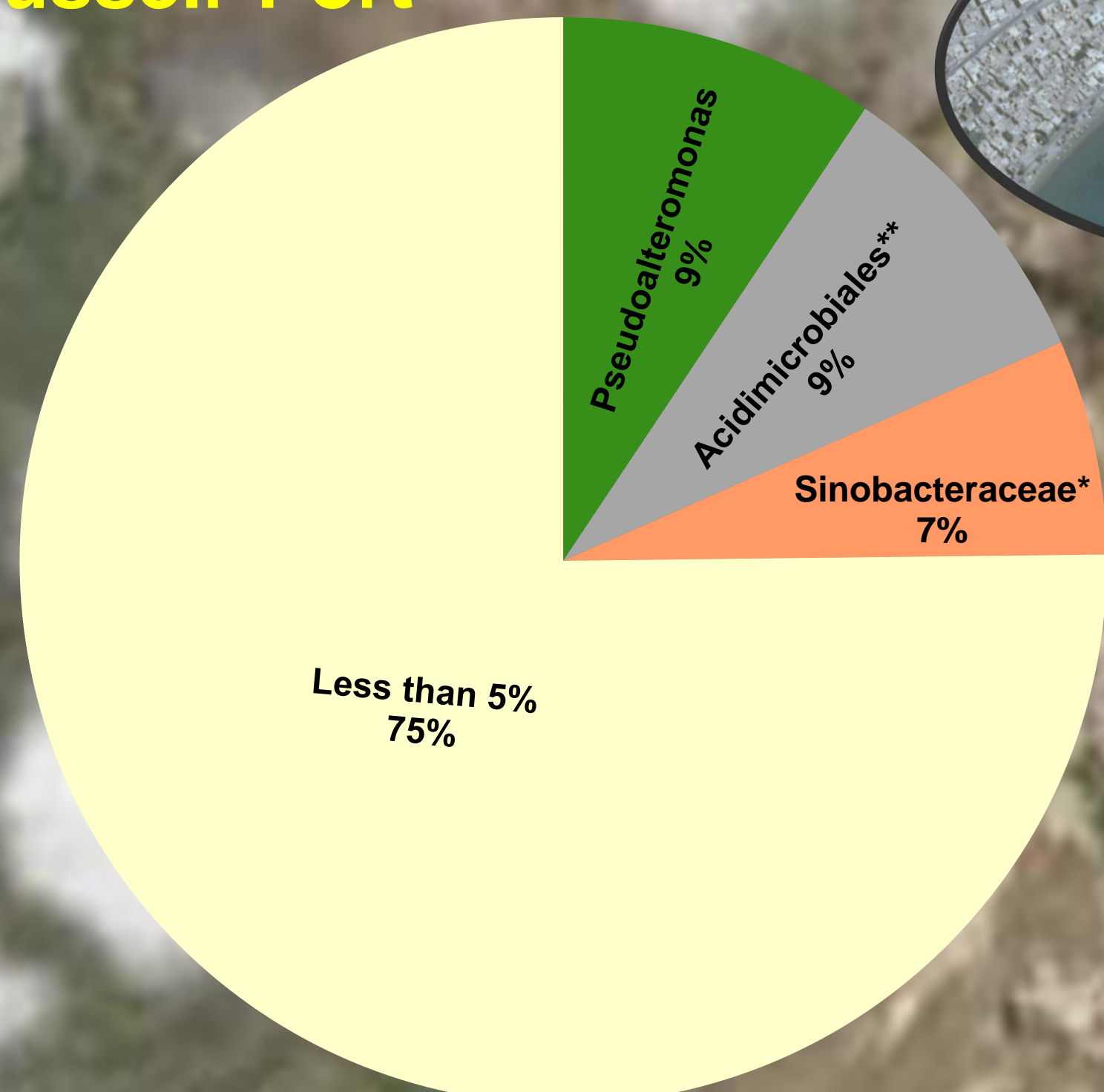
8- Hamrawein Port



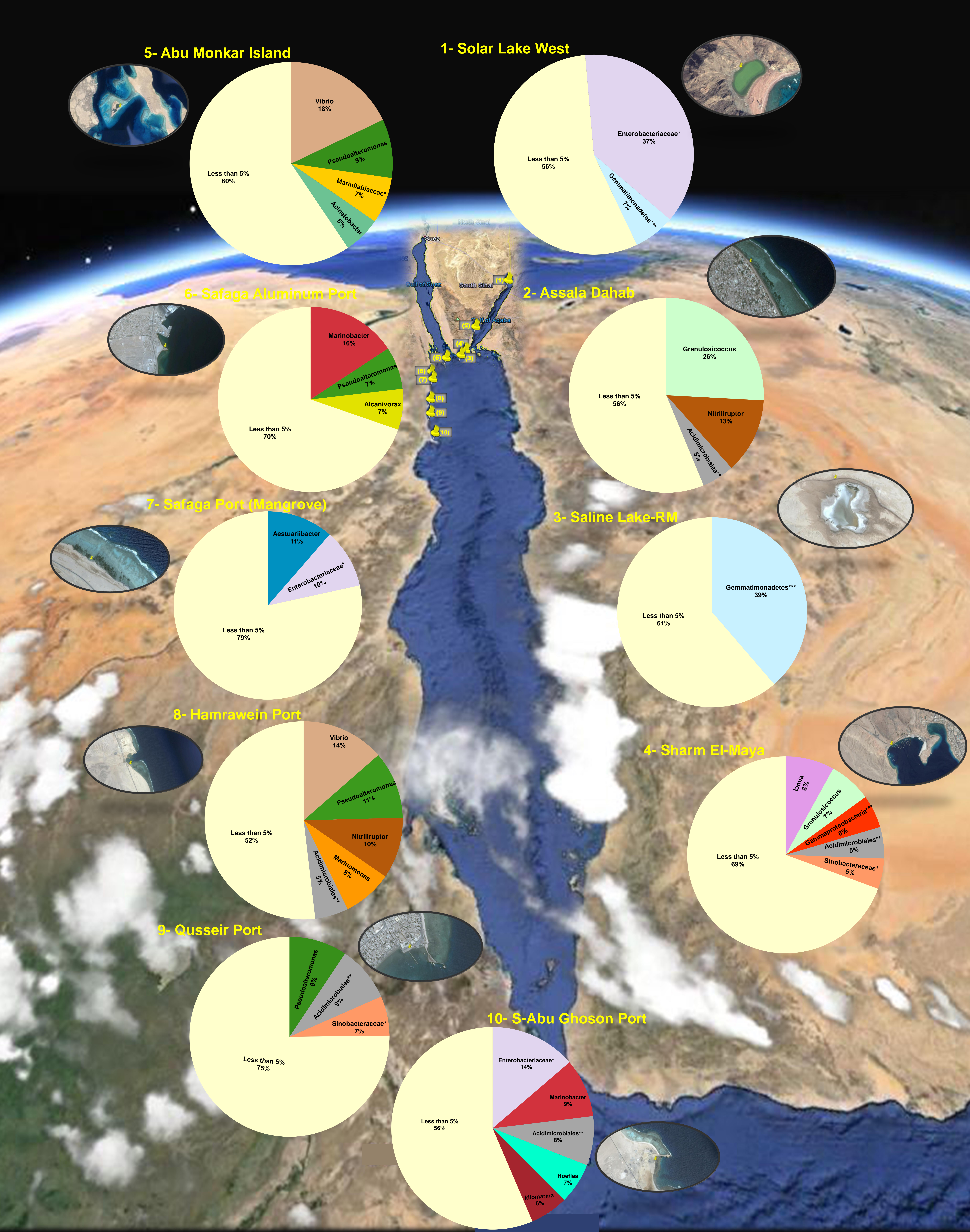
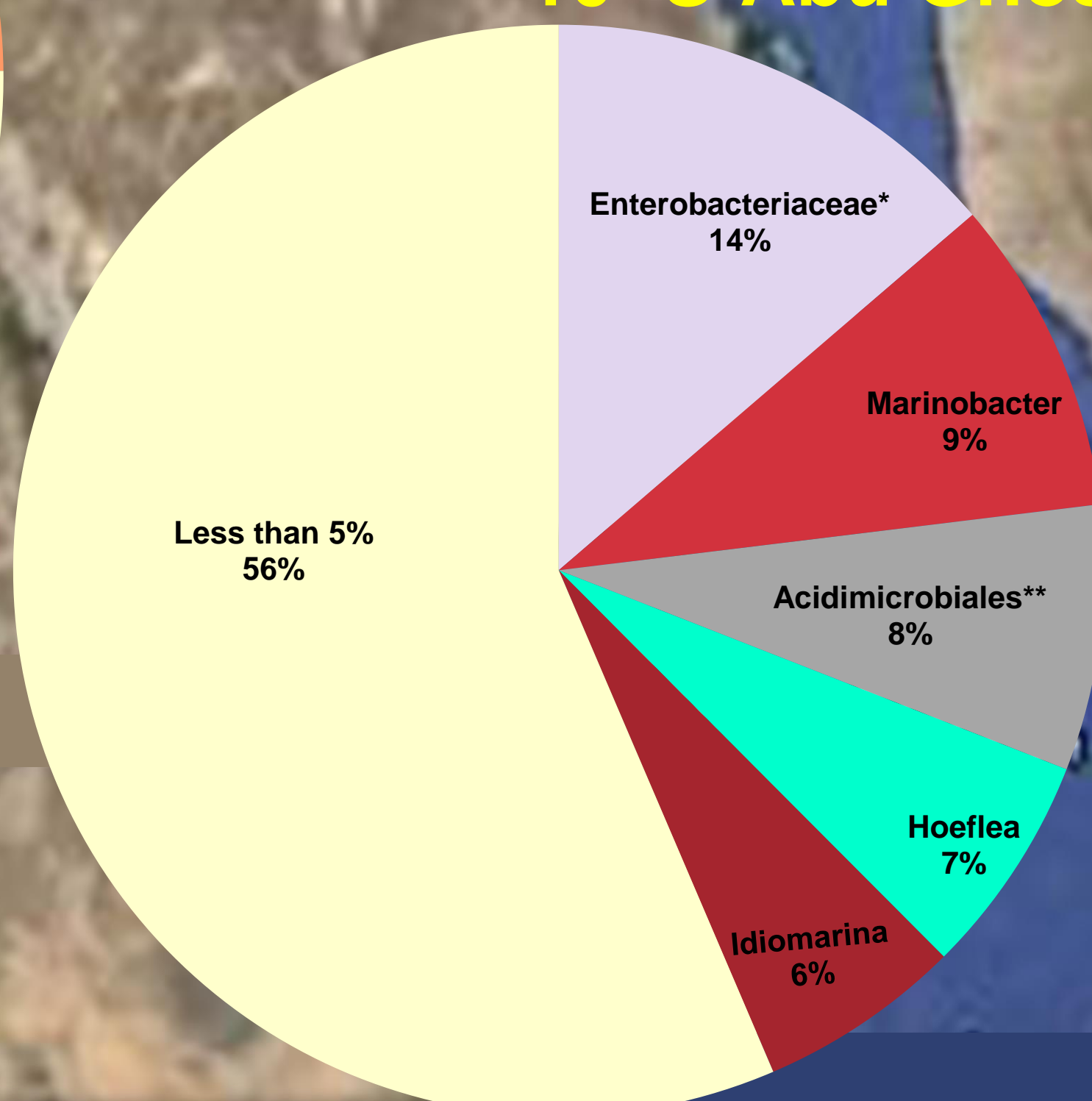
4- Sharm El-Maya



9- Qusseir Port



10- S-Abu Ghoson Port



	Abu Monkar Island	Assala Dahab	Hamawarein Port	Safaga Port (Mangrove)	Qusseir Port	Safaga Aluminum Port	Salne Lake-R	S-Abu Ghoson Port	Sharm El-Maya	Solar Lake-W
Bacteria;phylum_NA;class_NA;orderx_NA;family_NA;genus_NA;species_NA	500	8	43	32	35	56	497	53	17	66
Bacteria;Proteobacteria;Gammaproteobacteria;Xanthomonadales;Sinobacteraceae;genus_NA;species_NA	345	318	276	388	788	308	2	521	808	1
Unknown;phylum_NA;class_NA;orderx_NA;family_NA;genus_NA;species_NA	192	145	174	166	133	109	245	159	223	317
Bacteria;Actinobacteria;Actinobacteria;Acidimicrobiales;family_NA;genus_NA;species_NA	163	815	790	319	1114	109	10	934	834	7
Bacteria;Proteobacteria;Gammaproteobacteria;orderx_NA;family_NA;genus_NA;species_NA	105	497	288	222	239	184	112	144	876	38
Bacteria;Proteobacteria;Deltaproteobacteria;orderx_NA;family_NA;genus_NA;species_NA	104	18	43	120	60	35	165	400	105	43
Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacteriales;Enterobacteriaceae;genus_NA;species_NA	86	36	16	1228	2	19	3	1625	3	4751
Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Burkholderiaceae;Ralstonia;species_NA	69	249	42	41	49	32	11	45	10	67
Bacteria;Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;genus_NA;species_NA	66	242	244	144	556	71	361	218	249	68
Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;family_NA;genus_NA;species_NA	35	31	5	59	25	7	150	1	28	12
Bacteria;Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Rhodopirellula;species_NA	32	221	47	116	213	48	10	187	233	1
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;genus_NA;species_NA	20	168	97	58	83	90	67	71	265	7
Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Flammeovirgaceae;genus_NA;species_NA	20	100	14	13	67	20	18	14	11	2
Bacteria;Acidobacteria;Holophagae;orderx_NA;family_NA;genus_NA;species_NA	18	211	219	202	490	11	18	109	281	9
Bacteria;Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Blastopirellula;species_NA	17	254	28	32	200	21	8	134	247	6
Bacteria;Planctomycetes;Phycisphaerae;orderx_NA;family_NA;genus_NA;species_NA	14	2	5	27	23	31	53	14	9	30
Bacteria;Chloroflexi;class_NA;orderx_NA;family_NA;genus_NA;species_NA	13	4	79	55	106	11	2	30	2	2
Bacteria;Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;genus_NA;species_NA	11	27	10	93	95	5	42	212	5	5
Bacteria;Planctomycetes;Phycisphaerae;Phycisphaerales;Phycisphaerae;genus_NA;species_NA	11	15	27	27	16	25	61	54	94	9
Bacteria;Proteobacteria;Alphaproteobacteria;orderx_NA;family_NA;genus_NA;species_NA	9	58	18	19	9	38	31	15	127	14
Bacteria;Proteobacteria;Gammaproteobacteria;Chromatiales;family_NA;genus_NA;species_NA	6	571	76	27	103	14	132	24	184	62
Bacteria;Actinobacteria;Actinobacteria;Solirubrobacterales;Conexibacteraceae;Conexibacter;species_NA	6	90	31	8	14	10	687	6	71	1
Bacteria;Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Planctomyces;species_NA	5	149	32	30	90	23	60	82	90	12
Bacteria;Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Pirellula;species_NA	5	19	22	15	66	9	25	15	41	17
Bacteria;Proteobacteria;Alphaproteobacteria;Rickettsiales;family_NA;genus_NA;species_NA	5	3	46	1	1	35	1	3	56	1
Bacteria;Chloroflexi;Caldilineae;Caldilineales;family_NA;genus_NA;species_NA	4	160	73	101	191	40	9	18	368	21
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Roseovarius;species_NA	4	15	20	15	3	15	1	39	5	2
Bacteria;Proteobacteria;Gammaproteobacteria;Chromatiales;Chromatiaceae;Nitrosococcus;species_NA	3	68	21	79	30	30	18	45	95	13
Bacteria;Proteobacteria;Gammaproteobacteria;Legionellales;Coxiellaceae;Coxiella;species_NA	3	31	13	12	22	35	7	12	37	6
Bacteria;Planctomycetes;class_NA;orderx_NA;family_NA;genus_NA;species_NA	2	38	6	2	2	3	34	28	7	15
Total "Egyptian Red Sea Microbiome"	1873	4563	2805	3651	4825	1444	2840	5212	5381	5605
Bacteria;Firmicutes;Bacilli;Lactobacillales;Carnobacteriaceae;Alkalibacterium;species_NA	132	0	0	0	0	0	0	0	0	0
Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;Marinilabiaceae;Alkaliflexus;species_NA	41	0	0	0	0	0	0	0	0	0
Bacteria;Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Sphingopyxis;litoris	21	0	0	0	0	0	0	0	0	0
Bacteria;Verrucomicrobia;Opitutae;Puniceococcales;Puniceococcaceae;Pelagicoccus;species_NA	4	0	0	0	0	0	0	0	0	0
	198									
Bacteria;Actinobacteria;Actinobacteria;Actinomycetales;Nocardiodaceae;Marmoricola;species_NA	0	56	0	0	0	0	0	0	0	0
Bacteria;Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Haloferula;phyci	0	32	0	0	0	0	0	0	0	0
Bacteria;Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Haloferula;species_NA	0	26	0	0	0	0	0	0	0	0
Bacteria;Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Luteolibacter;species_NA	0	24	0	0	0	0	0	0	0	0
Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae;Deltia;species_NA	0	14	0	0	0	0	0	0	0	0
	152									
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinomonas;basaltis	0	0	1071	0	0	0	0	0	0	0
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinomonas;aquimarina	0	0	25	0	0	0	0	0	0	0
Bacteria;Firmicutes;Clostridia;Clostridiales;Veillonellaceae;Dialister;pneumosintes	0	0	15	0	0	0	0	0	0	0
Bacteria;Proteobacteria;Gammaproteobacteria;Pasteurellales;Pasteurellaceae;Aggregatibacter;species_NA	0	0	15	0	0	0	0	0	0	0
			1126							
Bacteria;Proteobacteria;Deltaproteobacteria;Desulfobacteriales;Desulfobacteraceae;Desulfobacterium;vacuolatum	0	0	0	46	0	0	0	0	0	0
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Neptuniibacter ;species_NA	0	0	0	29	0	0	0	0	0	0
Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Colwelliaceae;Thalassomonas;loyana	0	0	0	22	0	0	0	0	0	0
				97						
Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Psychromonadaceae;Psychromonas;arctica	0	0	0	0	23	0	0	0	0	0
Bacteria;Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;Actibacter;species_NA	0	0	0	0	13	0	0	0	0	0
Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Idiomarinaceae;genus_NA;species_NA	0	0	0	0	13	0	0	0	0	0
					49					
Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Congregibacter;litoralis	0	0	0	0	0	91	0	0	0	0
Bacteria;Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;Tenacibaculum;species_NA	0	0	0	0	0	53	0	0	0	0
Bacteria;Proteobacteria;Deltaproteobacteria;Desulfobacteriales;Desulfobacteraceae;Desulfofrigus;species_NA	0	0	0	0	0	22	0	0	0	0
Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Alteromonas;genovensis	0	0	0	0	0	21	0	0	0	0
Bacteria;Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;Psychroserpens;mesophilus	0	0	0	0	0	18	0	0	0	0
Bacteria;Firmicutes;Clostridia;Clostridiales;Unassigned;Thermaerobacter;subterraneus	0	0	0	0	0	17	0	0	0	0
Bacteria;Verrucomicrobia;Opitutae;Puniceococcales;Puniceococcaceae;Lentimonas;marisflavi	0	0	0	0	0	14	0	0	0	0
						236				
Bacteria;Firmicutes;Clostridia;Halanaerobiales;Halobacteroidaceae;genus_NA;species_NA	0	0	0	0	0	0	550	0	0	0
Bacteria;Firmicutes;Clostridia;Halanaerobiales;Halobacteroidaceae;Orenia;marismortui	0	0	0	0	0	0	284	0	0	0
Bacteria;Firmicutes;Clostridia;Clostridiales;Clostridiaceae;Caloranaerobacter;species_NA	0	0	0	0	0	0	35	0	0	0
Bacteria;Firmicutes;Bacilli;Bacillales;Thermoactinomyetaceae;Planifilum;species_NA	0	0	0	0	0	0	23	0	0	0
Bacteria;Proteobacteria;Gammaproteobacteria;Methylococcales;Methylococcaceae;Methylohalobius;crimeensis	0	0	0	0	0	0	23	0	0	0
Bacteria;Firmicutes;Clostridia;Halanaerobiales;Halobacteroidaceae;Halobacteroides;halobius	0	0	0	0	0	0	22	0	0	0
Bacteria;Firmicutes;Clostridia;Halanaerobiales;Halobacteroidaceae;Halanaerobacter;species_NA	0	0	0	0	0	0	20	0	0	0
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Halomonadaceae;Halovibrio;denitrificans	0	0	0	0	0	0	18	0	0	0
							975			
Bacteria;Proteobacteria;Gammaproteobacteria;Thiotrichales;Piscirickettsiaceae;Methylophaga;marina	0	0	0	0	0	0	0	31	0	0

Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Idiomarinaeae;Pseudidiomarina;sediminum	0	0	0	0	0	0	0	24	0	0
								55		
Bacteria;Proteobacteria;Alphaproteobacteria;Sphingomonadales;Erythrobacteraceae;Altererythrobacter;species_NA	0	0	0	0	0	0	0	0	139	0
Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaeae;Marinimicrobium;species_NA	0	0	0	0	0	0	0	0	18	0
									157	
Bacteria;Proteobacteria;Gammaproteobacteria;Chromatiales;Halothiobacillaceae;Halothiobacillus;species_NA	0	0	0	0	0	0	0	0	0	98
Bacteria;Firmicutes;Clostridia;Halanaerobiales;Halanaerobiaceae;Halocella;cellulosilytica	0	0	0	0	0	0	0	0	0	22
										120
Bacteria;Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;Chloroacidobacterium;species_NA	0	0	0	0	0	0	0	3	90	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Loktanella;hongkongensis	0	0	0	0	0	0	0	1	17	0
Bacteria;Proteobacteria;Gammaproteobacteria;Unassigned;Unassigned;Thiohalorhabdus;species_NA	0	0	0	0	0	0	512	0	0	24
Bacteria;Firmicutes;Clostridia;Halanaerobiales;Halobacteroidaceae;Orenia;species_NA	0	0	0	0	0	0	111	0	0	2
Bacteria;Planctomycetes;Unassigned;Unassigned;Unassigned;Brocadia;anamoxidans	0	0	0	0	0	0	78	0	0	31
Bacteria;Firmicutes;Clostridia;Halanaerobiales;Halobacteroidaceae;Halobacteroides;species_NA	0	0	0	0	0	0	67	0	0	2
Bacteria;Firmicutes;Clostridia;Halanaerobiales;Halanaerobiaceae;Halanaerobium;species_NA	0	0	0	0	0	0	63	0	0	56
Bacteria;Firmicutes;Clostridia;Thermoanaerobacteriales;Thermoanaerobacteraceae;Thermacetogenium;species_NA	0	0	0	0	0	0	37	0	0	2
Bacteria;WS1;class_NA;orderx_NA;family_NA;genus_NA;species_NA	0	0	0	0	0	0	23	0	0	13
Bacteria;Fibrobacteres;Fibrobacteria;Fibrobacteriales;family_NA;genus_NA;species_NA	0	0	0	0	0	0	22	0	0	16
Bacteria;Lentisphaerae;Lentisphaeria;Victivallales;Victivallaceae;genus_NA;species_NA	0	0	0	0	0	0	22	0	0	10
Bacteria;Proteobacteria;Alphaproteobacteria;Sphingomonadales;Erythrobacteraceae;Erythrobacter;litoralis	0	0	0	0	0	0	2	0	88	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhodobiaceae;Parvibaculum;species_NA	0	0	0	0	0	0	1	22	0	1
Bacteria;Proteobacteria;Gammaproteobacteria;Salinisphaerales;Salinisphaeraceae;Salinisphaera;species_NA	0	0	0	0	0	0	1	15	48	0
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Hahellaceae;Hahella;species_NA	0	0	0	0	0	24	0	0	11	0
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Alcanivoracaceae;Alcanivorax;indicus	0	0	0	0	0	11	0	0	86	0
Bacteria;Spirochaetes;Spirochaetes;Spirochaetales;Leptospiraceae;genus_NA;species_NA	0	0	0	0	0	8	0	0	33	3
Bacteria;Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;Psychroflexus;species_NA	0	0	0	0	0	21	2	0	0	411
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oleiphilaceae;Oleiphilus;messinensis	0	0	0	1	0	0	0	0	33	0
Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Idiomarinaeae;Idiomarina;species_NA	0	0	0	0	1	0	0	716	0	338
Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Pseudoalteromonadaeae;Algicola;species_NA	0	0	0	0	348	0	0	28	5	0
Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Idiomarinaeae;Pseudidiomarina;species_NA	0	0	0	0	19	0	0	5	2	0
Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Flammeovirgaceae;Marinoscillum;species_NA	0	0	0	3	0	2	0	0	2	2
Bacteria;Proteobacteria;Gammaproteobacteria;Vibrionales;Vibrionaceae;Aliivibrio;species_NA	0	0	0	0	47	1	0	0	0	0
Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Moraxellaceae;Psychrobacter;species_NA	0	0	0	0	18	38	0	0	0	0
Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Psychromonadaceae;Psychromonas;species_NA	0	0	0	0	6	81	0	0	0	0
Bacteria;Proteobacteria;Gammaproteobacteria;Thiotrichales;Piscirickettsiaceae;Thiomicrospira;chilensis	0	0	0	0	6	24	0	0	0	0
Bacteria;Proteobacteria;Gammaproteobacteria;Thiotrichales;Piscirickettsiaceae;Thiomicrospira;species_NA	0	0	0	1	28	0	0	0	0	191
Bacteria;Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;Gilvibacter;species_NA	0	0	0	0	2	7	0	0	77	0
Bacteria;Deferribacteres;Deferribacteres;Deferribacterales;family_NA;genus_NA;species_NA	0	0	0	0	1	1	49	6	10	16
Bacteria;Proteobacteria;Deltaproteobacteria;Myxococcales;Nannocystaceae;genus_NA;species_NA	0	0	0	1	0	0	0	0	33	0
Bacteria;Tenericutes;Mollicutes;Haloplasmales;Haloplasmaeae;Haloplasma;contractile	0	0	0	2	0	0	72	0	0	0
Bacteria;Firmicutes;Clostridia;Halanaerobiales;Halanaerobiaceae;Halocella;species_NA	0	0	0	6	0	0	33	0	0	8
Bacteria;Planctomycetes;Phycisphaerae;Phycisphaerales;family_NA;genus_NA;species_NA	0	0	0	2	0	0	19	0	0	8
Bacteria;OP1;class_NA;orderx_NA;family_NA;genus_NA;species_NA	0	0	0	0	1	0	0	377	0	530
Bacteria;Spirochaetes;Spirochaetes;Spirochaetales;Spirochaetaceae;genus_NA;species_NA	0	0	0	4	0	0	4	0	5	25
Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Hyphomicrobiaceae;Prosthecomicrobium;species_NA	0	0	0	21	0	0	1	1	1	0
Bacteria;Proteobacteria;Deltaproteobacteria;Syntrophobacteriales;Syntrophaceae;Desulfomonile;limimaris	0	0	0	2	0	14	0	0	0	0
Bacteria;Fusobacteria;Fusobacteria;orderx_NA;family_NA;genus_NA;species_NA	0	0	0	4	0	19	0	0	0	25
Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Chitinophagaceae;Balneola;species_NA	0	0	0	19	0	1	0	0	26	2
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;Rhodovibrio;species_NA	0	0	0	2	0	13	50	0	0	7
Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaeae;Aestuariusbacter;saalexigens	0	0	0	1374	4	0	0	0	0	0
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Saccharospirillaceae;Saccharospirillum;species_NA	0	0	0	3	27	0	0	4	0	1
Bacteria;Gemmatimonadetes;Gemmatimonadetes;Gemmatimonadales;Gemmatimonadaeae;Gemmatimonas;species_NA	0	0	0	4	9	0	0	21	7	5
Bacteria;Tenericutes;Mollicutes;Haloplasmales;Haloplasmaeae;Haloplasma;species_NA	0	0	0	13	4	0	75	0	0	0
Bacteria;Proteobacteria;Deltaproteobacteria;Desulfobacteriales;Desulfobulbaceae;Desulfurivibrio;species_NA	0	0	0	5	4	0	75	0	0	32
Bacteria;Proteobacteria;Deltaproteobacteria;Myxococcales;Polyangiaceae;genus_NA;species_NA	0	0	0	21	7	0	11	0	8	0
Bacteria;Planctomycetes;Phycisphaerae;Phycisphaerales;Phycisphaeraceae;Phycisphaera;species_NA	0	0	0	3	4	0	140	39	3	11
Bacteria;Actinobacteria;Actinobacteria;Actinomycetales;Propionibacteriaceae;Jiangella;species_NA	0	0	0	16	43	14	0	0	0	0
Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Psychromonadaceae;Psychromonas;agarivorans	0	0	0	7	7	28	0	0	0	0
Bacteria;Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;Lutibacter;species_NA	0	0	0	3	1	90	0	0	0	0
Bacteria;Proteobacteria;Alphaproteobacteria;Caulobacteriales;Hyphomonadaceae;Hyphomonas;Henriciella	0	0	0	8	2	7	0	0	59	0
Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;genus_NA;species_NA	0	0	0	13	12	25	8	0	0	0
Bacteria;Proteobacteria;Deltaproteobacteria;Desulfobacteriales;Desulfobacteraceae;Desulfonema;species_NA	0	0	0	43	9	21	1	0	0	6
Bacteria;TA06;class_NA;orderx_NA;family_NA;genus_NA;species_NA	0	0	0	29	3	29	1	0	0	82
Bacteria;Nitrospirae;Nitrospira;Nitrospirales;Nitrospiraceae;genus_NA;species_NA	0	0	0	25	1	37	122	0	0	203
Bacteria;Proteobacteria;Deltaproteobacteria;Desulfobacteriales;Desulfobulbaceae;Desulfobulbus;species_NA	0	0	0	12	73	17	37	0	0	12
Bacteria;Chlorobi;Chlorobia;Chlorobiales;family_NA;genus_NA;species_NA	0	0	0	6	27	9	13	0	0	9
Bacteria;OP8;class_NA;orderx_NA;family_NA;genus_NA;species_NA	0	0	0	3	7	33	9	0	0	30
Bacteria;Nitrospirae;Nitrospira;Nitrospirales;family_NA;genus_NA;species_NA	0	0	0	1	4	7	181	0	0	8
Bacteria;Proteobacteria;Epsilonproteobacteria;Campylobacteriales;Helicobacteraceae;Sulfurimonas;species_NA	0	0	0	7	10	294	1	0	2	0
Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Flammeovirgaceae;Reichenbachiella;species_NA	0	0	0	2	21	9	8	0	1	4
Bacteria;Proteobacteria;Deltaproteobacteria;Syntrophobacteriales;Syntrophobacteraceae;genus_NA;species_NA	0	0	0	51	2	51	7	1	0	15
Bacteria;Firmicutes;Clostridia;Clostridiales;family_NA;genus_NA;species_NA	0	0	0	1	1	4	16	2	0	5
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Alcanivoracaceae;Alcanivorax;species_NA	0	0	0	52	4	799	2	5	527	0
Bacteria;Firmicutes;Bacilli;Lactobacillales;Streptococcaceae;Streptococcus;species_NA	0	0	147	0	0	0	0	0	0	1

Bacteria;Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;Polaribacter;dokdonensis	0	0	6	0	0	13	0	0	0	0
Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;genus_NA;species_NA	0	0	4	0	0	17	0	0	0	0
Bacteria;Proteobacteria;Gammaproteobacteria;Legionellales;Coxiellaceae;genus_NA;species_NA	0	0	15	0	0	3	0	3	1	0
Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Alteromonas;hispanica	0	0	4	0	5	0	0	0	0	0
Bacteria;Proteobacteria;Gammaproteobacteria;Thiotrichales;Piscirickettsiaceae;Methylophaga;species_NA	0	0	110	0	4	0	0	49	0	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Thalassobacter;species_NA	0	0	98	0	2	0	0	45	0	0
Bacteria;Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;Persicivirga;species_NA	0	0	17	0	4	32	0	0	0	0
Bacteria;Firmicutes;Clostridia;Clostridiales;Peptostreptococcaceae;Fusibacter;species_NA	0	0	162	0	134	195	0	0	0	31
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Marivita;species_NA	0	0	1	0	1	30	0	0	20	0
Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Unassigned;Melitea	0	0	1	0	3	6	0	202	39	0
Bacteria;Cyanobacteria;Cyanobacteria;SubsectionI;family_NA;genus_NA;species_NA	0	0	14	5	0	0	0	0	0	0
Bacteria;Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;Croceibacter;atlanticus	0	0	5	13	0	0	0	0	0	0
Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Microbulbifer;species_NA	0	0	120	22	0	0	0	1	0	0
Bacteria;Gemmatimonadetes;Gemmatimonadetes;Gemmatimonadales;Gemmatimonadaceae;genus_NA;species_NA	0	0	8	3	0	0	0	11	0	0
Bacteria;Deferribacteres;Deferribacteres;Deferribacterales;SAR406;genus_NA;species_NA	0	0	5	4	0	0	209	0	0	178
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Jannaschia;species_NA	0	0	31	5	0	0	1	0	4	0
Bacteria;Proteobacteria;Gammaproteobacteria;Acidithiobacillales;family_NA;genus_NA;species_NA	0	0	13	25	0	0	100	4	5	4
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Oceaniserpentilla;species_NA	0	0	27	55	0	111	0	0	0	0
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinomonas;species_NA	0	0	73	45	0	149	0	0	0	4
Bacteria;Proteobacteria;Deltaproteobacteria;Desulfuromonadales;Desulfuromonadaceae;Pelobacter;species_NA	0	0	16	5	0	9	51	0	0	37
Bacteria;Cyanobacteria;Cyanobacteria;SubsectionI;Unassigned;Synechococcus;species_NA	0	0	16	4	6	0	0	3	0	0
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Marinobacterium;species_NA	0	0	1	1	17	0	0	5	0	0
Bacteria;Actinobacteria;Actinobacteria;Coriobacteriales;Coriobacteriaceae;genus_NA;species_NA	0	0	7	3	22	0	28	0	0	0
Bacteria;Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bacteriovoraceae;genus_NA;species_NA	0	0	10	4	4	0	59	0	10	20
Bacteria;Proteobacteria;Deltaproteobacteria;Desulfuromonadales;Desulfuromonadaceae;Desulfuromusa;species_NA	0	0	4	17	2	9	0	0	0	0
Bacteria;Proteobacteria;Gammaproteobacteria;Chromatiales;Chromatiaceae;genus_NA;species_NA	0	0	34	37	13	10	0	0	0	2
Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Haliea;species_NA	0	0	45	4	24	30	0	0	19	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Rhodovulum;marinum	0	0	8	6	1	21	0	0	11	0
Bacteria;Chloroflexi;Anaerolineae;Anaerolineales;Anaerolinaceae;Bellilinea;species_NA	0	0	1	22	20	25	0	2	0	0
Bacteria;Proteobacteria;Deltaproteobacteria;Desulfobacteriales;Desulfobacteraceae;Desulfosarcina;species_NA	0	0	91	32	30	26	2	0	0	0
Bacteria;Chloroflexi;Dehalococcoidetes;Unassigned;Unassigned;Dehalogenimonas;species_NA	0	0	1	20	1	66	2	0	0	347
Bacteria;Proteobacteria;Gammaproteobacteria;Unassigned;Unassigned;Sedimenticola;species_NA	0	0	17	33	15	3	1	0	17	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Rhodovulum;species_NA	0	0	19	2	5	4	35	0	45	3
Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Chitinophagaceae;Gracilimonas;species_NA	0	0	19	532	24	6	139	179	0	179
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;Pelagibius;species_NA	0	0	63	70	54	25	49	84	4	16
Bacteria;Acidobacteria;class_NA;orderx_NA;family_NA;genus_NA;species_NA	0	0	33	20	29	12	65	21	6	16
Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae;Roseateles;species_NA	0	26	0	0	0	0	0	0	2	0
Bacteria;Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Sphingopyxis;species_NA	0	7	0	0	0	0	0	0	57	0
Bacteria;Proteobacteria;Deltaproteobacteria;Desulfobacteriales;Nitrospiraceae;Nitrospiraceae;Entotheonella	0	3	0	0	0	0	0	6	22	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Lutimaribacter;species_NA	0	1	0	0	0	0	0	2	22	0
Bacteria;Chloroflexi;SAR202;orderx_NA;family_NA;genus_NA;species_NA	0	6	0	0	0	0	11	6	0	13
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;Inquilinus;species_NA	0	5	0	0	0	0	1	2	0	2
Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Rhodothermaceae;Salinibacter;species_NA	0	2	0	0	0	0	189	4	30	21
Bacteria;Firmicutes;Clostridia;Clostridiales;Clostridiaceae;Geosporobacter;species_NA	0	1	0	0	0	33	0	0	0	0
Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae;genus_NA;species_NA	0	1	0	0	0	15	0	0	0	0
Bacteria;Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;Muricauda;species_NA	0	10	0	0	0	17	0	1	49	0
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Alcanivoracaceae;Kangiella;species_NA	0	7	0	0	0	4	0	221	54	0
Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;Marinilabiaceae;Anaerophaga;species_NA	0	6	0	0	0	5	16	0	0	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Aurantimonadaceae;genus_NA;species_NA	0	5	0	0	16	0	0	0	0	4
Bacteria;Proteobacteria;Gammaproteobacteria;Thiotrichales;family_NA;genus_NA;species_NA	0	16	0	0	1	0	0	12	4	0
Bacteria;Proteobacteria;Deltaproteobacteria;Desulfobacteriales;Nitrospiraceae;Nitrospina;species_NA	0	2	0	0	3	0	11	0	0	90
Bacteria;Nitrospirae;Nitrospira;Nitrospirales;Nitrospiraceae;Nitrospira;species_NA	0	8	0	0	2	0	3	11	2	9
Bacteria;Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;Gramella;species_NA	0	4	0	0	8	3	0	19	1	0
Bacteria;Bacteroidetes;Flavobacteria;Flavobacteriales;Cryomorpaceae;Crocinitomix;species_NA	0	1	0	1	0	0	0	0	2	0
Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Aestuariibacter;species_NA	0	7	0	1	0	0	0	108	10	0
Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Flammeovirgaceae;Fulvirirga;species_NA	0	1	0	2	0	0	0	238	4	0
Bacteria;Verrucomicrobia;Opitutae;Puniceicoccales;Puniceicoccaceae;Coraliomargarita;species_NA	0	6	0	4	0	0	3	0	0	0
Bacteria;Proteobacteria;Alphaproteobacteria;Kordiimonadales;Kordiimonadaceae;Kordiimonas;species_NA	0	3	0	1	0	0	1	19	0	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;Thalassospira;species_NA	0	3	0	77	0	0	1	23	8	0
Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Flammeovirgaceae;Persicobacter;species_NA	0	29	0	11	2	0	0	0	0	0
Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Cytophagaceae;Flexibacter;species_NA	0	2	0	13	4	0	0	1	0	0
Bacteria;Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Subdivision3;genus_NA;species_NA	0	7	0	13	2	0	18	4	1	2
Bacteria;Chlamydiae;Chlamydiae;Chlamydiales;Chlamydiaceae;genus_NA;species_NA	0	10	0	3	4	23	0	0	0	0
Bacteria;Proteobacteria;Deltaproteobacteria;Desulfuromonadales;family_NA;genus_NA;species_NA	0	7	0	3	20	6	159	11	120	12
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Marinovum;algicola	0	26	13	0	0	0	0	0	0	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Tropicimonas;isoalkanivorans	0	2	15	0	0	0	0	0	44	0
Bacteria;Proteobacteria;Alphaproteobacteria;Caulobacteriales;Hyphomonadaceae;Hellea;species_NA	0	1	8	0	0	0	0	0	21	0
Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus;carboniphilus	0	18	25	0	0	0	0	34	0	0
Bacteria;Actinobacteria;Actinobacteria;Nitriliruptorales;Nitriliruptoraceae;Nitriliruptor;alkaliphilus	0	41	34	0	0	0	3	42	8	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Donghicola;species_NA	0	3	7	0	0	19	0	0	0	0
Bacteria;Chlamydiae;Chlamydiae;Chlamydiales;Criblamydiaceae;Criblamydia;species_NA	0	62	28	0	5	0	0	0	2	0
Bacteria;Verrucomicrobia;Spartobacteria;Chthoniobacter;family_NA;genus_NA;species_NA	0	216	9	0	1	0	0	2	0	0
Bacteria;Verrucomicrobia;Spartobacteria;orderx_NA;family_NA;genus_NA;species_NA	0	85	2	0	1	0	0	6	0	0
Bacteria;Chlamydiae;Chlamydiae;Chlamydiales;Parachlamydiaceae;Protochlamydia;species_NA	0	8	24	0	6	0	0	5	0	2

Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Cytophagaceae;Microscilla;species_NA	0	54	28	0	2	0	0	1	1	0
Bacteria;Proteobacteria;Gammaproteobacteria;Legionellales;Legionellaceae;genus_NA;species_NA	0	35	5	0	2	0	0	5	2	1
Bacteria;Proteobacteria;Gammaproteobacteria;Aeromonadales;Aeromonadaceae;Oceanisphaera;species_NA	0	4	1	0	297	25	0	0	0	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Labrenzia;species_NA	0	1	7	0	33	8	0	0	0	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Loktanela;species_NA	0	13	12	0	4	7	0	31	28	0
Bacteria;Proteobacteria;Gammaproteobacteria;Legionellales;Legionellaceae;Legionella;species_NA	0	117	81	0	38	19	1	6	10	0
Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Flammeovirgaceae;Flexithrix;species_NA	0	3	13	12	0	0	0	2	16	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhodobiaceae;Affella;species_NA	0	23	26	7	5	0	0	0	0	0
Bacteria;Firmicutes;Erysipelotrichi;Erysipelotrichales;Erysipelotrichaceae;genus_NA;species_NA	0	10	26	11	11	0	0	0	0	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhodobiaceae;Andersenella;species_NA	0	122	74	7	67	0	0	0	12	0
Bacteria;Proteobacteria;Gammaproteobacteria;Thiotrichales;Piscirickettsiaceae;Mariprofundus;species_NA	0	1	8	9	4	0	0	121	0	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Palleronia;species_NA	0	101	1	2	3	0	0	9	35	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Dinoroseobacter;species_NA	0	29	6	8	12	0	0	5	12	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Amaricoccus;species_NA	0	258	40	6	15	0	0	2	8	1
Bacteria;Chlamydiae;Chlamydiae;Chlamydiales;Waddliaceae;Waddlia;species_NA	0	33	62	2	45	0	7	24	13	0
Bacteria;Acidobacteria;Acidobacteria_Gp26;Unassigned;Unassigned;Gp26;species_NA	0	21	38	11	27	0	220	14	1	78
Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Phyllobacteriaceae;Mesorhizobium;species_NA	0	27	1	9	1	6	0	0	46	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Pseudoruegeria;aquimaris	0	14	10	1	6	8	0	0	1	0
Bacteria;Proteobacteria;Gammaproteobacteria;Vibrionales;Vibrionaceae;Photobacterium;species_NA	0	5	2	32	2	1	0	0	1	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Hyphomicrobiaceae;Hyphomicrobium;species_NA	0	7	23	4	9	5	0	20	0	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Roseobacter;species_NA	0	2	23	2	18	37	0	1	0	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodospirillales;Unassigned;Alysiosphaera;species_NA	0	74	17	26	1	14	0	15	9	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Phyllobacteriaceae;genus_NA;species_NA	0	40	83	9	15	2	0	7	72	0
Bacteria;Proteobacteria;Alphaproteobacteria;Sphingomonadales;Erythrobacteraceae;Erythrobacter;species_NA	0	11	7	13	4	12	0	67	369	0
Bacteria;Firmicutes;Clostridia;Clostridiales;Clostridiaceae;Clostridiisalibacter;species_NA	0	4	7	14	2	25	303	0	0	0
Bacteria;Firmicutes;Clostridia;Clostridiales;Clostridiaceae;Caminicella;species_NA	0	3	29	101	4	1	101	0	0	1
Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Geobacillus;species_NA	0	17	16	1	8	158	6	0	3	0
Bacteria;Firmicutes;Clostridia;Clostridiales;Clostridiaceae;Clostridium;species_NA	0	10	5	2	20	61	74	2	0	3
Bacteria;Proteobacteria;Gammaproteobacteria;Chromatiales;Ectothiorhodospiraceae;Thioalkalispira;species_NA	0	4	2	357	7	6	42	2	0	3
Bacteria;Actinobacteria;Actinobacteria;Nitrospirales;Nitrospiraceae;Nitrospirillum;species_NA	0	1852	1414	8	60	5	4	159	517	0
Bacteria;Actinobacteria;Actinobacteria;orderx_NA;family_NA;genus_NA;species_NA	0	5	37	18	20	98	86	30	6	0
Bacteria;TM6;class_NA;orderx_NA;family_NA;genus_NA;species_NA	0	121	96	4	90	168	236	42	10	51
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;genus_NA;species_NA	0	44	20	47	65	16	13	114	58	29
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;family_NA;genus_NA;species_NA	0	36	32	13	28	201	144	12	78	10
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;Defluviococcus;species_NA	0	12	48	36	31	16	7	13	9	19
Bacteria;Proteobacteria;Gammaproteobacteria;Unassigned;Unassigned;Thiohalophilus;species_NA	0	10	1	68	162	74	3	3	87	35
Bacteria;Proteobacteria;Gammaproteobacteria;Chromatiales;Ectothiorhodospiraceae;genus_NA;species_NA	0	4	9	11	10	40	784	12	60	155
Bacteria;Proteobacteria;Deltaproteobacteria;Desulfobacteriales;Desulfobacteraceae;Desulfocella;species_NA	531	0	0	0	0	0	0	1	0	0
Bacteria;Firmicutes;Clostridia;Clostridiales;Unassigned;Anaerococcus;prevotii	129	0	0	0	0	0	0	5	0	0
Bacteria;Proteobacteria;Gammaproteobacteria;Aeromonadales;Succinivibrionaceae;Succinivibrio;species_NA	127	0	0	0	0	0	0	6	0	0
Bacteria;Firmicutes;Clostridia;Clostridiales;Unassigned;Anaerococcus;species_NA	64	0	0	0	0	0	0	3	0	0
Bacteria;Proteobacteria;Alphaproteobacteria;Caulobacteriales;Caulobacteraceae;Brevundimonas;species_NA	61	0	0	0	0	0	0	11	0	0
Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Moraxellaceae;Acinetobacter;lwoffii	29	0	0	0	0	0	0	2	0	0
Bacteria;Firmicutes;Clostridia;Clostridiales;Veillonellaceae;genus_NA;species_NA	28	0	0	0	0	0	0	1	0	0
Bacteria;Proteobacteria;Deltaproteobacteria;Desulfovibrionales;Desulfohalobiaceae;Desulfovermiculus;species_NA	3	0	0	0	0	0	373	0	0	123
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Oceanospirillum;species_NA	425	0	0	0	0	1	0	0	0	0
Bacteria;Proteobacteria;Epsilonproteobacteria;Campylobacteriales;Campylobacteraceae;Arcobacter;species_NA	440	0	0	0	0	404	0	0	0	64
Bacteria;Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;Salegentibacter;mishustinae	3	0	0	0	14	0	0	0	0	0
Bacteria;Proteobacteria;Gammaproteobacteria;Xanthomonadales;Xanthomonadaceae;Pseudoxanthomonas;species_NA	54	0	0	0	5	0	0	4	0	0
Bacteria;Bacteroidetes;Flavobacteria;Flavobacteriales;Cryomorpaceae;genus_NA;species_NA	4	0	0	0	3	0	4	0	36	0
Bacteria;Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bdellovibrionaceae;Bdellovibrio;species_NA	7	0	0	0	5	0	15	1	39	10
Bacteria;Proteobacteria;Gammaproteobacteria;Thiotrichales;Piscirickettsiaceae;Cycloclasticus;species_NA	4	0	0	0	1	359	0	0	9	0
Bacteria;Firmicutes;Clostridia;Clostridiales;Veillonellaceae;Phascolarctobacterium;species_NA	96	0	0	2	0	0	0	4	0	0
Bacteria;Actinobacteria;Actinobacteria;Actinomycetales;Microbacteriaceae;Microbacterium;species_NA	4	0	0	18	0	0	0	3	0	4
Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacteriales;Enterobacteriaceae;Shigella;dysenteriae	1	0	0	5	0	0	0	5	0	19
Bacteria;Proteobacteria;Alphaproteobacteria;Caulobacteriales;Hyphomonadaceae;Hyphomonas;Hyphomonas	19	0	0	9	0	0	0	14	1	0
Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Alteromonas;species_NA	1	0	0	150	0	5	0	216	47	0
Bacteria;BRC1;class_NA;orderx_NA;family_NA;genus_NA;species_NA	1	0	0	15	0	14	51	20	0	7
Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Moraxellaceae;Acinetobacter;species_NA	784	0	0	3	57	0	0	49	0	2
Bacteria;Proteobacteria;Betaproteobacteria;Burkholderiales;Comamonadaceae;Aquabacterium;species_NA	8	0	0	6	18	0	0	1	0	8
Bacteria;Proteobacteria;Deltaproteobacteria;SAR324;family_NA;genus_NA;species_NA	4	0	0	24	1	0	40	1	0	15
Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Rhodothermaceae;Salisaeta;species_NA	2	0	0	4	5	0	84	8	39	62
Bacteria;Fusobacteria;Fusobacteria;Fusobacteriales;Fusobacteriaceae;Psychrilyobacter;species_NA	159	0	0	1	2	10	0	0	0	0
Bacteria;Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;Eudoraea;species_NA	46	0	0	14	102	36	0	0	0	0
Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Congregibacter;species_NA	4	0	0	1	8	26	0	0	0	0
Bacteria;Proteobacteria;Gammaproteobacteria;Thiotrichales;Piscirickettsiaceae;genus_NA;species_NA	5	0	0	9	20	33	0	1	44	0
Bacteria;Firmicutes;class_NA;orderx_NA;family_NA;genus_NA;species_NA	135	0	0	158	2	148	2	0	0	0
Bacteria;Proteobacteria;Deltaproteobacteria;Desulfobacteriales;Desulfobacteraceae;Desulfobacterium;species_NA	3	0	0	57	14	10	15	0	0	54
Bacteria;Proteobacteria;Deltaproteobacteria;Desulfobacteriales;family_NA;genus_NA;species_NA	8	0	0	25	32	2	18	3	0	8
Bacteria;Proteobacteria;Deltaproteobacteria;Desulfuromonadales;Geobacteraceae;Geobacter;species_NA	21	0	0	2	2	6	1	5	1	0
Bacteria;Spirochaetes;Spirochaetes;Spirochaetales;Spirochaetaceae;Spirochaeta;species_NA	88	0	0	79	36	63	182	9	3	154
Bacteria;OP3;class_NA;orderx_NA;family_NA;genus_NA;species_NA	23	0	0	23	6	29	140	26	8	151
Bacteria;Firmicutes;Clostridia;Clostridiales;Peptostreptococcaceae;Peptoniphilus;species_NA	54	0	0	8	0	0	0	1	0	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacteriales;Rhodobacteraceae;Stappia;meyerae	11	0	0	3	0	0	0	16	0	0

Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;Prevotellaceae;Prevotella;species_NA	542	0	11	0	0	0	0	31	3	0
Bacteria;Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;Maribacter;species_NA	8	0	22	0	0	24	0	0	0	0
Bacteria;Actinobacteria;Actinobacteria;Actinomycetales;Corynebacteriaceae;Corynebacterium;species_NA	150	0	8	0	15	0	1	18	5	0
Bacteria;Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;Pibocella;species_NA	4	0	32	0	1	18	0	0	0	0
Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Pseudoalteromonadaceae;Pseudoalteromonas;tetraodonis	12	0	5	0	22	4	0	0	3	0
Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Glaciecola;species_NA	46	0	3	64	0	0	0	0	0	0
Bacteria;Cyanobacteria;Cyanobacteria;SubsectionI;Unassigned;Cyanobacterium;species_NA	5	0	6	9	3	0	0	0	0	0
Bacteria;Actinobacteria;Actinobacteria;Solirubrobacterales;family_NA;genus_NA;species_NA	5	0	6	6	22	0	0	5	0	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Bradyrhizobiaceae;Bradyrhizobium;species_NA	25	0	5	39	7	0	0	9	0	34
Bacteria;Proteobacteria;Gammaproteobacteria;Unassigned;Unassigned;Thiohalomonas;species_NA	1	0	26	15	4	0	18	0	0	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Maritimibacter;species_NA	1	0	2	11	14	0	1	0	7	0
Bacteria;Deferribacteres;Deferribacteres;Deferribacterales;Unassigned;Caldithrix;species_NA	4	0	2	7	16	0	66	0	5	71
Bacteria;Proteobacteria;Gammaproteobacteria;Vibrionales;Vibrionaceae;Vibrio;kanaloae	36	0	54	1	1	1	0	0	0	0
Bacteria;Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;Arenibacter;echinorum	4	0	24	1	13	6	0	0	0	0
Bacteria;Proteobacteria;Gammaproteobacteria;Unassigned;Unassigned;Thiobios;species_NA	9	0	15	8	55	45	0	0	0	2
Bacteria;Proteobacteria;Deltaproteobacteria;Desulfobacteriales;Desulfobacteraceae;Desulfococcus;species_NA	3	0	5	14	6	1	0	0	0	2
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Halomonadaceae;Cobetia;species_NA	29	0	500	8	11	26	0	0	2	0
Bacteria;Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;genus_NA;species_NA	13	0	20	5	31	8	0	0	128	0
Bacteria;Proteobacteria;Deltaproteobacteria;Desulfobacteriales;Desulfobulbaceae;genus_NA;species_NA	10	0	97	281	71	43	0	0	1	0
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oleiphilaceae;Oleiphilus;species_NA	2	0	10	122	12	53	0	0	108	0
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Halomonadaceae;Halomonas;species_NA	236	0	203	25	209	95	0	10	0	229
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Thalassobius;species_NA	1	0	23	5	5	2	0	5	21	0
Bacteria;Acidobacteria;Acidobacteria_Gp22;Unassigned;Unassigned;Gp22;species_NA	22	0	26	23	92	35	0	26	29	2
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Oceanospirillaceae;Pseudospirillum;species_NA	8	0	10	28	5	13	0	1	122	5
Bacteria;Proteobacteria;Deltaproteobacteria;Desulfobacteriales;Desulfobulbaceae;Desulfopila;species_NA	330	0	10	6	5	14	9	0	0	1
Bacteria;Proteobacteria;Deltaproteobacteria;Desulfarculales;Desulfarculaceae;genus_NA;species_NA	3	0	1	25	16	55	32	0	0	53
Bacteria;Chloroflexi;Anaerolineae;Anaerolineales;Anaerolineaceae;genus_NA;species_NA	8	0	10	273	124	128	362	0	31	262
Bacteria;Proteobacteria;Deltaproteobacteria;Desulfobacteriales;Desulfobacteraceae;genus_NA;species_NA	44	0	21	109	200	140	75	2	0	84
Bacteria;WS3;class_NA;orderx_NA;family_NA;genus_NA;species_NA	64	0	12	60	34	17	21	33	8	10
Bacteria;OD1;class_NA;orderx_NA;family_NA;genus_NA;species_NA	22	0	15	1	16	16	77	7	2	45
Bacteria;Cyanobacteria;class_NA;orderx_NA;family_NA;genus_NA;species_NA	16	0	31	27	11	9	28	14	74	31
Bacteria;Firmicutes;Erysipelotrichi;Erysipelotrichales;Erysipelotrichaceae;Turicibacter;species_NA	194	9	0	0	0	0	0	0	0	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;Oceanibaculum;species_NA	2	19	0	0	0	0	0	6	1	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Paracoccus;species_NA	27	1	0	0	1	0	0	1	1	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Ruegeria;species_NA	37	2	0	0	1	1	0	2	1	0
Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Saprospiraceae;Lewinella;species_NA	4	32	0	0	3	7	0	2	107	0
Bacteria;Bacteroidetes;Bacteroidia;Bacteroidales;Marinilabiaceae;genus_NA;species_NA	1008	21	0	0	3	102	48	1	0	30
Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacteriales;Enterobacteriaceae;Escherichia;coli	3	2	0	36	0	0	0	63	0	185
Bacteria;Actinobacteria;Actinobacteria;Actinomycetales;Propionibacteriaceae;Propionibacterium;species_NA	1	2	0	14	1	0	0	8	0	8
Bacteria;Proteobacteria;Betaproteobacteria;Hydrogenophilales;Hydrogenophilaceae;Thiobacillus;species_NA	9	50	0	80	37	0	0	41	4	0
Bacteria;TM7;class_NA;orderx_NA;family_NA;genus_NA;species_NA	28	4	0	11	30	0	0	45	393	23
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodospirillales;family_NA;genus_NA;species_NA	2	70	0	17	2	0	0	33	14	9
Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Saprospiraceae;genus_NA;species_NA	20	17	0	6	29	1	0	8	70	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Oceanicola;species_NA	5	7	0	13	3	2	0	7	11	0
Bacteria;Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;Winogradskyella;species_NA	4	7	0	3	4	14	0	14	5	0
Bacteria;Proteobacteria;Alphaproteobacteria;Parvularculales;Parvularculaceae;Parvularcula;species_NA	3	7	0	2	2	1	0	34	11	0
Bacteria;Proteobacteria;Deltaproteobacteria;Myxococcales;Haliangiaceae;Haliangium;species_NA	4	28	0	13	11	13	5	0	20	0
Bacteria;Chloroflexi;Anaerolineae;Anaerolineales;family_NA;genus_NA;species_NA	4	8	0	25	35	15	550	1	0	324
Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Saprospiraceae;Haliscomenobacter;species_NA	42	23	0	7	41	14	20	38	400	1
Bacteria;Lentisphaerae;Lentisphaeria;orderx_NA;family_NA;genus_NA;species_NA	22	2	0	13	16	27	16	2	6	2
Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Teredinibacter;species_NA	40	99	7	0	0	0	0	0	0	0
Bacteria;Firmicutes;Clostridia;Clostridiales;Peptostreptococcaceae;Tepidibacter;species_NA	15	3	0	0	0	0	0	0	0	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Albidovulum;species_NA	12	17	0	6	0	0	0	0	22	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Phyllobacteriaceae;Ahrensia;species_NA	5	107	25	0	1	0	0	2	95	0
Bacteria;Deinococcus-Thermus;Deinococci;Deinococcales;Deinococcaceae;Deinococcus;species_NA	6	28	0	9	20	0	2	11	31	0
Bacteria;Chlamydiae;Chlamydiae;Chlamydiales;Parachlamydiaceae;Neochlamydia;species_NA	4	40	39	0	23	9	0	3	8	6
Bacteria;Chlamydiae;Chlamydiae;Chlamydiales;Simkaniaceae;Rhabdochlamydia;species_NA	1	14	5	0	4	16	15	6	0	2
Bacteria;Proteobacteria;Deltaproteobacteria;Desulfovibrionales;Desulfovibrionaceae;Desulfovibrio;species_NA	190	13	1	4	0	63	3	0	0	38
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Sediminimonas;species_NA	1	3	24	9	0	6	3	0	7	2
Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Phyllobacteriaceae;Nitratireductor;species_NA	8	47	11	12	1	0	0	0	108	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Phyllobacteriaceae;Hoefflea;species_NA	244	4	243	346	46	0	0	780	1	0
Bacteria;Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Roseibacillus;species_NA	13	42	18	1	21	0	0	11	24	0
Bacteria;Proteobacteria;Gammaproteobacteria;Chromatiales;Granulosicoccaceae;Granulosicoccus;species_NA	11	3910	135	6	7	0	0	2	1131	0
Bacteria;Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;Gemmata;species_NA	4	1	21	4	2	0	0	6	3	3
Bacteria;Actinobacteria;Actinobacteria;Acidimicrobiales;Iamiaceae;Iamia;species_NA	40	423	117	41	384	0	1	84	1296	0
Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus;species_NA	2	22	37	19	7	0	5	23	1	0
Bacteria;Proteobacteria;Deltaproteobacteria;Myxococcales;family_NA;genus_NA;species_NA	120	24	24	50	79	0	73	41	89	25
Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Rhodothermaceae;genus_NA;species_NA	53	75	8	33	27	0	83	169	599	11
Bacteria;Gemmatimonadetes;Gemmatimonadetes;orderx_NA;family_NA;genus_NA;species_NA	47	3	16	162	89	0	7493	173	43	843
Bacteria;Chloroflexi;Caldilineae;Caldilineales;Caldilineaceae;Caldilinea;species_NA	27	63	56	56	102	0	2	13	131	4
Bacteria;Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bdellovibrionaceae;genus_NA;species_NA	7	3	17	6	7	0	4	2	34	3
Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Methylobacteriaceae;Methylobacterium;species_NA	4	1	5	2	3	17	0	0	0	1
Bacteria;Cyanobacteria;Cyanobacteria;SubsectionII;SubgroupII;Pleurocapsa;species_NA	13	16	34	4	1	34	0	0	175	0
Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Unassigned;Haliea	3	4	12	23	42	31	0	0	6	0

Bacteria;Proteobacteria;Gammaproteobacteria;Thiotrichales;Thiotrichaceae;Leucothrix;species_NA	7	4	29	333	147	146	0	0	14	4
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Sulfitobacter;species_NA	121	1	152	227	13	111	0	34	150	0
Bacteria;Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;genus_NA;species_NA	46	38	41	27	48	20	0	20	97	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Hyphomicrobiaceae;Filomicrobium;species_NA	20	114	72	80	106	20	0	10	38	0
Bacteria;Actinobacteria;Actinobacteria;Acidimicrobiales;Acidimicrobiaceae;genus_NA;species_NA	7	73	129	30	140	11	0	9	150	0
Bacteria;Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;Zeaxanthinibacter;species_NA	7	70	31	29	10	34	0	16	58	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Rhodobiaceae;Rhodobium;species_NA	5	173	34	96	57	5	0	25	19	0
Bacteria;Actinobacteria;Actinobacteria;Acidimicrobiales;Acidimicrobiaceae;Ilumatobacter;species_NA	1	19	29	6	7	9	0	3	70	0
Bacteria;Proteobacteria;Gammaproteobacteria;Vibrionales;Vibrionaceae;Vibrio;species_NA	2414	106	1902	74	194	410	0	2	5	6
Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Pseudoalteromonadaceae;Pseudoalteromonas;species_NA	1264	29	1576	290	1124	837	0	4	376	5
Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Marinobacter;species_NA	258	1	14	299	396	1789	0	1110	13	227
Bacteria;Proteobacteria;Alphaproteobacteria;Sphingomonadales;Sphingomonadaceae;Sphingomonas;species_NA	21	18	16	6	8	6	0	8	44	2
Bacteria;Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;Robiginitalea;species_NA	16	16	13	27	31	11	0	6	160	1
Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;genus_NA;species_NA	10	4	2	23	13	15	0	9	110	4
Bacteria;Chlamydiae;Chlamydiae;Chlamydiales;Simkaniaceae;genus_NA;species_NA	7	73	26	6	48	41	0	15	19	4
Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas;species_NA	6	193	40	10	13	27	2	0	36	0
Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Chitinophagaceae;genus_NA;species_NA	10	4	3	3	7	16	86	9	18	0
Bacteria;Chlamydiae;Chlamydiae;Chlamydiales;Parachlamydiaceae;Parachlamydia;species_NA	6	30	36	2	14	6	1	16	12	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Tropicimonas;species_NA	4	43	43	18	18	10	7	9	97	0
Bacteria;Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bacteriovoraceae;Peredibacter;species_NA	4	43	14	29	6	12	1	3	14	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Wenxinia;species_NA	1	169	424	42	24	15	8	9	113	0
Bacteria;Proteobacteria;Gammaproteobacteria;Acidithiobacillales;Acidithiobacillaceae;Acidithiobacillus;species_NA	1	27	4	63	115	56	1	2	2	0
		10296	10448	8251	7340	9584	15487	6502	10521	6810

Bacteria;Planctomycetes;Unassigned;Unassigned;Unassigned;Brodacia;anamnoxidans	0	0	0	0	0	0	0	0.016455696	0	0	0.008573009
Bacteria;Proteobacteria;Alphaproteobacteria;Caulobacteriales;Caulobacteraceae;Brevundimonas;species_NA	0.006020529	0	0	0	0	0	0	0	0.001785424	0	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Lutimaribacter;species_NA	0	9.54472E-05	0	0	0	0	0	0	0.000324623	0.00244146	0
Bacteria;Verrucomicrobia;Opitutae;Punicococcales;Punicococcaceae;Lentimonas;marisflavi	0	0	0	0	0	0	0.001682895	0	0	0	0
Bacteria;Verrucomicrobia;Opitutae;Punicococcales;Punicococcaceae;Pelagicoccus;species_NA	0.000394789	0	0	0	0	0	0	0	0	0	0
Bacteria;Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Haloferrula;phycli	0	0.003054309	0	0	0	0	0	0	0	0	0
Bacteria;Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Haloferrula;species_NA	0	0.002481626	0	0	0	0	0	0	0	0	0
Bacteria;Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;Luteoilbacter;species_NA	0	0.002290732	0	0	0	0	0	0	0	0	0
TOTALS	0.097808922	0.151474659	0.127927266	0.117319255	0.194230176	0.133068878	0.311181435	0.140236975	0.231605815	0.121681416	0
Unidentified Genera											
Bacteria;Proteobacteria;Gammaproteobacteria;Enterobacteriales;Enterobacteriaceae;genus_NA;species_NA	0.006222	0.002358	0.001092	0.09974	0.000159	0.00165	0.000153	0.135372	0.000183	0.371753	0
Bacteria;Proteobacteria;Gammaproteobacteria;Xanthomonadales;Sinobacteraceae;genus_NA;species_NA	0.02496	0.020825	0.018838	0.031514	0.062739	0.026752	0.000102	0.043402	0.04925	7.80E-05	0
Bacteria;Actinobacteria;Actinobacteria;Acidimicrobiales;family_NA;genus_NA;species_NA	0.011793	0.053373	0.053921	0.02591	0.088694	0.009468	0.000509	0.077807	0.050835	0.000548	0
Bacteria;Proteobacteria;Deltaproteobacteria;Desulfobacteriales;Desulfobulbaceae;genus_NA;species_NA	0.000723	0	0.006621	0.022823	0.005653	0.003735	0	0	6.10E-05	0	0
Bacteria;Chloroflexi;Anaerolineae;Anaerolineales;Anaerolineaceae;genus_NA;species_NA	0.000579	0	0.000683	0.022173	0.009873	0.011118	0.018443	0	0.00189	0.020501	0
Bacteria;Proteobacteria;Gammaproteobacteria;orderx_NA;family_NA;genus_NA;species_NA	0.007597	0.032547	0.019657	0.018031	0.019029	0.015982	0.005706	0.011996	0.053395	0.002973	0
Bacteria;Acidobacteria;Holophagae;orderx_NA;family_NA;genus_NA;species_NA	0.001302	0.013818	0.014948	0.016407	0.039013	0.000955	0.000917	0.009098	0.017128	0.000704	0
Unknown;phylum_NA;class_NA;orderx_NA;family_NA;genus_NA;species_NA	0.013891	0.009496	0.011876	0.013483	0.010589	0.009468	0.012482	0.013246	0.013593	0.024804	0
Bacteria;Gemmatimonadetes;Gemmatimonadetes;orderx_NA;family_NA;genus_NA;species_NA	0.0034	0.000196	0.001092	0.013158	0.007086	0	0.381751	0.014412	0.002621	0.065962	0
Bacteria;Firmicutes;class_NA;orderx_NA;family_NA;genus_NA;species_NA	0.009767	0	0	0.012833	0.000159	0.012855	0.000102	0	0	0	0
Bacteria;Planctomycetes;Planctomycetacia;Planctomycetales;Planctomycetaceae;genus_NA;species_NA	0.004775	0.015848	0.016654	0.011696	0.044268	0.006167	0.018392	0.018161	0.015177	0.005321	0
Bacteria;Proteobacteria;Deltaproteobacteria;orderx_NA;family_NA;genus_NA;species_NA	0.007524	0.001179	0.002935	0.009747	0.004777	0.00304	0.008406	0.033322	0.0064	0.003365	0
Bacteria;Proteobacteria;Deltaproteobacteria;Desulfobacteriales;Desulfobacteraceae;genus_NA;species_NA	0.003183	0	0.001433	0.008853	0.015924	0.01216	0.003821	0.000167	0	0.006573	0
Bacteria;Chloroflexi;Caldilineae;Caldilineales;family_NA;genus_NA;species_NA	0.000289	0.010478	0.004983	0.008203	0.015207	0.003474	0.000459	0.0015	0.022431	0.001643	0
Bacteria;Acidobacteria;Acidobacteria;Acidobacteriales;Acidobacteriaceae;genus_NA;species_NA	0.000796	0.001768	0.000683	0.007554	0.007564	0.000434	0.00214	0.017661	0.000305	0.000391	0
Bacteria;WS3;class_NA;orderx_NA;family_NA;genus_NA;species_NA	0.00463	0	0.000819	0.004873	0.002707	0.001477	0.00107	0.002749	0.000488	0.000782	0
Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;family_NA;genus_NA;species_NA	0.002532	0.00203	0.000341	0.004792	0.00199	0.000608	0.007642	8.30E-05	0.001707	0.000939	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;genus_NA;species_NA	0.001447	0.011002	0.006621	0.004711	0.006608	0.007817	0.003413	0.005915	0.016153	0.000548	0
Bacteria;Chloroflexi;class_NA;orderx_NA;family_NA;genus_NA;species_NA	0.000941	0.000262	0.005392	0.004467	0.008439	0.000955	0.000102	0.002499	0.000122	0.000156	0
Bacteria;Proteobacteria;Deltaproteobacteria;Syntrophobacteriales;Syntrophobacteraceae;genus_NA;species_NA	0	0	0	0.004142	0.000159	0.00443	0.000357	8.30E-05	0	0.001174	0
Bacteria;Proteobacteria;Deltaproteobacteria;Myxococcales;family_NA;genus_NA;species_NA	0.008682	0.001572	0.001638	0.004061	0.00629	0	0.003719	0.003416	0.005425	0.001956	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodospirillales;Rhodospirillaceae;genus_NA;species_NA	0	0.002881	0.001365	0.003817	0.005175	0.00139	0.000662	0.009497	0.003535	0.002269	0
Bacteria;Proteobacteria;Gammaproteobacteria;Chromatiales;Chromatiaceae;genus_NA;species_NA	0	0	0.002321	0.003005	0.001035	0.000869	0	0	0	0.000156	0
Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Rhodothermaceae;genus_NA;species_NA	0.003834	0.004912	0.000546	0.00268	0.00215	0	0.004229	0.014079	0.036511	0.000861	0
Bacteria;phylum_NA;class_NA;orderx_NA;family_NA;genus_NA;species_NA	0.036174	0.000524	0.002935	0.002599	0.002787	0.004864	0.025321	0.004415	0.001036	0.005164	0
Bacteria;Actinobacteria;Actinobacteria;Acidimicrobiales;Acidimicrobiaceae;genus_NA;species_NA	0.000506	0.004781	0.000805	0.002437	0.011146	0.000955	0	0.00075	0.009143	0	0
Bacteria;TA06;class_NA;orderx_NA;family_NA;genus_NA;species_NA	0	0	0	0.002355	0.000239	0.002519	5.10E-05	0	0	0.006416	0
Bacteria;Cyanobacteria;class_NA;orderx_NA;family_NA;genus_NA;species_NA	0.001158	0	0.002116	0.002193	0.000876	0.000782	0.001427	0.001166	0.004511	0.002426	0
Bacteria;Planctomycetes;Phycisphaerae;orderx_NA;family_NA;genus_NA;species_NA	0.001013	0.000131	0.000341	0.002193	0.001831	0.002693	0.0027	0.001166	0.000549	0.002347	0
Bacteria;Planctomycetes;Phycisphaerae;Phycisphaerales;Phycisphaeraceae;genus_NA;species_NA	0.000796	0.000982	0.001843	0.002193	0.001274	0.002171	0.003108	0.004499	0.00573	0.000704	0
Bacteria;Proteobacteria;Gammaproteobacteria;Chromatiales;family_NA;genus_NA;species_NA	0.000434	0.037394	0.005187	0.002193	0.008201	0.001216	0.006725	0.001999	0.011215	0.004851	0
Bacteria;Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Verrucomicrobiaceae;genus_NA;species_NA	0.003328	0.002489	0.002798	0.002193	0.003822	0.001737	0	0.001666	0.005912	0	0
Bacteria;Chloroflexi;Anaerolineae;Anaerolineales;family_NA;genus_NA;species_NA	0.000289	0.000524	0	0.002031	0.002787	0.001303	0.028021	8.30E-05	0	0.025352	0
Bacteria;Nitrospirae;Nitrospirae;Nitrospirales;Nitrospiraceae;genus_NA;species_NA	0	0	0	0.002031	8.00E-05	0.003214	0.006216	0	0	0.015884	0
Bacteria;Proteobacteria;Deltaproteobacteria;Desulfarculales;Desulfarculaceae;genus_NA;species_NA	0.000217	0	6.80E-05	0.002031	0.001274	0.004777	0.00163	0	0	0.004147	0
Bacteria;Proteobacteria;Deltaproteobacteria;Desulfobacteriales;family_NA;genus_NA;species_NA	0.000579	0	0	0.002031	0.002548	0.000174	0.000917	0.00025	0	0.000626	0
Bacteria;Proteobacteria;Gammaproteobacteria;Acidithiobactiales;family_NA;genus_NA;species_NA	0	0	0.000887	0.002031	0	0	0.005095	0.000333	0.000305	0.000313	0
Bacteria;Proteobacteria;Deltaproteobacteria;SAR324;family_NA;genus_NA;species_NA	0.000289	0	0	0.001949	8.00E-05	0	0.002038	8.30E-05	0	0.001174	0
Bacteria;OP3;class_NA;orderx_NA;family_NA;genus_NA;species_NA	0.001664	0	0	0.001868	0.000478	0.002519	0.007133	0.002166	0.000488	0.011815	0
Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;genus_NA;species_NA	0.000723	0.000262	0.000137	0.001868	0.001035	0.001303	0	0.00075	0.006705	0.000313	0
Bacteria;Proteobacteria;Deltaproteobacteria;Myxococcales;Polyangiaceae;genus_NA;species_NA	0	0	0	0.001706	0.000557	0	0.00056	0	0.000488	0	0
Bacteria;Acidobacteria;class_NA;orderx_NA;family_NA;genus_NA;species_NA	0	0	0.002252	0.001624	0.002309	0.001042	0.003312	0.001749	0.000366	0.001252	0
Bacteria;Proteobacteria;Alphaproteobacteria;orderx_NA;family_NA;genus_NA;species_NA	0.000651	0.003798	0.001229	0.001543	0.000717	0.003301	0.001579	0.00125	0.007741	0.001095	0
Bacteria;Actinobacteria;Actinobacteria;orderx_NA;family_NA;genus_NA;species_NA	0	0.000327	0.002525	0.001462	0.001592	0.008512	0.004381	0.002499	0.000366	0	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhodospirillales;family_NA;genus_NA;species_NA	0.000145	0.004584	0	0.001381	0.000159	0	0	0.002749	0.000853	0.000704	0
Bacteria;BRC1;class_NA;orderx_NA;family_NA;genus_NA;species_NA	7.20E-05	0	0	0.001218	0	0.001216	0.002598	0.001666	0	0.000548	0
Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Flammeovirgaceae;genus_NA;species_NA	0.001447	0.006549	0.000956	0.001056	0.005334	0.001737	0.000917	0.001166	0.00067	0.000156	0
Bacteria;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae;genus_NA;species_NA	0	0	0	0.001056	0.000955	0.002171	0.000408	0	0	0	0
Bacteria;Lentisphaerae;Lentisphaeria;orderx_NA;family_NA;genus_NA;species_NA	0.001592	0.000131	0	0.001056	0.001274	0.002345	0.000815	0.000167	0.000366	0.000156	0
Bacteria;Proteobacteria;Gammaproteobacteria;Oceanospirillales;family_NA;genus_NA;species_NA	0	0.002358	0.002184	0.001056	0.002229	0.017459	0.007336	0.001	0.004754	0.000782	0
Bacteria;Verrucomicrobia;Verrucomicrobiae;Verrucomicrobiales;Subdivision3;genus_NA;species_NA	0	0.000458	0	0.001056	0.000159	0	0.000917	0.000333	6.10E-05	0.000156	0
Bacteria;Firmicutes;Erysipelotrichi;Erysipelotrichales;Erysipelotrichaceae;genus_NA;species_NA	0	0.000655	0.001775	0.000893	0.000876	0	0	0	0	0	0
Bacteria;Proteobacteria;Gammaproteobacteria;Chromatiales;Ectothiorhodospiraceae;genus_NA;species_NA	0	0.000262	0.000614	0.000893	0.000796	0.003474	0.039943	0.001	0.003657	0.012128	0
Bacteria;TM7;class_NA;orderx_NA;family_NA;genus_NA;species_NA	0.002026	0.000262	0	0.000893	0.002389	0	0	0.003749	0.023955	0.0018	0
Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Phyllobacteriaceae;genus_NA;species_NA	0	0.00262	0.005665	0.000731	0.001194	0.000174	0	0.000583	0.004389	0	0
Bacteria;Proteobacteria;Gammaproteobacteria;Thiotrichales;Piscirickettsiaceae;genus_NA;species_NA	0.000362	0	0	0.000731	0.001592	0.002866	0	8.30E-05	0.002682	0	0
Bacteria;Actinobacteria;Actinobacteria;Solirubrobacteriales;family_NA;genus_NA;species_NA	0.000362	0	0.00041	0.000487	0.001752	0	0	0.000417	0	0	0
Bacteria;Bacteroidetes;Sphingobacteria;Sphingobacteriales;Saprosiraceae;genus_NA;species_NA	0.001447	0.001113	0	0.000487	0.002309	8.70E-05	0	0.000666	0.004267	0	0
Bacteria;Chlamydiae;Chlamydiae;Chlamydiales;Simkaniaceae;genus_NA;species_NA	0.000506	0.004781	0.001775	0.000487	0.003822	0.003561	0	0.00125	0.001158	0.000313	0
Bacteria;Chlorobi;Chlorobia;Chlorobiales;family_NA;genus_NA;species_NA	0	0	0	0.000487	0.00215	0.000782	0.000662	0	0	0.000704	0
Bacteria;Proteobacteria;Deltaproteobacteria;Bdellovibrionales;Bdellovibrionaceae;genus_NA;species_NA	0.000506	0.000196	0.00116	0.000487	0.000557	0	0.000204	0.000167	0.002072	0.000235	0
Bacteria;Bacteroidetes;Flavobacteria;Flavobacteriales;Flavobacteriaceae;genus_NA;species_NA	0.000941	0	0.001365	0.000406	0.002468	0.000695	0	0	0.007802	0	0
Bacteria;											

Bacteria;Chloroflexi;SAR202;orderx_NA;family_NA;genus_NA;species_NA	0	0.000393	0	0	0	0	0.00056	5.00E-04	0	0.001017
Bacteria;Deferrribacteres;Deferrribacteres;Deferrribacterales;family_NA;genus_NA;species_NA	0	0	0	0	8.00E-05	8.70E-05	0.002496	5.00E-04	0.00061	0.001252
Bacteria;Fibrobacteres;Fibrobacteria;Fibrobacterales;family_NA;genus_NA;species_NA	0	0	0	0	0	0	0.001121	0	0	0.001252
Bacteria;Firmicutes;Bacilli;Bacillales;Bacillaceae;genus_NA;species_NA	0	0	0.000273	0	0	0.001477	0	0	0	0
Bacteria;Firmicutes;Clostridia;Clostridiales;Veillonellaceae;genus_NA;species_NA	0.002026	0	0	0	0	0	0	8.30E-05	0	0
Bacteria;Firmicutes;Clostridia;Halanaerobiales;Halobacteroidaceae;genus_NA;species_NA	0	0	0	0	0	0	0.028021	0	0	0
Bacteria;Lentisphaerae;Lentisphaeria;Victivallales;Victivallaceae;genus_NA;species_NA	0	0	0	0	0	0	0.001121	0	0	0.000782
Bacteria;Proteobacteria;Alphaproteobacteria;Rhizobiales;Aurantimonadaceae;genus_NA;species_NA	0	0.000327	0	0	0.001274	0	0	0	0	0.000313
Bacteria;Proteobacteria;Gammaproteobacteria;Alteromonadales;diomarinaceae;genus_NA;species_NA	0	0	0	0	0.001035	0	0	0	0	0
Bacteria;Proteobacteria;Gammaproteobacteria;Legionellales;Coxiellaceae;genus_NA;species_NA	0	0	0.001024	0	0	0.000261	0	0.00025	6.10E-05	0
Bacteria;Proteobacteria;Gammaproteobacteria;Legionellales;Legionellaceae;genus_NA;species_NA	0	0.002292	0.000341	0	0.000159	0	0	0.000417	0.000122	7.80E-05
Bacteria;Proteobacteria;Gammaproteobacteria;Thiotrichales;family_NA;genus_NA;species_NA	0	0.001048	0	0	8.00E-05	0	0	0.001	0.000244	0
Bacteria;Spirochaetes;Spirochaetes;Spirochaetales;Leptospiraceae;genus_NA;species_NA	0	0	0	0	0	0.000695	0	0	0.002011	0.000235
Bacteria;Verrucomicrobia;Spartobacteria;Chthoniobacter;family_NA;genus_NA;species_NA	0	0.014145	0.000614	0	8.00E-05	0	0	0.000167	0	0
Bacteria;Verrucomicrobia;Spartobacteria;orderx_NA;family_NA;genus_NA;species_NA	0	0.005566	0.000137	0	8.00E-05	0	0	5.00E-04	0	0
Bacteria;WS1;class_NA;orderx_NA;family_NA;genus_NA;species_NA	0	0	0	0	0	0	0.001172	0	0	0.001017
	0.254158	0.296921	0.23821	0.41837	0.456372	0.255799	0.741899	0.467181	0.429602	0.69788

2011 Culture-Dependent	Saline Lake-RM
Bact;Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Marinobacter	0.235399
Bact;Proteobacteria;Gammaproteobacteria;Oceanospirillales;Halomonadaceae;Halomonas	0.183291
Bact;Firmicutes;Clostridia;Clostridiales;Clostridiaceae;Clostridiisalibacter	0.033267
Bact;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus	0.027669
Bact;Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;Alteromonas	0.024762
Bact;Firmicutes;Clostridia;Halanaerobiales;Halanaerobiaceae;Halanaerobium	0.001561
Bact;Firmicutes;Clostridia;Clostridiales;Clostridiaceae	0.000915
Bact;Proteobacteria;Deltaproteobacteria;Desulfovibrionales;Desulfovibrionaceae;Desulfovibrio	0.000592
Bact;Firmicutes;Clostridia;Clostridiales	0.000161
Bact;Firmicutes;Clostridia;Clostridiales;Clostridiaceae;Clostridium	0.285837
Bact;Proteobacteria;Gammaproteobacteria;Alteromonadales;Idiomarinaceae;Idiomarina	0.057114
Bact;Firmicutes;Clostridia;Clostridiales;Clostridiaceae;Clostridiisalibacter;paucivora	0.04371
Bact;Proteobacteria;Deltaproteobacteria;Desulfovibrionales;Desulfovibrionaceae;Desulfovibrio;halophilus	0.043495
Bact;Bacteroidetes;Bacteroidia;Bacteroidales;Marinilabiaceae;Anaerophaga	0.018356
Bact;Firmicutes;Bacilli;Bacillales;Bacillaceae;Bacillus;chandigarhensis	0.017549
Unknown	0.007859
Bact;Firmicutes;Clostridia;Halanaerobiales;Halobacteroidaceae;Orenia;marismortui	0.005222
Bact;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae	0.00323
Bact;Firmicutes;Clostridia;Clostridiales;Clostridiaceae;Caloranaerobacter	0.001453
Bact;Firmicutes;Clostridia;Halanaerobiales;Halanaerobiaceae;Halocella	0.001238
Bact;Firmicutes;Bacilli;Bacillales;Bacillaceae;Paraliobacillus;quinghaiensis	0.001184
Bact;Firmicutes;Bacilli;Bacillales;Bacillaceae;Paraliobacillus	0.000861
Bact;Firmicutes;Clostridia;Clostridiales;Clostridiaceae;Caminicella	0.000861
Bact;Bacteroidetes;Sphingobacteria;Sphingobacteriales	0.000269
Bact;Firmicutes;Clostridia;Clostridiales;Peptostreptococcaceae;Fusibacter	0.000269
Bact;Firmicutes;Clostridia;Clostridiales;Clostridiaceae;Alkaliphilus	0.000161
Bact;Firmicutes;Clostridia;Clostridiales;Clostridiaceae;Clostridium;caminithermale	5.40E-05
Bact;Firmicutes;Clostridia;Clostridiales;Clostridiaceae;Geosporobacter	5.40E-05

COLOR KEY

Taxa shared with other datasets for the same site

2011 Cultured-Dependent dataset	Saline Lake-RM	Halophilic
Bact;Proteobacteria;Deltaproteobacteria;Desulfovibrionales;Desulfovibrionaceae;Desulfovibrio	0.044087	Sulfur Metabolizers
Bact;Proteobacteria;Gammaproteobacteria;Oceanospirillales;halomonadaceae;halomonas	0.183291	
Bact;Firmicutes;Clostridia;Halanaerobiales;halobacteroidaceae;Orenia;marismortui	0.005222	
Bact;Firmicutes;Clostridia;Halanaerobiales;Halanaerobiaceae;halocella	0.001238	
Bact;Proteobacteria;Gammaproteobacteria;Alteromonadales;Alteromonadaceae;marinobacter	0.235399	
Bact;Proteobacteria;Gammaproteobacteria;Alteromonadales;Idiomarinaceae;Idiomarina	0.057114	
Bact;Firmicutes;Clostridia;Clostridiales;Clostridiaceae;Clostridiisalibacter	0.076977	
Bact;Bacteroidetes;Bacteroidia;Bacteroidales;marinilabiaceae;Anaerophaga	0.018356	
Bact;Firmicutes;Clostridia;Halanaerobiales;Halanaerobiaceae;Halanaerobium	0.001561	
Bact;Firmicutes;Clostridia;Clostridiales;Clostridiaceae;Caloranaerobacter	0.001453	
Total	0.624698	

2011 Culture-Dependent dataset	Solar Lake-W	COLOR KEY
Bact;Proteobacteria;Deltaproteobacteria;Desulfovibrionales;Desulfovibrionaceae;Desulfovibrio	0.071825	Taxa shared with other datasets for the same site
Bact;Firmicutes;Clostridia;Clostridiales;Clostridiaceae;Clostridiisalibacter	0.041737	
Bact;Proteobacteria;Gammaproteobacteria;Vibrionales;Vibrionaceae;salinivibrio	0.010029	
Bact;Proteobacteria;Gammaproteobacteria;Oceanospirillales;halomonadaceae;halomonas	0.000062	
Bact;Proteobacteria;Gammaproteobacteria;Vibrionales;Vibrionaceae;Vibrio	0.545755	
Bact;Firmicutes;Clostridia;Clostridiales;Clostridiaceae	0.001807	
Bact;Firmicutes;Clostridia;Clostridiales	0.000249	
Bact;Bacteroidetes;Bacteroidia;Bacteroidales;marinilabiaceae	0.070952	
Bact;Firmicutes;Clostridia;Clostridiales;Clostridiaceae;Clostridium	0.021616	
Bact;Firmicutes;Clostridia;Clostridiales;Clostridiaceae;Caminicella	0.017255	
Unknown	0.012646	
Bact;Proteobacteria;Gammaproteobacteria;Alteromonadales;Pseudoalteromonadaceae;Pseudoalteromonas	0.000374	
Bact;Firmicutes;Clostridia;Clostridiales;Peptostreptococcaceae;Fusibacter	0.000311	
Bact;Bacteroidetes;Sphingobacteria;Sphingobacteriales	0.000249	
Bact;Bacteroidetes;Bacteroidia;Bacteroidales;marinilabiaceae;Anaerophaga	0.013269	
Bact;Proteobacteria;Deltaproteobacteria;Desulfobacterales;Desulfobacteraceae;Desulfocella	0.010029	
Bact;Proteobacteria;Gammaproteobacteria;Vibrionales;Vibrionaceae;salinivibrio;costicola	0.001495	
Bact;Proteobacteria;Gammaproteobacteria;Vibrionales;Vibrionaceae;salinivibrio;proteolyticus	0.001184	
Bact;Firmicutes;Clostridia;halanaerobiales;halobacteroidaceae;Orenia;marismortui	0.00081	
Bact;Firmicutes;Clostridia;Clostridiales;Clostridiaceae;Geosporobacter	0.046596	
Bact;Firmicutes;Clostridia;Clostridiales;Clostridiaceae;Clostridium;botulinum	0.040553	
Bact;Firmicutes;Clostridia;Clostridiales;Clostridiaceae;Clostridium;caminithermale	0.031271	
Bact;Proteobacteria;Gammaproteobacteria;Vibrionales;Vibrionaceae;Vibrio;parahaemolyticus	0.024917	
Bact;Bacteroidetes;Bacteroidia;Bacteroidales;Porphyromonadaceae	0.01277	
Bact;Proteobacteria;Gammaproteobacteria;Vibrionales;Vibrionaceae;Vibrio;vulnificus	0.004236	
Bact;Spirochaetes;Spirochaetes;Spirochaetales;Spirochaetaceae;Spirochaeta;bajacaliforniensis	0.002803	
Bact;Firmicutes;Clostridia;Clostridiales;Lachnospiraceae	0.002243	
Bact;Proteobacteria;Gammaproteobacteria;Vibrionales;Vibrionaceae;Vibrio;alginolyticus	0.001807	
Bact;Proteobacteria;Gammaproteobacteria;Vibrionales;Vibrionaceae;Aliivibrio	0.00162	
Bact;Proteobacteria;Gammaproteobacteria;Vibrionales;Vibrionaceae;Vibrio;harveyi	0.001495	
Bact;Proteobacteria;Gammaproteobacteria;Vibrionales;Vibrionaceae	0.001246	
Bact;Proteobacteria;Alphaproteobacteria;Rhodobacterales;Rhodobacteraceae;Nesiotobacter;exalbescens	0.001184	
Bact;Proteobacteria;Gammaproteobacteria;Vibrionales;Vibrionaceae;Photobacterium	0.000374	
Bact;Proteobacteria;Gammaproteobacteria;Vibrionales;Vibrionaceae;Photobacterium;leiognathi	0.000187	
Bact;Firmicutes;Clostridia;Clostridiales;Clostridiaceae;Caloranaerobacter	0.000125	
Bact;Firmicutes;Clostridia;Clostridiales;Unassigned;Anaerovorax	6.20E-05	

2011 Culture-Dependent Dataset	Solar Lake-W	Halophilic
Bact;Proteobacteria;Deltaproteobacteria;Desulfovibrionales;Desulfovibrionaceae;Desulfovibrio	0.071825	Sulfur Metabolizers
Bact;Bacteroidetes;Bacteroidia;Bacteroidales;marinilabiaceae	0.070952	
Bact;Firmicutes;Clostridia;Clostridiales;Clostridiaceae;Clostridiisalibacter	0.041737	
Bact;Bacteroidetes;Bacteroidia;Bacteroidales;marinilabiaceae;Anaerophaga	0.013269	
Bact;Proteobacteria;Gammaproteobacteria;Vibrionales;Vibrionaceae;salinivibrio	0.012708	
Bact;Proteobacteria;Deltaproteobacteria;Desulfobacterales;Desulfobacteraceae;Desulfocella	0.010029	
Bact;Firmicutes;Clostridia;halanaerobiales;halobacteroidaceae;Orenia;marismortui	0.00081	
Bact;Proteobacteria;Gammaproteobacteria;Oceanospirillales;halomonadaceae;halomonas	0.000062	
Total	0.221392	