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SYMPHONY OF THE SEAS: UNVEILING METAGENOMIC BIODIVERSITY THROUGH 16S rRNA IN THE RED SEA

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SUMMARY

The Red Sea is the habitat of rare microbial communities that have evolved in extreme environmental conditions, but their potential for antibiotic production has not been fully explored. The aim of the study was to examine the distribution and diversity of antibiotic-producing bacteria and actinomycetes in the six sites (A1-A6) of the Saudi Arabian Red Sea. With the help of the sequencing of 16S rRNA genes and bioinformatic analysis, the individual bacterial communities have been identified. The most diverse location was location A5, which had the largest number of antibiotic-producing families, such as Arcobacteraceae, Vibrionaceae, and Pseudoalteromonadaceae. The relative abundance of the Micrococcaceae was statistically determined, and its presence was constant (ranging between 0.1 and 0.3) at all locations. The findings indicated that there was variability in the potential of producing antibiotics among all the sites, and Location A5 was found to be the most promising site in bioprospecting. Conversely, sites A1 and A4 had moderate antibiotic-producing potentials, with sites A2, A3, and A6 having lesser diversity and having fewer antibiotic-producing bacteria. These results indicate the exceptional importance of environmental factors in the development of microbial communities and designate Location A5 as a key location to find new antibiotics. The experiment offers information on the microbial biodiversity of the Red Sea and offers a significant source of guidance to future studies on bioprospecting in extreme marine environments.

Key words: *metagenomic analysis, marine actinobacteria, red sea biodiversity, 16s RRNA, environmental adaptation.*

INTRODUCTION

The marine environment is inhabited by Actinobacteria, which are particularly important in biotechnology and drug discovery. Various communities of actinobacteria are present in deep-sea sediments of the Arctic and Atlantic Ocean basins, and have great potential for different biological activities [1]. Actinobacteria are abundant in marine algae and a rich source of bioactive metabolites, promising a great potential for their bioactive metabolites as novel drugs [2]. Furthermore, actinobacteria associated with marine invertebrates have revealed their potential to produce secondary metabolites with diverse biological activities such as antibiotics, cytotoxic compounds, and enzymes. These compounds have utility in biotechnology, agriculture, and in pharmaceuticals [3]. Furthermore, marine actinomycetes possess the longevity of viability and antibiotic activity even during prolonged storage, making them potential sources of novel antibiotics [4]. Actinobacteria from marine sources are a rich and largely unexplored reservoir of potentially bioactive compounds with important implications for future progress in biotechnology and pharmaceutical development. This paper deals with the deficiency in knowledge about the potential of microorganisms that inhabit the extreme environment of the Red Sea as antibiotic producers. It also shows the significance of investigating this area to obtain new bioactive compounds, especially due to the increasing resistance to antibiotics, and provides useful insights for future bioprospecting and pharmaceutical studies.

Because of the tremendous potential of actinobacteria in the discovery of bioactive compounds, they are a rich resource for drug development. As a result, they have gained attention as a substantial reservoir of bioactive compounds of vital importance in pharmaceutical applications. Of note, the actinobacteria of the Micrococcus genus possess impressive biosynthetic prowess and the ability to exhibit antibacterial, antifungal, cytotoxic, antioxidant, and anti-inflammatory activities despite relatively small genomes [5].

Endophytic actinobacteria associated with ethnobotanical medicinal plants have been demonstrated to be especially vibrant sources of bioactive compounds with antimicrobial activity [6]. Actinomycetes have shown a consistent ability to produce novel bioactive compounds, and their formidable capacity is especially noted when they grow in extreme natural environments, such as deep-sea biotopes and hot springs [1][7]. In addition, habitat-specific marine actinobacteria from the Arctic have been explored for the discovery of bioactive metabolites [8].

The Red Sea is a unique marine ecosystem due to its exceptional biodiversity. A recent study has demonstrated remarkable thermal tolerance for reef corals [9], another study has established enduring stability and health of Red Sea waters [10], and similarly, another study has shown flourishing macroalgal communities supported by mangroves [11]. In another recent study, it has been shown that four to five hundred of the Red Sea can support more than 1,500 species, and the dynamics of circulation and the abundance of coral reefs [12], all characterize this ecosystem with a very high biodiversity, making it unique. These peculiarities create a refuge for reef corals and favor the spread of macroalgae, changing the genetic diversity of reef species. In addition, low heavy metal pollution and its exceptional ecosystem, coupled with high biological biodiversity, are also outstanding in the Red Sea.

Due to its unique attributes, the Red Sea ecosystem is a fascinating metagenomic focus. The sea is an exceptional and unique habitat involving extreme salinity, high temperature, oligotrophy, and continuous emissions of hydrocarbons from deep-sea vents and those of heavy oil tanker traffic [13]. It has been reported that the Red Sea is located in the Global Dust Belt, which means that it is susceptible to substantial dust accumulations in neighboring arid regions [14][15]. The Red Sea, an appealing site to investigate the influence of dust deposition on the diverse microbiota, is a semi-enclosed ‘’megacosm.

In addition, Red Sea mangrove sediments serve as reservoirs for exceptional concentrations and microbial diversity. It has also been indicated that the Red Sea microbiota is highly active from a biosynthetic point of view since they produce bioactive compounds with antibacterial, cytotoxic, and/or inhibitory activities. Such distinctiveness favors studying microbial adaptation, diversity, and changes in marine ecosystems induced by environmental alterations in the Red Sea. The Red Sea Littoral Regions and coasts surrounding Jeddah, Saudi Arabia, have been reported to be subject to a variety of ecological factors. While the mangrove habitats have been degraded by coastal urban growth and the mangrove vegetation depleted, so too have the risks of heavy metal contamination risen [16]. From slightly to moderately disturbed macrobenthic species in the Gulf waters of Saudi Arabia, there is less stress from open waters than inner harbors [17]. Heavy metals have been reported to contaminate sediments on the eastern coast of the Red Sea near Jeddah, which are notorious, and are concentrated mostly in the central and southern areas [18]. The most predominant phylum at the littoral of the Red Sea near Jeddah is Annelida [19]. This area harbors diverse marine benthic micro-eukaryotic communities. There are regions in the coastal areas bordering the Red Sea at Jeddah that are known to be prone to harmful algal outbreaks and worsen the biofouling risks to desalination plants during winter [20].

Unexplored actinobacterial species may colonize coasts along the Red Sea near Jeddah, Saudi Arabia. The molecular composition of antibiotic-producing microbes associated with soft corals in the Red Sea has been studied. Two isolates, belonging to the class Actinobacteria, indicate the possibility of the occurrence of previously unknown actinobacterial species in this region. In addition, a study of the microbial diversity in mangrove sediments of the Red Sea isolated a novel species, *Nitratireductor thuwali* [13]. Adapted to oligotrophic, extreme conditions, this aerobic, heterotrophic bacterium survives. This also suggests that the mangrove sediments in the Red Sea may host yet unknown bacterial species, some of which may be actinobacteria [13]. Firstly, these studies highlight the necessity for more biotechnological potential and microbial diversity studies in the coastal Red Sea regions of Jeddah.

Several factors limit the understanding of microbial diversity in the Red Sea of Saudi Arabia. First of all, there is a noticeable absence of extensive research efforts to examine the microbial community living inside this kind of niche. Moreover, microbiome studies in Saudi Arabia have mainly concentrated on environmental and ecological aspects with little or no practical implications for the results, strictly limiting the scope of research [16]. The medical applications of thermophilic microorganisms in hot springs of Saudi Arabia are another unexplored area in research. Also, the microbial diversity of the understudied mangrove habitat in Saudi Arabia is still not effectively studied, hiding some very specialized microbial strains that are only present in this environment. Uncovering the potential applications for microbial diversity in the Red Sea and other unique ecosystems in Saudi Arabia will necessitate a deeper, more comprehensive examination of this incredibly diverse microbial landscape.

Extraction and analysis of microbial diversity were once limited to a handful of bacterial 'model' species by molecular markers such as the 16S rRNA gene, but the application of these molecular markers is vital to study the evolutionary relationships and patterns of the microbial community across a range of habitats [12]. Because the 16S rRNA gene is used so commonly to explore bacterial biogeographical patterns while retaining taxonomic and diversity information across multiple datasets, this is an appropriate use case. Nevertheless, estimations of microbial diversity by the 16S rRNA gene alone can be clouded by its intrinsic limitations of low taxonomic resolution and intragenomic variability. While those limitations exist, the examination of variable regions in the 16S rRNA gene provides a valuable basis on which to study the composition and structure of microbial communities. These analyses can provide answers to some important questions about how biodiversity is driven and how microbial communities are organized across different habitats.

To the best of our knowledge, the metagenomic diversity of the Red Sea in Saudi Arabia has not been studied definitively. Consequently, this study aims to enhance the metagenomic analysis by using 16S rRNA gene sequences to investigate actinobacteria biodiversity in the Red Sea ecosystems of Saudi Arabia.

Key Contribution

1. Determination of the most diverse antibiotic-producing bacterial families, such as Arcobacteraceae, Vibrionaceae, Pseudoalteromonadaceae, and Micrococcaceae, among six sites of the Red Sea, and Location A5 exhibits the best potential for bioprospecting new antibiotics.

2. The identification of Micrococcaceae as the most important, stable family found in all locations, with implications of its ecological flexibility and stable position in the harsh environment of the Red Sea.

3. Evidence of the high variability of production potential of antibiotics among different sites, which shows the relevance of site-relevant environmental conditions in determining the microbial community structure and applications in biotechnology.

Based on the literature review, marine ecosystems, especially in extreme environments, such as the Red Sea, are a good source of antibiotic-producing bacteria, such as actinobacteria and other microbial families. Recent research has pointed out how marine microorganisms, in particular deep-sea sediment and mangrove microorganisms, have the potential to produce bioactive compounds with antibacterial, antifungal, and cytotoxic properties. The studies have established that salinity, temperature, and nutrient levels, as environmental factors, have an effect on the microbial diversity and antibiotic production. The rare ambient conditions in the Red Sea, such as salinity levels, temperature limits, and limitation of nutrients, favor the abundance of hardy microbial communities that pose a high biotechnological potential. Past research indicates that more research is required on these ecosystems, since they could provide new strains of microbes with potential uses in fighting antibiotic resistance.

This paper will discuss the peculiar microbial communities associated with the Red Sea with respect to their potential in the production of new antibiotics. It starts with an emphasis on the extreme environmental conditions and biodiversity of the Red Sea, and then gives a description of the sampling strategy, DNA extraction, 16S rRNA gene amplification, and bioinformatic analysis, which were used to measure the microbial diversity. The findings have identified unique families of bacteria, such as Arcobacteraceae, Vibrionaceae, Pseudoalteromonadaceae, and Micrococcaceae, with statistical information about their relative abundance and ability to produce antibiotics, especially in Location A5. These findings are interpreted and discussed in the context of the ecological role of the identified families and the impact of the environmental factors on the diversity of microbes. The paper has concluded with the recommendation of Location A5 as a good bioprospecting potential site, and more needs to be explored to overcome the challenges of antibiotic resistance.

MATERIALS AND METHODS

This section reveals the methods and procedures that were used to investigate the distribution and diversity of antibiotic-producing bacteria and actinomycetes in the Red Sea. The experiment was carried out in six sampling sites (A1-A6) along the Saudi Arabian coast of the Red Sea, which were chosen based on the respective environmental factors. This study was carried out in a number of phases that include sampling of the sediments, DNA extraction and amplification, sequencing, bioinformatics analysis, and identification of bacteria.

Sampling

The experiment started with a collection of 30 samples from six locations (A1-A6) with 5 replicates in each sample location (Table 1), each sampling a unique maritime ecosystem in Saudi Arabia's marine sediments and seawater. There was 1 month of periods for sampling over two days in each region; five ecosystems near the Red Sea in Jeddah were selected as sampling points (Figure 7). The microbial integrity was preserved via transport to the laboratory for analysis of samples, which were stored in ice boxes immediately after collection.

DNA Extraction and Amplification

The samples were submitted to standardized extraction protocols optimized for actinobacteria for total genomic DNA extraction. Universal bacterial primers were used to PCR amplify the 16S rRNA gene. Optimization was performed for thermal cycling conditions and reagents to achieve a high yield and specificity of the target gene.

Purification and Sequencing

Purification of the PCR products was performed using a commercial kit, following the manufacturer's instructions. Automated DNA sequencing technology (Sanger method) was used to sequence the purified PCR products, and sequence quality was validated before analyzing the sequence data.

Bioinformatics Analysis

Using the AL 16S program, the resulting 16S rRNA gene sequences were manually aligned against actinobacterial sequences from databases such as DDBJ, EMBL, and GenBank. Phylogenetic estimation was performed using least squares, maximum likelihood, and neighbor joining of the PHYLIP suite. Evolutionary distance matrices were calculated. To evaluate the robustness of tree topologies, bootstrap analyses using 1000 resampling iterations were performed.

Physiological and Biochemical Characterization

Physiological and biochemical analyses of the isolates were performed following established protocols for actinobacterial taxonomy. These strains were taxonomically classified, and the phenotypic and genetic characteristics of the resulting strain were interpreted.

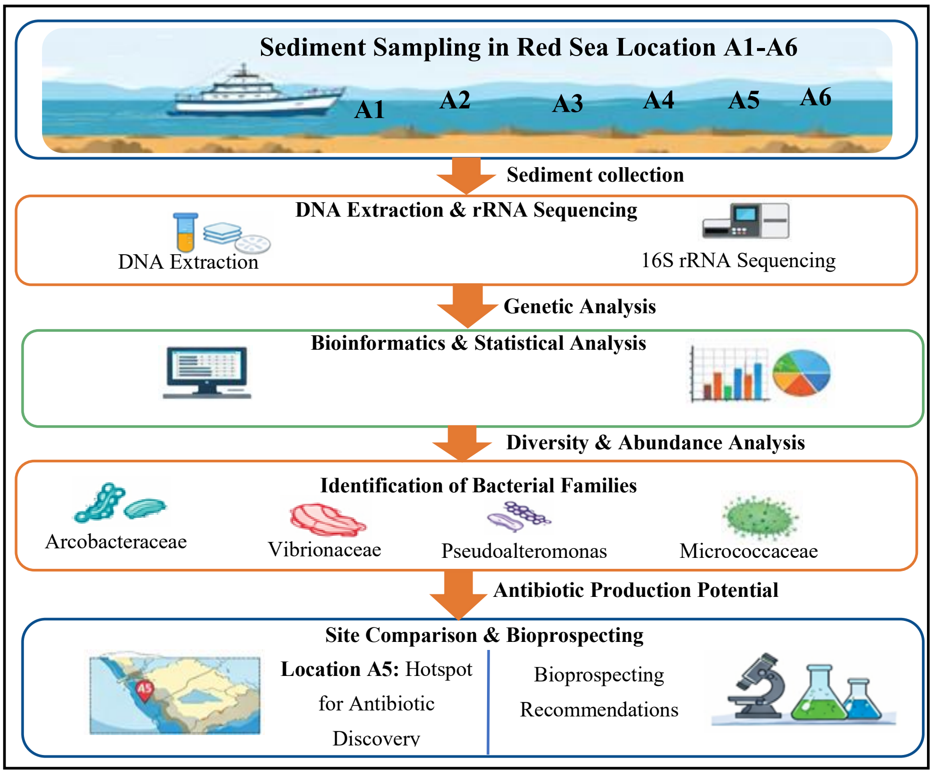

Figure 1. Methodological flow diagram of antibiotic-producing bacteria identification in red sea marine sediments

The general methodology that was used in this research is shown in figure 1. The flowchart gives a visualization of the procedures to be taken in the research process, starting with the sampling of the sediment at the six Red Sea sites (A1-A6) through the eventual bioprospecting suggestions. The process has been indicated in the diagram with all the main steps involved: DNA extraction, 16S rRNA sequencing, bioinformatic analysis, bacterial family identification, and site comparison, which finally led to the discovery of Location A5 as the best antibiotic discovery site.

RESULTS

This study was analyzed through bioinformatics and the available software tools to perform the required processing and analysis on the 16S rRNA gene sequences. Alignment of sequences was done by the AL

16S program, and the estimation of phylogenetic relationships was performed by PHYLIP least squares and maximum likelihood application together with the neighbor-joining algorithm. To get an accurate interpretation of the microbial composition of various sampling sites, statistical analyses (bacterial diversity and abundance) of the samples (using the hierarchical clustering and comparative analysis) were performed using software programs such as R and other pertinent bioinformatics sites as well.

In figure 2 shows the bacterial family phylogenetic distribution and relative abundances of samples A1-A6 as determined from this hierarchical clustering analysis. The clustering based on the bacterial community composition is represented by the dendrogram, and each bar represents the proportional abundance of the different bacterial families. This shows niche-specific microbial diversity where noteworthy families include *Bradymonadaceae* and *Flavobacteriaceae*, which are variably dominant in different samples. This clustering pattern points to the influence of community assembly by environmental selection pressures, perhaps related to habitat characteristics in each of the sampled environments that were studied.

Micrococcaceae had variable abundance levels across samples A1 to A6, between ~0.1 and 0.3 relative abundance (Figure 2). These samples consistently showed the presence of the family and a trend of increasing concentration in specific samples. For instance, an abundance of *Micrococcaceae* was seen to rise from sample A1 to between sample A5 (0.3) and sample A11 (0.27) before decreasing again slightly in sample A6. The presence of this *Micrococcaceae* pattern indicates that localized environmental conditions in the Red Sea may be suitable for the growth of this family, suggesting ecological niches where this family can live, under specific conditions due to its metabolic adaptability to different marine stressors.

Moraxellaceae was noticed to be dominant across many locations, especially locations A2, A3, and A6. However, Saccharospirillaceae indicated a strong presence in A1 and A4, but Flavobacteriaceae was notably seen in A1. By considering the families of antibiotic-producing bacteria found in each of the locations, Actinobacteriaceae were detected in A5, Pseudoalteromonadaceae were found in A4, while Flavobacteriaceae were detected in A1. A5 showed the highest bacterial diversity, while A3 and A6 showed the lowest diversity. Each location displayed a unique structure of the microbial community.

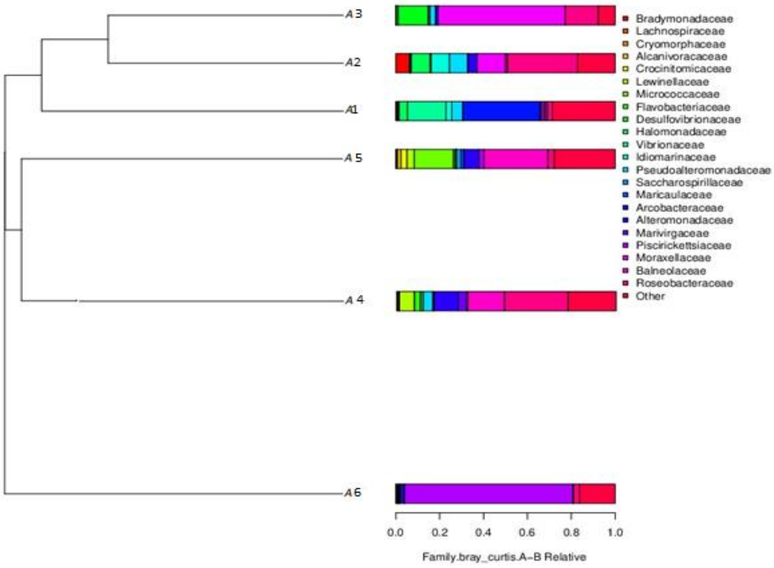

Figure 2. Hierarchical clustering of bacterial family abundances in environmental samples a6-a11: phylogenetic distribution and composition

This stacked bar chart details the bacterial family composition in the total Red Sea, with a quantitative breakdown of relative abundance (range: 0-1.0) (Figure 3). A large number of notable families (*Flavobacteriaceae*, for example) and significant contributions from other groups characterize the bacterial majority. These families are distributed in a stratified community structure adaptive to the marine environment. However, distinct stratification like this could reflect particular roles in nutrient cycling (presumably because each role requires a different set of enzymes), suggesting that the Red Sea microbial ecosystem may have evolved along different tracks.

The Microbial biodiversity of the Red Sea showed *Micrococcaceae* maintaining a stable relative abundance of 0.1 and their presence throughout this part of the sample (Figure 3). Such a consistent low-to-moderate abundance indicates that although resilient across disparate samples, *Micrococcaceae* is sensitive to local habitat factors in relative abundance. The location under study from the Red Sea showed a steadier level, perhaps due to less favorable conditions among other environmental locations. This suggests that *Micrococcaceae* might be playing a less dominant role in this microbial community and evolving according to different ecological pressures.

Considering the relative abundance of the antibiotic-producing microbiome, *Actinobacteriaceae* identified in location A5 had ~5% abundance; *Pseudoalteromonadaceae* in locations A4 and A5 had 10% and 5%, respectively; *Flavobacteriaceae* found in A1 had 15%; *Vibrionaceae* detected in A5 had 10%. Location A5 indicated the highest diversity of antibiotic-producing families. The distribution pattern found in each of the locations suggests that location A5 may be a promising site for microbiomes that produce antibiotics, considering the fact that some families of microbiomes known for producing antibiotics are found in the location.

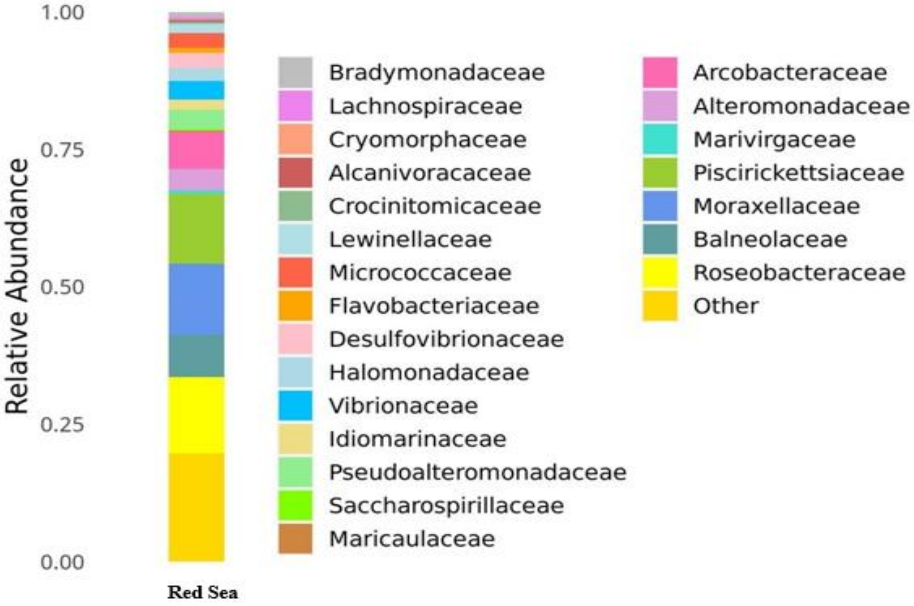

Figure 3. Bacterial family composition and relative abundance in red sea marine sediment

A stacked bar plot suggests a direct comparison of bacterial family distributions among locations A1–A6 (Figure 4). The diversity at the family level also varies a lot, with *Piscirickettsiaceae* being nearly restricted to sample A6 (Figure 4) and *Moraxellaceae* fluctuating in abundance among samples. In yellow, the consistent (and many) presence of "Other" bacteria suggests taxa that are present, but at very low abundance and infrequently across samples. A comparative analysis of this kind suggests that environmental factors can guide microbial diversity, with a few families that become dominant in distinct habitats due to selection pressures applied by local conditions.

A detailed look at samples A1 to A6 revealed that *Micrococcaceae* varied as follows: Minimal presence was noted for A6, while A1 (0.15-0.2), A2 (0.1), A3 (0.15), A4 (0.2), and A5 (0.25-0.3) showed presence (Figure 4). This variation indicates that the abundance of *Micrococcaceae* appears to shift in response to habitat, with higher relative concentrations of this family in samples A1 to A5. The fact that A6 decreased is consistent with the idea that, instead, environmental factors or competitive dynamics are controlling the persistence of *Micrococcaceae* in these habitats.

Location A5 is the site that revealed the highest likelihood for antibiotics due to the diverse community of antibiotic-producing bacteria comprising the families *Arcobacteraceae*, *Vibrionaceae*, and *Pseudoalteromonadaceae*, and a small proportion of *actinomycete*-related families (Figure 4). Locations A1 and A4 demonstrated moderate suitability, where the *Flavobacteriaceae* comprised approximately 15%, with the presence of the *Pseudoalteromonadaceae* family located at A4, approximately 10%. In contrast, Locations A2, A3, and A6 showed low levels of diversity and had few, if any, antibiotic-producing bacteria or *actinomycetes*. From the current study, it emerges that Location A5 holds the greatest potential for bioprospecting novel antibiotics in addition to Locations A1 and A4.

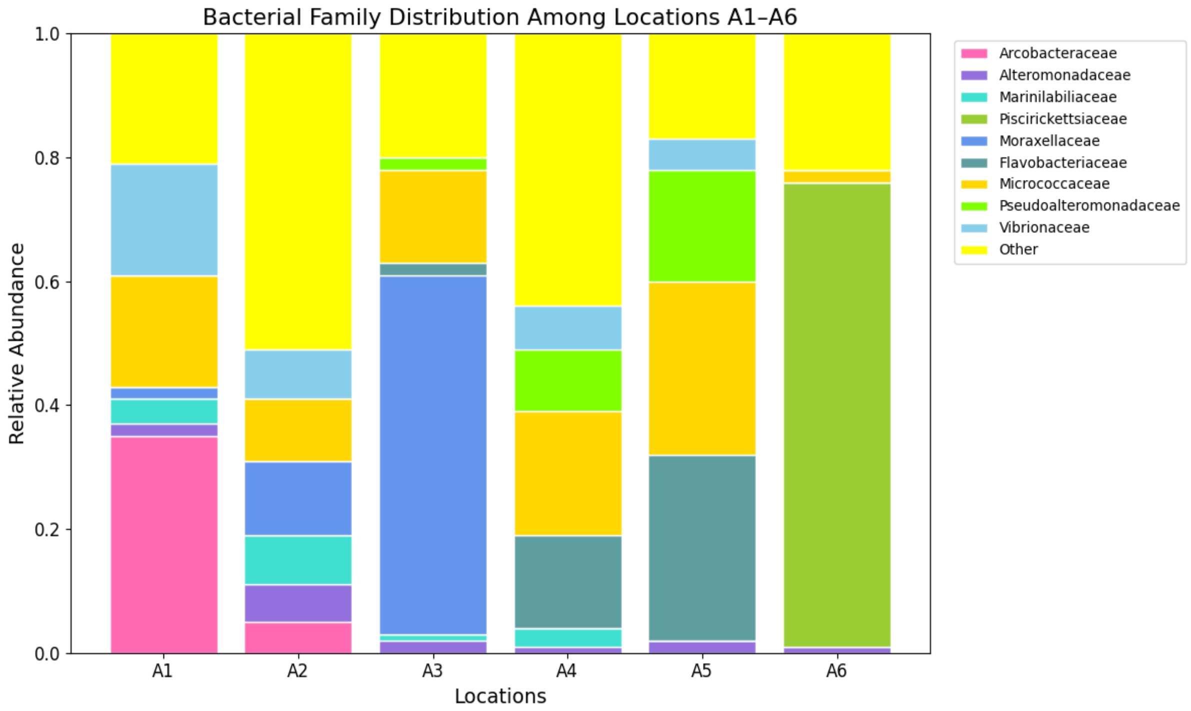

Figure 4. Comparative bacterial family composition in environmental locations A1-A6: family-level diversity and abundance

The microbial community relationships among samples A1–A5 are expressed by this Bray-Curtis dissimilarity heatmap, after adding hierarchical clustering (Figure 5). The community composition variation among samples is shown by a color gradient from red (high similarity) to blue (high dissimilarity). Outlier samples have unique microbial profiles, and sample groupings are revealed according to ecological or spatial proximity. Variation in environmental conditions between sample sites could lead to such dissimilarity patterns due to the impact on microbial adaptation and community structure.

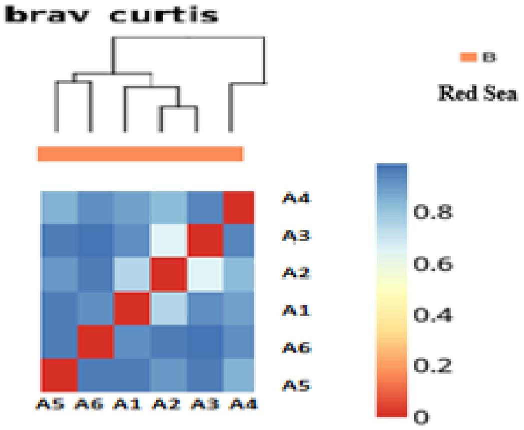

Figure 5. Bray-Curtis's dissimilarity heatmap, after adding hierarchical clustering

In the log-transformed abundance data (Figures 6 and 7), *Micrococcaceae* has a high abundance, shown by a red coloring, with a log10 relative abundance of about 1. The above dominance in log10 scaling clearly suggests that *Micrococcaceae* is present in a greater fraction in some samples. The transformation of the heatmap shows the coherent detection of *Micrococcaceae* over other Actinobacteria, which further points to their potential resilience and ecological relevance in these sampled zones. These results highlight that it is straightforward for log-transformed visualizations to enhance dominance patterns for the microbial community, which may be challenging to discern using raw abundance values, especially when groups have high prevalence but moderate raw abundance values.

A log10-transformed heatmap of bacterial family distributions across Red Sea environmental gradients is shown with hierarchical clustering to show relative abundance (Figure 6). Families, such as the *Flavobacteriaceae* and *Alteromonadaceae,* are differentiated by prevalence using the color scale (blue = low, red = high abundance). A complex microbial community structure is underscored by intermediate abundances (yellow) and rare taxa. This fine-scale view supports a habitat-driven adaptation, and the dominant families may provide insights into selective pressures operating in the Red Sea environment.

Microbacteriaceae was observed with medium representation in all of the sites and was slightly more frequently represented in yellow to light blue squares (Figure 6). On the other hand, *Micrococcaceae* was abundant in some sites to a certain extent, especially in red-orange spots, which indicated that they might have localized high density.

Bacillaceae showed moderate colonization at different sites, which are marked in yellow. One found family was *Pseudoalteromonadaceae*, which appeared in several places; areas colored in red-orange suggested they were a major part of the microbial population. Vibrionaceae was also isolated at a high frequency, with higher distribution in the central regions, with the orange–red stating the same. Also, *Staphylococcaceae* was found to be at a moderate network, and *Erythrobacteraceae* had low to moderate distribution in all the investigated sites.

The *Pseudoalteromonadaceae* family was found to be the most diverse at all the surveyed sites and highly associated with red-marked locations. The distribution of *Vibrionaceae* appeared more intense at the central zones, while *Micrococcaceae* was also found at some discrete points, marked in red here. *Bacillaceae* were moderate throughout the year and did not dominate in any specific region; whereas *Microbacteriaceae* remained moderate throughout the study period.

The most promising candidates for antibiotic production are *Pseudoalteromonadaceae*-rich sites and sites with strong Vibrionaceae representatives. In addition, sites showing co-occurrence of different antibiotic producers belonging to several families are ideal for bioprospecting. From the heatmap analysis, it can be suggested that perhaps these areas, where several potential bacterial families are found densely (yellow to red fields), should be considered as containing diverse microbial populations, which may be of great interest for new antibiotics.

In figure 6, *Micrococcaceae* are present with moderate abundance (orange or salmon in color with a log10 relative abundance near 0). Additionally, the abundance of *Micrococcaceae* is intermediate relative to high-abundance samples, allowing a glimpse into the ability of *Micrococcaceae* to maintain a stable but non-dominant role across a wide range of habitat types. The relative abundance was moderate, indicating that *Micrococcaceae* succeeded in an ecological niche in this part of the Red Sea, which allowed it to persist without outcompeting other taxa, a trait that is consistent with the ability of many *Micrococcaceae* to exploit diverse metabolic pathways under nutrient-limited conditions.

In figure 7 shows this heatmap of log10-transformed relative abundances of bacterial families in sample B from marine environments, going from -3 (low in blue) to 1 (high in red). Some taxa are dominated by key families, for example, *Alteromonadaceae* and *Roseobacteraceae*, and other taxa are observed in low abundance (e.g., *Oceanospirillaceae*). Families with moderate presence appear in the yellow and show a diverse microbial ecosystem. Bacterial family dominance and adaptation are described from this analysis with implications for nutrient cycling and ecosystem function in marine environments.

The bacterial identification of the Red Sea displays a large and diverse group of prospective antibiotic-producing bacteria (Figure 7). *Micrococcae* and relatives of *Actinobacteria* comprised high-abundance families with antimicrobial activity. Higher density of *Pseudoalteromonadaceae, Flavobacteriaceae, Vibrionaceae*, and *Bacillaceae* also improves the antibiotic index, which is comprised of diverse bioactive molecules. Nevertheless, there are potentially untapped antimicrobial potentialities presented by families like *Balneolaceae*, *Roseobacteraceae, Arcobacteraceae*, and *Moraxellaceae* based on the relative abundance observed here. Families of moderate population density include *Erythrobacteraceae*, *Saccharospirillaceae*, and *Marivirgaceae* among the low-density families. Such a diverse and rich bacterial population places the Red Sea as a potential resource for identifying new antibiotics.

In all figure 2-4 (non-log-transformed data) and figure 6–7 (log-transformed heatmaps), *Micrococcaceae* consistently proved to be the most abundant Actinobacterial family with typical relative abundances of 0.1–0.3. The consistent detection of *Micrococcaceae* across all samples further underscores the ecological stability of the family in the microbial community and the potential of the family to serve as a keystone family in the Red Sea's unique marine ecosystem. Across methods, the presence of *Micrococcaceae* is relatively stable, indicating that this genus is well adapted to a variety of environmental stresses in this marine setting.

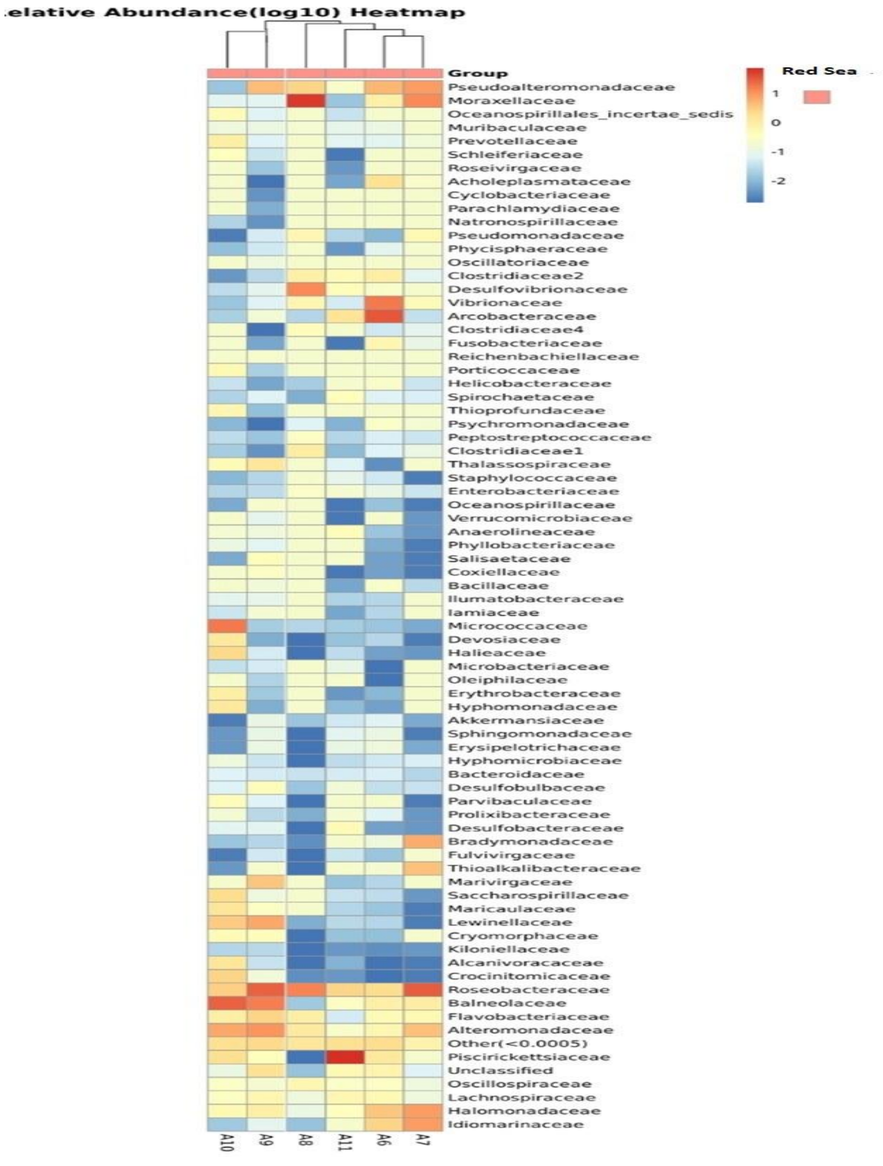

Figure 6. Relative abundance (log 10) heatmap of bacterial family abundance in marine microbiome sample B: log10-transformed heatmap analysis

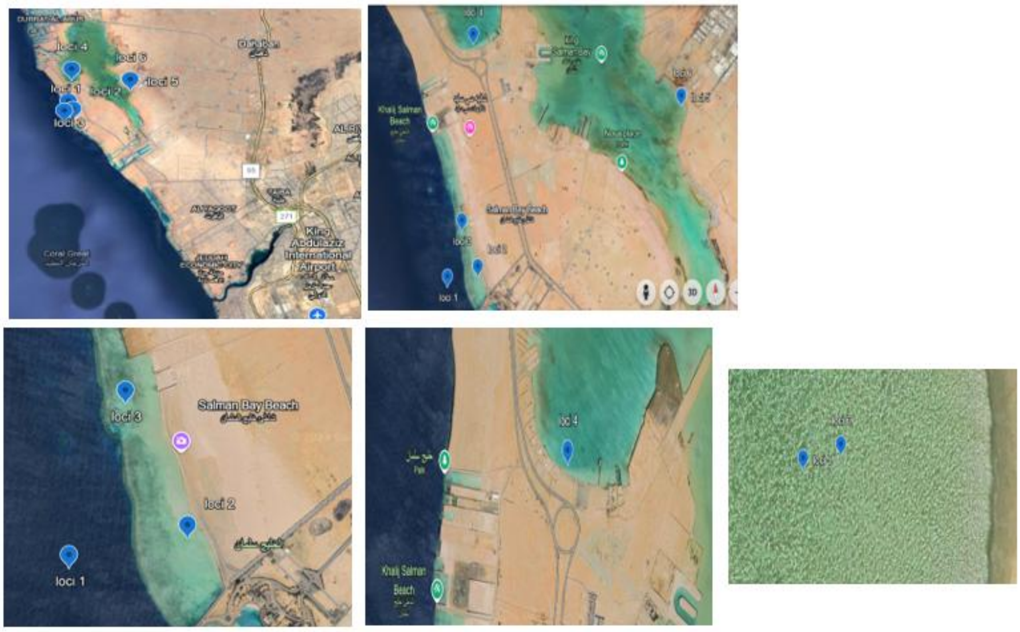

Figure 7. Maps of the samples' locations along the red sea

Table 1. Sampling sites along the red sea

Sample Name	Geographical Coordination
A1	21.8590660,38.970901
A1	21.8590660,38.970901
A1	21.8590660,38.970901
A1	21.8590660,38.970901
A1	21.8590660,38.970901
A2	21.8605948,38.9775245
A2	21.8605948,38.9775245
A2	21.8605948,38.9775245
A2	21.8605948,38.9775245
A2	21.8605948,38.9775245
A3	21.8674615, 38.9740600
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A3	21.8674615, 38.9740600
A3	21.8674615, 38.9740600
A4	21.8954161,38.9765387
A4	21.8954161,38.9765387
A4	21.8954161,38.9765387
A4	21.8954161,38.9765387
A4	21.8954161,38.9765387
A5	21.8861652,39.0218396
A5	21.8861652,39.0218396
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A5	21.8861652,39.0218396
A5	21.8861652,39.0218396
A6	21.8863092,39.0220096
A6	21.8863092,39.0220096

A6	21.8863092,39.0220096
A6	21.8863092,39.0220096
A6	21.8863092,39.0220096

DISCUSSION

Analysis of microbial diversity of the Red Sea marine sediment showed that the sample sites A1- A6 had a great variation in the bacterial species that produce antibiotics and actinomycetes. The site A5 turned out to be the most promising site, indicating the maximum richness of the families of antibiotic producers, such as Arcobacteraceae, Vibrionaceae, and Pseudoalteromonadaceae, and other families of actinomycetes. This observation is consistent with the past research that identified the physicochemical factors of the Red Sea, which promote the richness and diversity of microbes [13]. The uniformity of Micrococcaceae in all sites, where the undue abundance varied between 0.1 and 0.3, is an indication of its ability to adjust to the harsh environment of the Red Sea. It indicates a constant ecological place of Micrococcaceae in this distinctive ecosystem, which is also in agreement with previous studies on the stability of actinobacteria in the marine environment [7]. The presence of Micrococcaceae in the log and raw data further shows that it is dominant in such marine environments.

The results of the current study not only broaden the knowledge on microbial diversity in the Red Sea, but also highlight its possibility to be used in biotechnological applications, especially in antibiotic discovery. The high antibiotic-producing potential at Location A5, coupled with significant populations of Flavobacteriaceae (15%) and Pseudoalteromonadaceae (10%) at A1 and A4, highlights the variability in microbial distribution driven by environmental and selective pressures. The additional indication of local ecological conditions or human activities on the microbial diversity is the presence of lower antibiotic-producing bacteria in the locations of A2, A3, and A6, dominated by the Moraxellaceae [16]. The results support the idea of further research on A5 as one of the hotspots in bioprospecting new bioactive compounds, which may result in new antibiotics. The paper corroborates the potential of the Red Sea as a source of future microbial resources in biotechnology with particular reference to antibiotic resistance.

CONCLUSIONS

This is a critical account of biodiversity and richness of bacteria and actinomycetes that produce antibiotics in the Red Sea sediments. The results have indicated that Location A5 has a potential bioprospective site of novel antibiotics where the array of antibiotic-producing bacterial families, such as Arcobacteraceae, Vibrionaceae, Pseudoalteromonadaceae, and Micrococcaceae, is diverse. Statistics were used to indicate that Micrococcaceae was always observed at all sites with relatively abundant proportions ranging between 0.1 and 0.3, indicating its adaptability to the extreme environment of the Red Sea. This uniform existence highlights its possible contribution to the ecosystem and indicates its significance in antibiotic production within the microbial communities of the Red Sea. The findings of this research play a significant role in marine microbial research, especially in the field of the distribution of antibiotic-producing microbes in harsh environments. The discovery of A5 as a hot spot in the discovery of new antibiotics is important due to the problem of antibiotic resistance in the world.

Future studies must be conducted to further study A5 and other promising sites to isolate and characterize new bioactive compounds. The study of the mechanisms of the high potential of A5 in the production of antibiotics may shed more light on understanding the adaptations of the microorganisms to severe environments. Moreover, the study should be extended to other parts of the Red Sea, and the results should be compared with the findings of other marine ecosystems, which may provide more comprehensive results of biotechnology and pharmaceutical applications.

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REFERENCES

[1] Ribeiro I, Antunes JT, Alexandrino DA, Tomasino MP, Almeida E, Hilário A, Urbatzka R, Leão PN, Mucha AP, Carvalho MF. Actinobacteria from Arctic and Atlantic deep-sea sediments—Biodiversity and bioactive potential. Frontiers in microbiology. 2023 Mar 30;14:1158441. https://doi.org/10.3389/fmicb.2023.1158441

[2] Xiong Z, Wang R, Xia T, Zhang S, Ma S, Guo Z. Natural products and biological activity from actinomycetes associated with marine algae. Molecules. 2023 Jun 30;28(13):5138. https://doi.org/10.3390/molecules28135138

[3] Majithiya VR, Gohel SD. Marine Invertebrates: Diversity and Biological Significance. Actinobacteria: Diversity, Applications and Medical Aspects. 2022 Nov 9:23. https://doi.org/10.5772/intechopen.106642

[4] Sankarapandian V, Jothirajan B, Arasu SP, Subramaniam S, Venmathi Maran BA. Marine biotechnology and its applications in drug discovery. InMarine biotechnology: Applications in food, drugs and energy 2023 May 30 (pp. 189-208). Singapore: Springer Nature Singapore. https://doi.org/10.1007/978-981-99-0624-6_9

[5] Tizabi D, Hill RT. Micrococcus spp. as a promising source for drug discovery: A review. Journal of Industrial Microbiology and Biotechnology. 2023;50(1):1-13. https://doi.org/10.1093/jimb/kuad017

[6] Lang ME, Sibanda T, Louw S, Uzabakiriho JD. Antimicrobial potential of the endophytic actinobacteria isolated from Harpagophytum procumbens: a Southern African medicinal plant. South African Journal of Botany. 2023 May 1;156:268-77. https://doi.org/10.1016/j.sajb.2023.03.030

[7] De Simeis D, Serra S. Actinomycetes: A never-ending source of bioactive compounds—An overview on antibiotics production. Antibiotics. 2021 Apr 22;10(5):483. https://doi.org/10.3390/antibiotics10050483

[8] Schneider YK, Hagestad OC, Li C, Hansen EH, Andersen JH. Selective isolation of Arctic marine actinobacteria and a down-scaled fermentation and extraction strategy for identifying bioactive compounds. Frontiers in microbiology. 2022 Nov 21;13:1005625. https://doi.org/10.3389/fmicb.2022.1005625

[9] Terraneo TI, Ouhssain M, Castano CB, Aranda M, Hume BC, Marchese F, Vimercati S, Chimienti G, Eweida AA, Voolstra CR, Jones BH. From the shallow to the mesophotic: a characterization of Symbiodiniaceae diversity in the Red Sea NEOM region. Frontiers in Marine Science. 2023 Apr 14;10:1077805. https://doi.org/10.3389/fmars.2023.1077805

[10] Rashedy SH, El-Manawy IM, Pereira L. Stability of macroalgal assemblage in the mangrove swamp as an indicator for the health and quality of the Red Sea waters. Egyptian Journal of Aquatic Research. 2023 Jun 1;49(2):245-52. https://doi.org/10.1016/j.ejar.2023.01.004

[11] Diem A, Tesfaldet YT, Hocherman T, Hoon V, Zijlemans K. Marine litter in the Red Sea: Status and policy implications. Marine Pollution Bulletin. 2023 Feb 1;187:114495. https://doi.org/10.1016/j.marpolbul.2022.114495

[12] Wang Y, Raitsos DE, Krokos G, Zhan P, Hoteit I. A Lagrangian model-based physical connectivity atlas of the Red Sea coral reefs. Frontiers in Marine Science. 2022 Oct 28;9:925491. https://doi.org/10.3389/fmars.2022.925491

[13] Marasco R, Michoud G, Sefrji FO, Fusi M, Antony CP, Seferji KA, Barozzi A, Merlino G, Daffonchio D. The identification of the new species Nitratireductor thuwali sp. nov. reveals the untapped diversity of hydrocarbon-degrading culturable bacteria from the arid mangrove sediments of the Red Sea. Frontiers in Microbiology. 2023 May 2;14:1155381. https://doi.org/10.3389/fmicb.2023.1155381

[14] Androsiuk L, Shay T, Tal S. Characterization of the environmental plasmidome of the red sea. Microbiology spectrum. 2023 Aug 17;11(4):e00400-23. https://doi.org/10.1128/spectrum.00400-23

[15] Behzad H, Ohyanagi H, Alharbi B, Ibarra M, Alarawi M, Saito Y, Duarte CM, Bajic V, Mineta K, Gojobori T. A cautionary signal from the Red Sea on the impact of increased dust activity on marine microbiota. BMC genomics. 2022 Apr 7;23(1):277. https://doi.org/10.1186/s12864-022-08485-w

[16] Sohaib M, Al-Barakah FN, Migdadi HM, Alyousif M, Ahmed I. Ecological assessment of physico-chemical properties in mangrove environments along the Arabian Gulf and the Red Sea coasts of Saudi Arabia. Egyptian Journal of Aquatic Research. 2023 Mar 1;49(1):9-16. https://doi.org/10.1016/j.ejar.2022.11.002

[17] Joydas TV, Qurban MA, Borja A, Manokaran S, Manikandan KP, Rabaoui LJ, Garmendia JM, Asharaf TT, Ayranci K, Shemsi AM, Mohammed S. Ecological status of macrobenthic communities in the Saudi waters of the western Arabian Gulf. Regional Studies in Marine Science. 2023 Jan 1;57:102751. https://doi.org/10.1016/j.rsma.2022.102751

[18] Halawani RF, Wilson ME, Hamilton KM, Aloufi FA, Taleb MA, Al-Zubieri AG, Quicksall AN. Spatial distribution of heavy metals in near-shore marine sediments of the Jeddah, Saudi Arabia region: Enrichment and associated risk indices. Journal of Marine Science and Engineering. 2022 Apr 30;10(5):614. https://doi.org/10.3390/jmse10050614

[19] Abuzahrah SS, Baeshen MN, Alkaladi A, Bataweel NM, Alhejen AM, Abdelkader H. Exploring the taxonomic and functional diversity of marine benthic micro-Eukaryotes along the Red Sea coast of Jeddah city. Saudi Journal of Biological Sciences. 2022 Aug 1;29(8):103342. https://doi.org/10.1016/j.sjbs.2022.103342

[20] Gomaa MN, Mulla DJ, Galzki JC, Sheikho KM, Alhazmi NM, Mohamed HE, Hannachi I, Abouwarda AM, Hassan EA, Carmichael WW. Red sea modis estimates of chlorophyll a and phytoplankton biomass risks to Saudi Arabian coastal desalination plants. Journal of Marine Science and Engineering. 2020 Dec 23;9(1):11. https://doi.org/10.3390/jmse9010011