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Microorganisms and Halophytes Attracted to the Northeast Coast of Qatar for Potential Phytoremediation: A Case Study and Analysis

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ABSTRACT

The area of Northeast coast of Qatar has been actively engaged in industrial oil and gas activities for many years. It is part of the northern basin of underground water and is affected by many environmental and industrial factors, such as the intrusion of polluted saline seawater, which could greatly impact the levels of inorganic and organic components of the land. Moreover, industrial expansions could add more impacts to the pollution levels in this area. The high salinity levels in the sabkha of this area are attributed to ions that are normally found in saline patches, such as sodium, chloride, and calcium. However, other heavy elements are found, such as boron and strontium. This study discusses the role of the industrial activities in the accumulation of these elements in the soil. Analyses show that the sabkha of Al Ghariya contains four *Bacillus* species: *B. vallismortis*, *B. subtilis*, *B. licheniformis*, and *B. cereus* (reliability codes: 1.80, 1.91, 1.99, and 2.01, respectively). Among these species, *B. licheniformis* is identified as a new record of polyextremophiles that are adapted to various environmental conditions. The possible roles of these *Bacillus* spp. at the polluted area are discussed. Some fungi genera were also identified, and the results reflect how the harsh environment negatively impacts microbial counts and attract more candidates for bioremediation and phytoremediation. The biological roles of halophytes and their associated microorganisms in these saline lands are discussed. The gene bank of these organisms offers opportunities for further studies to develop a biological system capable of remediating and sustaining agricultural lands, as well as improving the production of many biological components in health, agriculture, and the economy.

Introduction

Knowing the soil properties is important for agricultural productivity and depends on the soil biota, organic and inorganic soil components, and biological soil crusts (BSCs). These factors interact with environmental conditions and determine the stability and sustainability of the soil (Adenan *et al.,* 2020). Microorganisms such as bacteria and fungi play crucial roles in nutrient cycling and energy transfer (Oja *et al.,* 2020). The soil texture strongly correlates with microbial diversity. Understanding the relationship between chemical parameters of soil and the abundance of microorganisms is needed to plan the remediation of polluted soils and solve problems related agriculture and wildlife in arid and saline habitats (Skariah *et al.,* 2023).

The environmental conditions at the Arabian Gulf region in general and in Qatar have unique peculiarities. The region is arid or semi-arid, it is among the warmest regions of the world, and rain is scarce at less than 152 mm per year (Yasseen and Al-Thani, 2013). When this low rainfall coincides with a high evaporation rate or the presence of salt water, dry and highly saline lands occur. The electrical conductivity of the saturated soil extracts (EC_e at 25ºC) can reach 200 dSm^{-1}. Moreover, the intrusion of seawater into the mainland could create saline patches and sabkhas. This situation might be worsened and cause great pollution by the pumping of industrial wastewater (IWW) underground from oil and gas activities, as well as the harsh environmental conditions in this region (Al-Thani and Yasseen, 2020; Al-Thani and Yasseen, 2021a; Manawi *et al.,* 2024).

This ecosystem has only a small number of native plants (about 400 species), which include xerophytes and halophytes that can adapt to the severe environment (Abdel-Bari, 2012). Previous studies have listed a limited number of native plants (about 50 species), including 25 halophytes, that could be used to remediate lands polluted with Na, Cl, and heavy metals (Al-Thani and Yasseen, 2021a; Yasseen and Al-Thani, 2022). These plants have mechanisms to absorb and metabolize inorganic and organic components of petroleum hydrocarbons (Yasseen, 2014).

IWW and its organic and inorganic components have negative impacts on various aspects of human life. Organic components accumulate in soil during the extraction, processing, and production of energy components. These components might be degraded by microorganisms through bioremediation methods or detoxification processes, which have been referred to as the "green liver" model adopted by plants. Al-Thani and Yasseen (2020) give some examples and details about the metabolic pathways of degradation of some petroleum hydrocarbons, which lead to useful metabolites such as acetyl Co-A and fumaric acid. These metabolites are incorporated in metabolic pathways such as the Krebs cycle, fatty acid metabolism, and amino acid interconversions. Native plants and their associated microbes can work together to reduce the risk of organic pollution by petroleum hydrocarbons.

Trace elements also have great negative impacts on biological systems and disturb the environment, ecosystems, and food chains, and these inorganic components are non-degradable. The best way to remediate soil or water contaminated with heavy metals is by phytoremediation and phyco-remediation using plants and algae that can absorb them in a process called "phytomining" (Wuana and Okieimen, 2011; Yasseen, 2014; Al-Thani and Yasseen, 2020; Al-Thani and Yasseen, 2024). Assessments of heavy metal contents in the soil of halophyte habitats revealed that these elements were in acceptable ranges for normal plant growth (Chapman and Pratt, 1961; McGee *et al.,* 2006; Yasseen and Abu-Al-Basal, 2010). Thus, the trace elements found in these soils might not be involved to any extent in the harsh characteristics of soils of the coastal zone (Milner and Kochian, 2008; Al-Thani and Yasseen, 2021a).

Some recent studies have revealed additional information about the accumulation of heavy metals in lands near the Ras Laffan industrial area, which is involved in the gas industry in Qatar. For example, Usman *et al.,* (2019) found that *Tetraena qatarensis* accumulates many heavy metals that are commonly found in the inorganic components (Cd, Cr, Cu, and Ni) of IWW. This native plant is a good candidate for the phyto-stabilization of toxic metals and efficient in phytoremediation methods. They concluded that the presence of native plants in a polluted area is a clear sign that they are tolerant of the heavy metals that are present.

Alsafran *et al.,* (2021) and Al-Thani and Yasseen (2023) recently suggested that the levels of some heavy metals such as As, Cr, and Ni are significantly higher than the normal levels in agricultural lands in various parts of Qatar. These high levels might be hazardous for human health. More recent studies have shown that halophytes in Qatar can remediate soil polluted with heavy metals (Yasseen and Al-Thani, 2022), including *Halopeplis perfoliata*, *Salicornia europaea*, *Salsola soda*, and *Tetraena qatarensis.* These plants can work together with associated endophytes to remediate soil polluted with heavy metals and metabolize organic components. Endophytes such as *Bacillus* spp. and *Pseudomonas* spp. have been reported to play important roles in many aspects of life.

Activities in the northern oil and gas fields are affecting the seawater in the Arabian Gulf and lands of the Qatari peninsula, which is negatively affecting the northern basin of underground water.

These organic and inorganic pollutants could affect the biota, including bacteria and wildlife. Abulfatih *et al.,* (2001) and Yasseen and Al-Thani (2022) reported on most of the native plants in North-eastern Qatar, including halophytes.

Therefore, this study aims to describe the soil properties at the North-east coast of Qatar (Al Ghariya area) that attract some new recognized microorganisms including bacteria species that can work together with halophytes to remediate polluted soils in this area.

Materials and Methods

Study location

Al Ghariya is a village on the northeast coast of Qatar in the municipality of Ash-Shamal (Fig. 1). In the past, the inhabitants lacked direct access to the groundwater, and water that could be obtained was saline and not suitable for drinking.

Therefore, the residents of this village established commercial relations with nearby villages to obtain water by bartering for access to drinkable water in exchange for marine goods such as fish and pearls. This area used to be the largest settlement in this part of the Qatari coast but is now deserted. At present, however, local people visit this area for recreational purposes.

Near this village, there are some ridges close to Fuwairet village, which is approximately 6.5km to the south. These villages and the Ras Laffan area are affected by the harsh environment, which has high temperature, scarce rainfall, and the intrusion of polluted seawater. These villages are the main center of industrial oil and gas activities in Qatar (Al-Siddiqi and Dawe, 2007; https://en.wikipedia.org/wiki/Al_Ghariyah) (Fig. 2).

The north field encompasses the main industrial gas center of the State of Qatar, which contains Al Shaheen and Al Rayyan oil fields. Fig. 3 shows the basins of underground water. This area is part of the northern basin, which encompasses the northern part of the country and deep seawater in the Arabian Gulf (https://water.fanack.com/qatar/water-resources-in-qatar/Fanack Water 2021).

Soil analysis

Soil samples from 17 sites around the Al Ghariya sabkha (North-east Qatar) were randomly collected in September 2022 (Fig. 4) from the surface and subsurface across the sabkha. The soil samples were sieved using a 2-mm mesh to separate plant debris and visible fauna and then stored at room temperature. The samples were used for various analyses, including physical, chemical, and microbial analyses. Several parameters were measured, including pH, salinity, bacteria count, fungal colonies, and various surface-level elements.

The physical and chemical characteristics of soil samples were determined using standard methods, such as the soil texture triangle. The soil of Al Ghariya sabkha is classified as sandy loam or loamy sand. Some of these samples were digested with acid, and minerals were analyzed for mineral content at the Central Laboratory of Qatar University (ICP-OES model Optima 7300 DV). Chemical analysis of the soil was carried out in collaboration with the laboratory. The total C, H, and N, total organic components (TOC), and major and trace elements were determined using a Flash 2000 CHN analyzer, Flash 2000 NC soil analyzer, ICP-OES, and ion chromatography. More details about the methods used can be found in previous studies (Gaudino *et al.,* 2007; Sarojam, 2010-2012).

Only soil samples that showed high concentrations of elements were chosen for further analysis. To analyze and visualize the spatial distribution of the elements, inverse distance weighting (IDW) was employed as an interpolation method. IDW is a widely used geostatistical technique for estimating values in unobserved locations based on the weighted average of nearby observed values. It assumes that values obtained from areas closer to an unknown point exert a greater influence on the estimated value compared to those situated farther away (Isaaks and Srivastava, 1989; Armstad, 2020). Soil samples were analyzed for salinity by measuring the electrical conductivity (dSm^{-1}) of suspensions of soil samples using deionized water at a ratio of 1:2 (soil:water). Saturated mixtures were prepared and then allowed to settle down for salinity and pH measurements. A pH conductivity TDS meter (Hanna Equipment India PVT, LTD, Hanna Instruments) with a potentiometric glass electrode was used. More details about the methods used are reported elsewhere (Gartley, 2011; https://northsearegion.eu/media/19831/water-and-soil-salinity.pdf).

Cultures for isolation of bacteria and fungi

A serial dilution method was used for all soil samples.

Briefly, one gram of soil sample was suspended in 9 ml of sterile distilled water to make serial dilutions (10^{-1} to 10^{-3}), and 1 ml from each dilution was placed on nutrient agar medium at 37ºC for 24h for bacterial growth and isolation. Bacterial colonies were counted, and the number of bacteria per gram of sample was calculated in colony-forming units (CFU) per gram of dry weight.

Bacterial colonies were also examined macroscopically and microscopically after Gram staining. Next, 19 selected and purified bacterial colonies were streaked on nutrient agar (NA) media with salt (10% NaCl) and without salt and incubated at two different temperatures (37ºC and 60ºC) for 48 h to isolate various bacterial extremophiles.

Potato dextrose agar was used as culture media for the isolation of fungi. Plates were incubated at 28°C for up to one week for fungal growth. The fungal morphology was studied macroscopically by observing the colony features (color, shape, size, and hyphae) and microscopically. The microscope analysis was done using a compound microscope with a digital camera and a lactophenol cotton blue (LCB)-stained slide that was mounted with a small portion of the mycelium.

Identification of bacteria using MALDI-TOF MS

Bacteria were identified using the method reported by Saleh (2021). A direct colony method with on-plate protein extraction was adopted. Briefly, a single bacterial colony from a fresh culture (incubated overnight in Luria-Bertani (LB) medium) was transferred to a MALDI Biotarget plate (48 spots). Once air-dried, 1 µL of 70% formic acid was placed on the colony, followed by 1 µL of MALDI matrix solution. The matrix solution was prepared using α-cyano-4-hydroxycinnamic acid (HCCA) in 50% acetonitrile and 2.5% trifluoroacetic acid.

Once the sample spot had dried, the plate was loaded into a MALDI-TOF MS instrument (Bruker Daltonic, Germany). Bruker Flex control software was used to compile and analyze the protein spectra, for which 240 laser shots were obtained in 40-shot steps for each spectrum and analyzed using default algorithms. The acceleration and source voltage were kept at 20 kV and 18.7 kV, respectively.

MALDI Biotyper RTC 3 software was used for comparison of the obtained protein spectra with a reference database, as well as bacterial identification and report generation. Flex Analysis software was used for pre-processing (baseline correction and smoothing) of the spectra and peak identification in protein profiles. The identification results were interpreted according to the manufacturer's guidelines and previous reports (Ashfaq *et al.,* 2022). Briefly, a score of 1.70–1.99 was interpreted as highly probable genus-level identification, a score of 2.00–2.29 was considered as genus-level and highly probable species-level identification, and a score of 2.30–3.00 was interpreted as species-level identification.

Results and Discussion

As shown in Table 1, the land in North-east Qatar in the Al Ghariya area has acceptable levels of C, H, N, and TOC (Chapman and Pratt, 1961; Chenchouni, 2017; Al-Mohannadi, 2022-2023).

However, there are very high salinity levels, and out of 17 sites, only one site has low salinity. The pH values of most sites are above 8 (Table 2), which disrupts the availability of many elements, such as B, Cu, Fe, K, Mg, Mn, P, and Zn, which are essential to most plants, including crops (Mengel *et al.,* 2001). Thus, the alkaline soil with high salinity levels in this sabkha might affect the biodiversity of not only native plants, but also other organisms. The microbial flora showed a variety of extremophiles, including mesophilic-non-halophilic, mesophilic-halophilic, thermophilic-non-halophilic, and thermophilic-halophilic species.

The bacteria count in this sabkha was compared with previous works, and the results showed that pure sandy soils have higher bacteria counts than Al Ghariya sabkha soil. The mean total bacteria count in pure sand was 24.4 x 10^4 CFU/g (Al-Sulaiti *et al.,* 2013), while in the present study, the bacteria count was 0.5-2.4 x 10^4 CFU/g. Some colonies of fungi were found in fungal cultures, which reflected how the harsh environment in the Al Ghariya sabkha negatively impacts the microbial counts. However, the bacteria and fungi in the sabkha are well adapted to the saline desert habitats (Fig. 5). The main inorganic elements of soils in the Al Ghariya sabkha were analyzed. Table 3 shows the results for various essential and non-essential elements, including heavy metals. The data show that Al, K, Li, and Pb had low levels, and Ba, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, and Zn had normal levels. Recent work of Hasna *et al.,* (2024) has confirmed that the degree of contamination at the sediments and Pollution Load Index off Doha, the Capital of Qatar, was low.

The high salinity level affected B, Ca, Na, and Sr and very likely Cl^- (not measured) (Abdel-Bari *et al.,* 2007). IDW was employed for the elements that were found at high levels (Fig. 6). The high levels of these elements might cause toxicity to most plants in this area, especially crops. Data from previous studies revealed that other trace elements such as As, Hg, Mo, Se, and V and anions such as Cl^- had high levels in soils and native plants in various parts of Qatar, including the North-east (Abdel-Bari *et al.,* 2007; Yasseen and Al-Thani, 2007; Yasseen and Abu Al-Basal, 2010; Al-Thani and Yasseen, 2020; 2021a; 2023; Yasseen and Al-Thani, 2022).

Boron (B) is an essential micronutrient for plants, and its normal range in most soils is 5-30 ppm (μgg^{-1} of dry soil) (Brdar-Jokanović, 2020). The concentrations of B in the soil at Al Ghariya sabkha were 126.37 ± 37.03 and 130.76 ± 38.18 μgg^{-1} of dry soil at the surface and subsurface, respectively. Notably, high levels of this element in the soil can negatively impact plant growth and the soil biota (Grieve *et al.,* 2010; Bhupenchandra *et al.,* 2022). High levels of boron in the soil can cause toxicity symptoms in plants, such as stunted growth and yellowing of leaves, and ultimately lead to their death (Mengel *et al.,* 2001). More details are available in other studies (Landi *et al.,* 2019).

Nutrient imbalance also has a negative impact on various physiological activities and causes a reduction in plant growth and productivity (Bhupenchandra *et al.,* 2022). Both salinity and high boron levels often co-occur in natural and agricultural environments (Pandey *et al.,* 2019). Bilen *et al.,* (2011) found that high concentrations of boron in soils cause not only toxicity to plants, but also have negative impacts on soil microbial populations. These impacts include the inhibition of microbial enzyme activity, reduction of the breakdown of organic matter, and reduction of nutrient cycling. The reduction in respiration in the soil in terms of soil CO_2-C production was higher in superficial layers that have lower boron contents than the sub-superficial layers of soils, especially during drying episodes (Mengel *et al.,* 2001; Arredondo and Bonomelli, 2023).

Interestingly, industrial oil and gas activities have great impacts on boron levels in soil and underground water. Such activities can release boron into the environment through various mechanisms, such as drilling, fracking, and production operation (Sari and Chellam, 2015; Acharya *et al.,* 2020). Notably, wastewater can add significant amounts of boron to soil and underground water if not properly disposed during spills and leaks (Al-Thani and Yasseen, 2020; 2021a; 2023; Yasseen and Al-Thani, 2022).

Calcium (Ca) is a major element for the nutrition of plants and other organisms, including humans. The normal range of concentrations in most soils is 0.4-0.5 mg^{-1} (Chapman and Pratt, 1961; Mengel *et al.,* 2001). However, the Ca levels in the surface and subsurface of Al Ghariya sabkha soil ranged between 22.64 ± 11.29 and 26.32 ± 11.29 mgg^{-1} dry soil, respectively. This element is among the dominant elements along with Na, Mg, and K in saline patches and sabkhas (Abdel-Bari *et al.,* 2007; Al-Yousef, 2023).

Calcium can also be present at high levels in polluted soils due to natural its abundance in the Earth's crust and its mobility in soil. Moreover, it can be released into the soil from weathering of calcium-rich rocks or contaminated water that has been used in the oil and gas industries. Such activities could add more calcium during pumping of IWW deep into the soil (Al-Thani and Yasseen, 2021a).

High levels of Ca in soils can have various impacts on microorganisms (Li *et al.,* 2018; Finley *et al.,* 2022). A high level of Ca inhibits microbial growth by interfering with the cell membranes by disrupting their structure and function. Furthermore, it can alter microbial communities as some microbes are sensitive to high levels of Ca, while others are more tolerant. It also disrupts microbial enzyme activities. Calcium can bind to enzymes and alter their structure or function, resulting in reduced enzyme activity, which leads to some changes in soil fertility.

Furthermore, Ca activates stress responses, which include changes in gene expression, production of stress-related proteins, and possibly other cellular responses. It also displaces some essential elements, such as Mg and K, as well as trace elements, causing ion imbalance. Lastly, it restricts the physical movement of microorganisms and limits their access to nutrients and water. Therefore, understanding the levels and behavior of Ca in saline and polluted soils is important for managing agricultural lands and contaminated sites, as well as mitigating potential impacts on plant growth and ecosystem health. Further investigations are needed to explore the influence of high Ca levels on microorganisms that are tolerant of highly saline soils and sabkhas.

Sodium (Na) is a major element in all saline soils and sabkhas and is normally found in non-saline soils at levels below 40 μgg^{-1} dry weight. However, the amount can be substantially higher in saline environments. For example, the concentration in Al Ghariyah sabkha soil ranged between 4.80 ± 2.43 and 17.34 ± 13.23 mgg^{-1} dry soil (4800-17,340 μgg^{-1}) in subsurface and surface soils, respectively. Previous reports confirmed that both Na and Cl are present at high levels in the saline habitats in Qatar (Abdel-Bari *et al.,* 2007). There are two main sources of sodium in the sabkhas and saline patches in Qatar: the intrusion of seawater into the mainland and IWW produced during oil and gas activities. These activities include exploration, drilling, extraction, production, refining, and spills during transportation, military exercises, and wars (Al-Thani and Yasseen, 2021a).

Sodium chloride (NaCl) and sodium hydroxide (NaOH) are major components of IWW during these activities. Therefore, efficient management and monitoring of sodium-containing wastewater is important for preventing environmental contamination and complying with regulatory requirements. Research centers, scientists, and decision-makers should take the initiative to solve the problem of sodium accumulation in soil and underground water to mitigate the negative impacts on wildlife and humans in many aspects of health, economy, and agriculture.

Notably, high levels of Na in soil have significant impacts on microbial activity in many ways (Rath *et al.,* 2016). These include osmotic stress, disruption of soil structure, and interference with the availability and uptake of essential nutrients by organisms, including microbes. Sodium can compete with other cations, such as Ca, Mg, and K. Nutrient imbalance can impair many activities, such as nitrogen fixation, nutrient mineralization, and decomposition of organic matter. It also increases the pH of soil, which could affect the availability of certain nutrients and the activity of enzymes in many microbes that breakdown organic matter.

Strontium (Sr) is a naturally occurring element in the earth's crust and is found in varying levels in soil. The normal concentration of this element is a few parts per million or µg/g of dry soil weight, but the level in the Al Ghariyah sabkha is between 1907.8 ± 766.3 and 2048.6 ± 1123.1 µg/g dry soil weight in the surface and subsurface, respectively. High levels of Sr in the soil can have both positive and negative effects on plants and the soil biota. The effects vary between stimulation of plant growth, toxicity, and other negative impacts, such as reducing growth, chlorosis, and leaf necrosis, which lead to reduction in seed production (Dresler *et al.,* 2018). Studies have reported more details about the negative impact of Sr on plants and humans (Agency for Toxic Substances and Disease Registry, 2004)

Restoration of polluted lands

In this area of Qatar, there has been great expansion in the energy sector, especially in the gas industry with concurrent urbanization and establishment and development of infrastructure. This has motivated universities and research centers to restore polluted lands that have been contaminated due to these activities. In recent years, comprehensive discussions have led to three strategies to treat polluted soils, water, and saline lands: (a) environmental manipulation, (b) genetic manipulation, and (c) biological approaches. The details of these solutions are reported in recent review studies (Al-Thani and Yasseen, 2021a; Yasseen and Al-Thani, 2022).

However, environmental manipulation does not provide real solutions to solve these problems. Notably, since the 1980s, the strategy of obtaining better soil for crops through environmental manipulation has been replaced by the strategy of using better crops for the soil with genetic manipulation (Epstein *et al.,* 1980). This approach has included many methods, such as conventional breeding methods, modern biotechnology, and genetic engineering (Alkorta *et al.,* 2004; Czako *et al.,* 2005; Liu *et al.,* 2015). More discussions can be found in recent publications discussing the progress of using transgenic technologies to improve abiotic stress tolerance in plants (Esmaeili *et al.,* 2022).

The aim of many studies has been the identification of traits, genes, and proteins at the cellular level to develop transgenic plants that can remediate polluted soils (Al-Thani and Yasseen, 2021a; Yasseen and Al-Thani, 2022). Recent works have discussed the main features of most wild plants on the Qatari peninsula, which are grouped into three main topics: (1) water, (2) solutes, and (3) structures (Yasseen and Al-Thani, 2023). There has been a great deal of achievement in identifying plants with morphological, physiological, and biochemical characteristics associated with resistance to various environmental stresses (Hasegawa *et al.,* 2000; Zhu, 2000; Hawkesford and Buchner, 2001; Xiong and Zhu, 2001; Flowers, 2004; Munns *et al.,* 2006; Redillas *et al.,* 2012; Hanin *et al.,* 2016; Al-Thani and Yasseen, 2018a), including pollution (Svoboda and Reenstra, 2002; Yasseen, 2014; Liu *et al.,* 2015; Yan *et al.,* 2020; Al-Thani and Yasseen, 2021a; Yasseen and Al-Thani, 2022). However, the costs of such technology have been very high, and discovering novel genes can cost millions of dollars given the multigenic nature of abiotic stresses in plants (NRC, 2000; Esmaeili *et al.,* 2022).

There has been limited success in this field, which has led to the introduction of more options to solve pollution issues. Biological approaches have emerged over the last two decades to solve problems related to multiple types of pollution, including salinity, trace elements, and organic components of IWW from various sources. In the State of Qatar, scientists, decision-makers, and energy companies are fully aware of the problems regarding the hazardous impacts of contaminants resulting from the accumulation of wastewater during gas and oil industrialization. Gas companies have pledged to the Supreme Council for Environmental Nature Reserve (SCENR) to implement environmental impact assessment (EIA) for any project that changes natural habitats. EIA is defined as a process of evaluating the positive and negative impacts and consequences of projects on various aspects of human life (Al-Ansi *et al.,* 2004; Yasseen and Abu-Al-Basal, 2008; Yasseen and Al-Thani, 2013).

To achieve successful restoration and conservation of endangered habitats, five steps should be considered and taken before conducting any real practical work: (1) collecting information, (2) recognizing problems, (3) setting plans, (4) finding solutions, and (5) maintaining monitoring. Few works have been done regarding the restoration of endangered habitats in Qatar (Yasseen and Abu-Al-Basal, 2008). However, a pioneer project to restore part of the coastal vegetation habitat was carried out during pipeline installation in 2002-2004 in the Ras Laffan area (see the maps in Figures 2 and 3). Al-Ansi *et al.,* (2004) and Yasseen and Al-Thani (2013) provide more details on this project. This project was considered as a successful attempt to restore part of the coastal habitat. It included mechanical works to remove the top of the land and keep some vegetation safe until the project ended.

During that period, native herbs and small shrubs were bred, cared for, and returned to the same area after the installation of the pipeline was completed.

Scientists have suggested such approaches as environmentally friendly solutions for many problems facing the ecosystem, health, agriculture, and the economy.

Many studies have discussed the cooperation between native plants and their associated microorganisms to remediate polluted environments efficiently and leading to beneficial interactions. Multiple mechanisms have been adopted to alleviate harsh abiotic stresses, including pollution stress. In the long term, horizontal gene transfer (HGT) between microbes and native plants could take place, which might lead to mutual beneficial activities between plants and their associated microorganisms (Al-Thani and Yasseen, 2018a, b).

Secretion of exudates from plants in the rhizosphere could also stimulate some metabolic pathways that utilize organic components of petroleum hydrocarbons. This process could degrade them to produce metabolites for the plants' metabolic pathways and to immobilize trace elements. Modern biotechnology can also be adopted to improve, develop, and create transgenic plants and microorganisms that are able to deal with polluted water and soils (Al-Thani and Yasseen, 2021a; Yasseen and Al-Thani, 2022).

It seems that collecting information and recognizing problems might require serious and substantial work before setting plans and finding solutions for any pollution issues. A significant number of studies have examined the collection of necessary information and the recognition of problems resulting from abiotic stresses and pollution of soil and water.

For example, Karakas *et al.,* (2020) suggested using halophyte plants to remove toxic salt ions such as Na and Cl from saline soils since modern molecular and biochemical approaches have reached a plateau. Therefore, new methods of using biological approaches have been strongly suggested.

Such approaches might reduce the generation of salt-tolerant plants by modern biotechnology. Instead, crop plants could be cultivated after the land has been remediated. Previous studies have reported a significant number of native plants, including halophytes, to remediate polluted soil with heavy metals found in Al Ghariya sabkha (Al-Thani and Yasseen, 2021a; 2023; Yasseen and Al-Thani, 2022).

Sodium and calcium can be remediated using plants such as *Acacia tortilis*, *Aeluropus lagopoides*, *Caroxylon imbricatum*, *Halopeplis perfoliate*, *Limonium axillare*, *Salsola setifera*, *Suaeda aegyptiacam*, *T. qatarensis*, and possibly others (Abdel-Bari *et al.,* 2007; Alhaddad *et al.,* 2021; Al-Thani and Yasseen, 2023). Intensive industrial activities are carried out in the eastern parts of Saudi Arabia in Al-Jubail province, which is not very far from Qatar. These areas were compared with other sites with little or no industrial activities. Many native plants were used successfully to remediate heavy metals like Cu, Fe, Mn, and Zn, which had accumulated in some sites because of oil production activities. The plants included *Anabasis setifera*, *Cyperus conglomeratus*, *Halocnemum strobilaceum*, *Haloxylon salicornicum*, *Panicum turgidum*, *Pennisetum divisum*, *Salsola baryosma*, *Seidlitzia rosmarinus*, *Suaeda vermiculata*, and *Zygophyllum coccineum* (Al-Khateeb and Leilah, 2005).

More data were collected regarding the efficiency of some halophytes in phytoremediating polluted soils with many heavy metals in various parts of the world, including Qatar. *Salsola* spp. and *T. qatarensis* proved efficient in the phytoremediation of many heavy metals such as Cd, Cr, Cu, Ni, Pb, and possibly others.

The contamination of agricultural lands with heavy metals, such as As, Cd, Cu, Cr, Ni, Pb, V, and Zn, might result in serious human health risks, including cancer (Alsafran *et al.,* 2021; Alsafran *et al.,* 2024). Recent work of Manawi *et al.,* (2024) have suggested an assessment of health risks that could be beneficial in building a baseline of heavy metals level in ground water in Qatar, which might help in the determination of future pollution of groundwater.

High levels of boron in Al Ghariya sabkha can be mitigated by biological approaches, which are a new trend in solving the problems of pollution from IWW. For example, Dragovic *et al.,* (2014) and Bañuelos *et al.,* (2022) concluded that *S. soda*, a species that is very common among Serbian flora and other parts of the world, can remediate heavy metals including B, Cd, Co, Cr, and Pb. This halophyte species is very common among the flora of Qatar and can remediate soil that is heavily polluted with salinity and trace elements such as Se and B (Centofanti and Bañuelos, 2015).

Two interesting studies from Turkey and Tunisia examined Sr in the Gafsa-Metlaoui basin in Tunisia and in the west of Kütahya, Turkey (between 38°96′–39°48′N latitude and 29°48′–29°71′E longitude) (Galfati *et al.,* 2011; Kislioglu and Sasmaz, 2017). They concluded that Sr can be removed from polluted soil using native xerophytes and halophytes, such as *Citrullus* sp., *Nerium oleander*, *Salicornia* sp., *T. qatarensis*, *Zygophyllum* sp., and possibly others among the flora of Qatar. The concentration of Sr in these lands is 145 to 1310 $\mu g g^{-1}$ dry soil. Other studies show that many native plants are efficient in absorbing Sr in mining soils (Sasmaz, 2017). These plants include *Alyssum saxatile*, *Anchusa arvensis*, *Carduus nutans*, *Centaurea cyanus*, *Cynoglossum officinale*, *Glaucium flavum*, *Isatis* sp., *Phlomis*, *Onosma* sp., *Silene compacta*, and *Verbascum thapsus,* which are good or acceptable candidates for Sr remediation of saline soils.

Microbiology of Al Ghariya

The microbial data from the Al Ghariya sabkha revealed that 1 in 19 isolates was considered as a polyextremophile that is adapted to various environmental conditions (i.e., mesophiles, halophiles, thermophiles, and thermo-halophiles; Table 4, Group A) (Fig. 7). This group contains various *Bacillus* spp. with different shapes and characteristics that can be adapted to various environmental conditions. The most common *Bacillus* species in this group was *Bacillus licheniformis* with a high reliability code of 1.99. This species can breakdown a variety of pollutants (Jeong *et al.,* 2020).

Groups B, C, and D represent bacilli that are able to grow only at 37ºC (mesophiles; 3 isolates), at 37ºC with salt (mesophiles and halophiles; 5 isolates), and at 60ºC (mesophiles, halophiles, and thermophiles; 10 isolates), respectively. Notably, three isolates of group B contained two mesophile bacteria species; *B. licheniformis* and *B. subtilis*, which grew best at 37ºC. These species had high reliability codes of 1.99 and 1.91, respectively. These bacteria can resist extreme environmental conditions by producing endospores, and under favorable conditions, these spores are able to restore growth by producing vegetative cells and colonies at normal lab conditions. Group C was represented by *B. licheniformis* with a reliability code of 1.99, as mentioned above. Group D contained 10 isolates, among which *Bacillus* species were recognized: *B. cereus*, *B. licheniformis*, and *B. subtilis* with reliability codes of 2.01, 1.99, and 1.91, respectively.

Thus, one *Bacillus* species in the Qatari sabkhas namely *Bacillus licheniformis* is identified for the first time as polyextremophile bacteria that can survive the harsh environmental conditions in this country. Notably, other species such as *B. cereus*, *B. subtilis* & *B. vallismortis* can survive some extreme environmental conditions, these species seemed dormant (as endospores) at a high temperature (60ºC), however, these endospores resumed growth by giving vegetative cells and colonies when transferred to more favorable conditions (Ulrich *et al.,* 2018).

In this work, some bacterial isolates from groups B & C seemed produce endospores or show very low growth rate which were not identified by the technique used, i.e., MALDI-TOF MS. However, when these isolates were transferred to normal lab conditions, these isolates produced cells and colonies which were identified by the technique used as reported in Table 4.

Further investigations are needed to obtain more information about these species and those that were not identified with the techniques used in the present study. Al-Thani and Yasseen (2020) reviewed the use of native plants and their associated microorganisms to remediate polluted soils with petroleum hydrocarbons, including heavy metals. They revealed some metabolic pathways and methods to degrade, extract, and stabilize these components. Bacteria species were identified using the VITEK system (bioMérieux-Vitek, Hazelwood, Mo.), and the API 20 C AUX identification System (REF 20 210 of bioMérieux SA) was used to identify yeasts, which were described in previous reports (Al-Sulaiti *et al.,* 2013; Al-Thani and Yasseen, 2020; 2021b).

These methods proved successful for the identification of these microorganisms in soils polluted with IWW. Among these microorganisms, bacteria species such as *Bacillus megaterium*, *Burkholderia* spp., *Enterobacter cloacae*, *Kocuria kristinae*, *Lactococcus lactis*, *Micrococcus luteus*, *Pseudomonas* spp., *Sphingomonas paucimobilis*, *Staphylococcus* spp., and *Stenotrophomonas maltophilia*, and yeast species such as *Candida* spp., *Cryptococcus* spp., *Kloeckera* spp., *Pichia angusta*, and *Trichosporon mucoides*.

Most of the bacteria species in Al Ghariya sabkha belonged to the genus *Bacillus* and included *B. cereus*, *B. licheniformis*, *B. subtilis*, and *B. vallismortis.* These were identified with high reliability with codes ranging from 1.76 for *B. subtilis* to 2.01 for *B. cereus*. The presence of *Bacillus* species in this sabkha confirmed previous reports that most of the bacteria species were rod shaped and Gram-positive with pointed ends that occurred singly, in pairs, and as short chains (Al-Thani and Yasseen, 2017; 2018b). These bacteria were mesophilic, halophilic, thermophilic, and thermo-halophilic and were described as polyextremophiles that can survive in extreme environmental conditions of salinity, desert, and extremely high temperatures.

These bacteria species play important roles in phytoremediation methods, which have been reported in many studies. Al-Thani and Yasseen (2018a) listed many mechanisms that microorganisms use to endure the abiotic stress of drought, salinity, and other conditions in natural and crop-plant habitats. The production of antibiotics and secondary metabolites has been reported in many countries around the world and could contribute positively to serious health issues (Huck *et al.,* 1991; Srividya *et al.,* 2008; Ahmed *et al.,* 2013; Bizuye *et al.,* 2013; Al-Thani and Yasseen, 2018b). Moreover, plants might immobilize heavy metals in the soil or inside the plant body, roots, and shoots (Ibrahim *et al.,* 2013). Yasseen and Al-Thani (2022) provide more details.

In this study, we tried to separate the roles of microbes from those of native plants (Kremer, 2013; Yasseen and Al-Thani, 2013). Notably, many bacteria species play various roles in remediating polluted soils and waters. Their functions include biodegradation of organic components of petroleum hydrocarbons, which could reduce spilled contaminants significantly by degrading them completely or partially (Das and Chandran, 2011).

Such processes could happen naturally in polluted lands and need continuous monitoring. Some microorganisms might also insolubilize heavy metals as they secrete exudates to prevent the absorption of these elements and to keep them immobilized in the soil (in the rhizosphere and non-rhizosphere) (Chen *et al.,* 2017; Mishra *et al.,* 2017; Al-Thani and Yasseen, 2021a).

Bacillus species in Al Ghariya sabkha may remediate trace elements such as B and Sr. The presence of *Bacillus* species such as *B. cereus*, *B. licheniformis*, *B. subtilis*, and *B. vallismortis* in this sabkha suggest that they could play some roles in remediating soil polluted with these trace elements. The data revealed some information about the concurrent accumulation of these trace elements with the presence of *Bacillus* species (Figs. 5 and 7).

Table.1 Elements composition of the main organic matter in Al Ghariya soil (% of dry soil).

Variables	Surface		Subsurface	
	Mean± S.D.	Range	Mean ± S.D.	Range
C %	4.12 ± 2.04	1.48-10.44	5.09 ± 2.78	1.48-9.80
H %	0.82 ± 0.28	0.35-1.62	0.90 ± 0.49	0.46-2.78
N %	0.19 ± 0.12	0.09-0.59	0.23 ± 0.12	0.08-0.51
TOC %	0.38 ± 0.19	0.12-0.74	0.31 ± 0.12	0.17-0.59
C/N Ratio	23.0 ± 4.6	13.3-33.3	22.6 ± 3.2	18.3-28.0

Table.2 Salinity levels, pH, bacteria count, and number of fungal colonies in the sabkha in the Al Ghariya area.

Site	Salinity status*			pH (Mean of surface and subsurface) **	Bacteria count CFU/g (X 10^4) ***	No. of fungi colonies/plate
	Surface	Subsurface	Level			
1	12.5	8.2	Very high	8.2	1.6	1
2	18.2	15.4	Very high	8.2	1.6	5
3	10.3	9.6	Very high	8.2	1.2	1
4	20.0	11.0	Very high	8.0	1.0	0
5	20.0	8.5	Very high	8.1	0.5	0
6	20.0	10.1	Very high	7.9	1.4	3
7	9.8	6.7	Very high	8.2	1.6	0
8	20.0	12.7	Very high	8.2	1.9	8
9	17.1	9.8	Very high	8.2	2.0	0
10	4.1	6.0	Very high	8.3	1.7	2
11	16.2	7.3	Very high	8.2	2.4	0
12	20.0	18.3	Very high	8.1	1.7	0
13	10.5	11.4	Very high	8.3	2.0	0
14	0.9	0.6	Low	8.6	2.0	30
15	20.0	20.0	Very high	8.1	1.7	16
16	20.0	20.0	Very high	8.1	1.3	14
17	20.0	15.2	Very high	8.3	0.7	8

*According to the method adopted, all sites of Al Ghariya area have high salinity (>3) except one site (14) with a low salinity level (<1). ** All sites have alkaline soil. The pH data are averages of data for surface and subsurface soil (range: 7.9-8.6). *** Bacterial count range: 0.5-2.4 x10^4 CFU/g.

Table.3 Elements including heavy metals in soil samples in Al Ghariya area.

Element	Surface		Subsurface	
	Mean±S.D.	Range	Mean±S.D.	Range
Al	205.37±96.60 (Low)	56.75-417.64	218.07±134.66 (Low)	29.18-620.20
B	126.21±37.03 (High)	60.83-198.92	130.76±38.18 (High)	79.16-220.18
Ba	18.79±6.43 (Normal)	7.52- 30.27	17.29±8.69 (Normal)	4.32-34.27
Ca *	22.64±11.29 (High)	10.10-54.70	26.32±14.31 (High)	7.38-71.27
Cd	0.328±0.148 (Normal)	0.11-0.58	0.381±0.229 (Normal)	0.10-1.03
Co	2.485±0.614 (Normal)	1.36-3.79	2.741±0.998 (Normal)	1.74-5.18
Cr	13.72±7.88 (Normal)	4.74-40.60	15.33±8.40 (Normal)	5.63-40.03
Cu	8.604±3.071 (Normal)	4.24-15.39	8.409±2.816 (Normal)	3.86-14.47
Fe *	1.688±0.873 (Normal)	0.50-3.24	1.903±1.039 (Normal)	0.56-4.27
K *	3.751±1.515 (Low)	1.43-5.96	2.991±1.023 (Low)	1.37- 4.62
Li	6.249±3.490 (Low)	0.48-13.27	5.952±3.534 (Low)	0.88-13.96
Mg *	6.014±2.613 (Normal)	2.77-11.65	7.281±3.090 (Normal)	3.54-15.13
Mn	76.16±20.63 (Normal)	44.60-110.59	82.72±36.12 (Normal)	40.60-188.88
Na *	17.34±13.23 (High)	0.96-41.63	4.80±2.43 (High)	0.56-10.69
Ni	12.83±3.23 (Normal)	7.16-19.74	14.42±4.74 (Normal)	7.79-23.64
Pb	2.282±0.597 (Low)	1.31-3.49	2.100±0.796 (Low)	1.07-4.22
Sr	1907.8±766.3 (High)	819.0-3213.2	2048.6±1123.1 (High)	427.0-4917.9
Zn	11.34 ±3.32 (Normal)	6.51-19.32	10.61±3.12 (Normal)	5.13-16.30

17 soil samples were collected from sites in Al Ghariya sabkha. * mg/g dry weight, all other elements in µg/g dry weight. S.D.: standard deviation of the means.

Table.4 Growth of bacterial isolates under different environmental conditions.

Group	No. of bacterial isolates out of the 19 isolates	Environmental conditions				Observations/ Reliability code #
		37ºC *	37ºC + 10 % NaCl **	60ºC***	60ºC + 10 % NaCl ****	
A	1	√	√	√	√	Polyextremophiles, e.g. *B. licheniformis* (1.99)#
B	3	√	x	x	X	Mesophiles, e.g. *B. subtilis* (1.91) & *B. licheniformis* (1.99) #. N.B.1. High temperature (60ºC) prevented growth of some bacteria, but these bacteria thrived when transferred to lab conditions. Further investigation is needed, e.g. *B. cereus* (2.01) & *B. vallismortis* (1.80) #
C	5	√	√	x	x	Mesophiles, halophiles, e.g. *B. licheniformis* (1.99)#. N.B. 2. High temperature (60ºC) prevented growth of some bacteria, but these bacteria thrived when transferred to lab conditions, *B. subtilis* (1.91) #. Further investigation is needed.
D	10	√	√	√	X	Mesophiles, halophiles, and thermophiles, most of the isolates found in this Sabkha, e.g. B. cereus (2.01, *B. licheniformis* (1.99) & *B. subtilis* (1.91) #

*Mesophilic-Non-halophilic,**Mesophilic-halophilic,***Thermophilic-non-halophilic, ****Thermophilic-halophilic. √ Growth, ˣ No growth, All the bacteria genera were identified as *Bacillus*. #Reliability codes: (a) 2.3–3.000: highly probable species-level identification, (b) 2.00–2.299: shows genus identification and probable species-level identification, (c) 1.70–1.999: probable genus level identification, N.B.1: 3 isolates, N.B. 2: 8 isolates.

Figure.1 Part of the Al Ghariya sabkha in North-east Qatar, including part of the village at the coastal-line.

Figure.2 Locations of oil and gas fields on the mainland and sea boundaries around the peninsula of Qatar.

Figure.3 Basins of underground water of Qatar showing Al Ghariya in the northern basin.

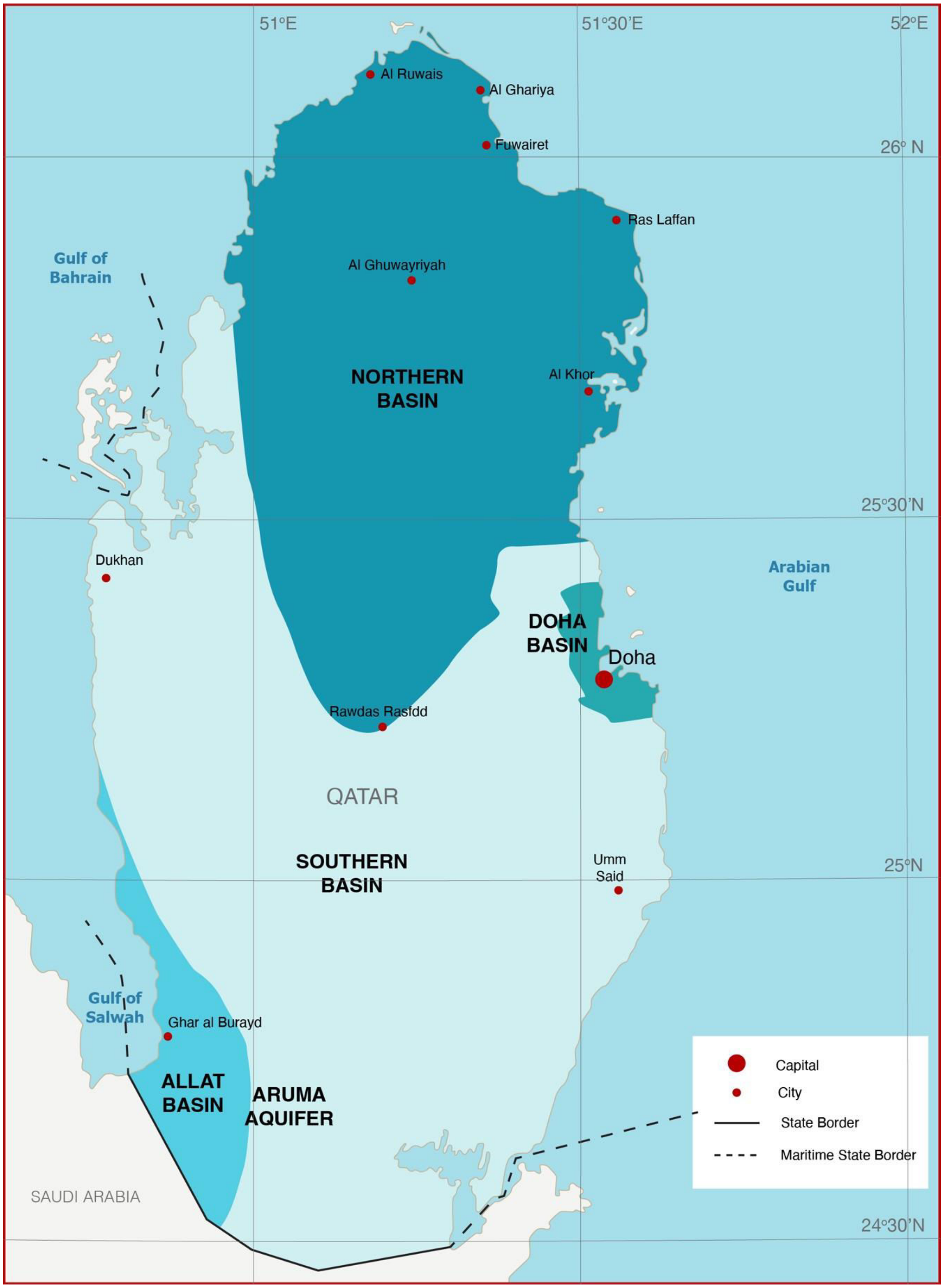


Figure.4 Map of Qatar showing the study sites in the north-east of the peninsula.

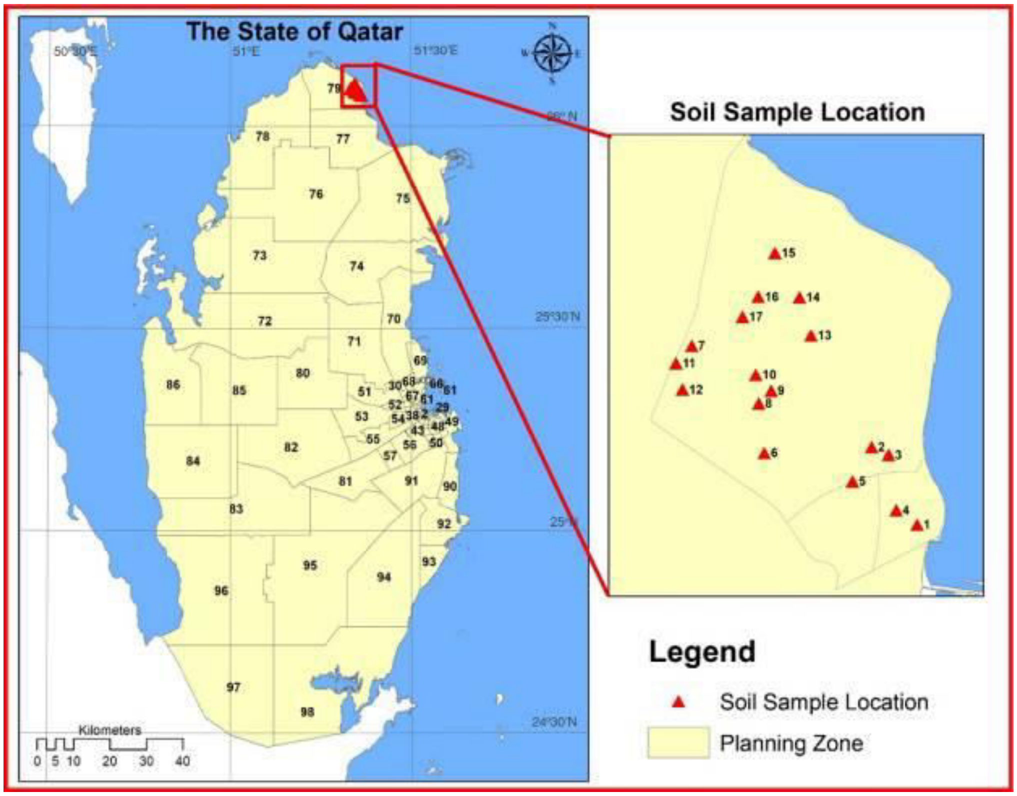


Figure.5 Bacteria and fungi distribution in Al Ghariya sabkha accounting to IDW.

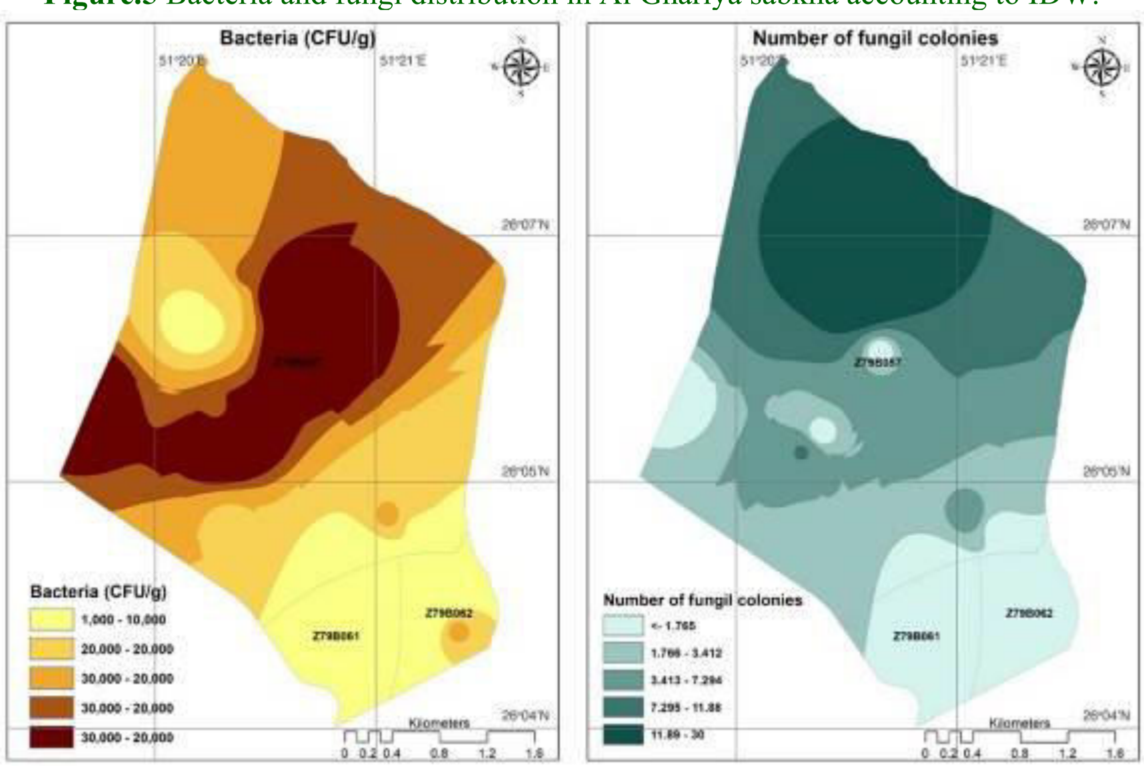


Figure.6 Trace elements found at high levels in Al Ghariya sabkha using IDW.

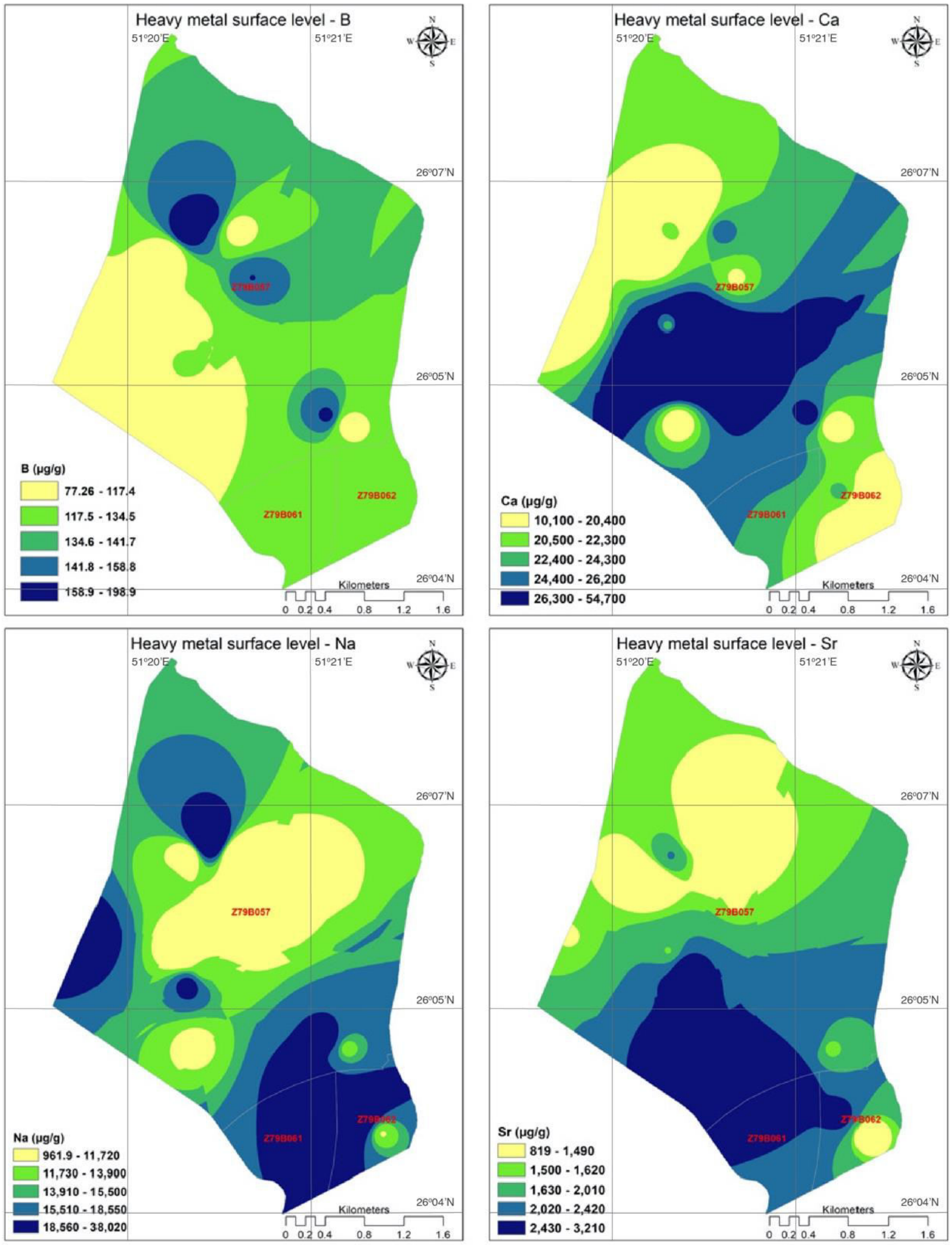
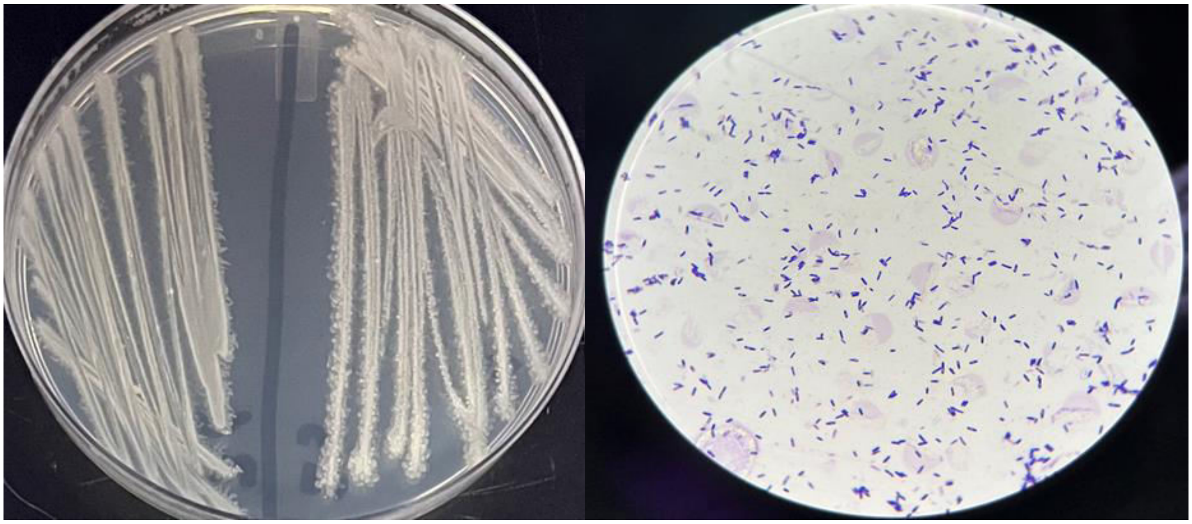


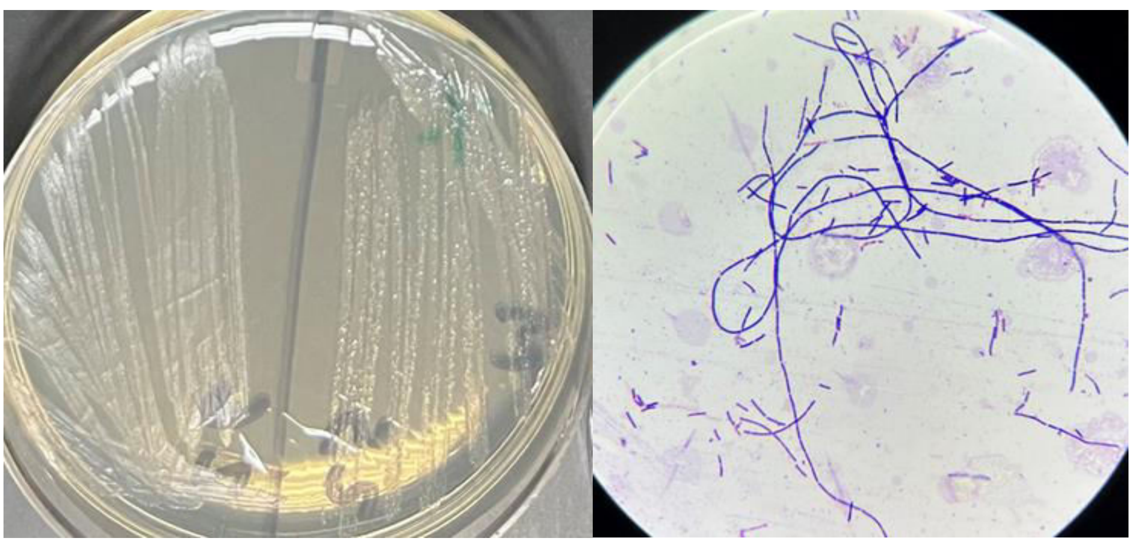
Figure.7 Colonies and cell morphology of *Bacillus licheniformis* (left), which grew in all different conditions.

A



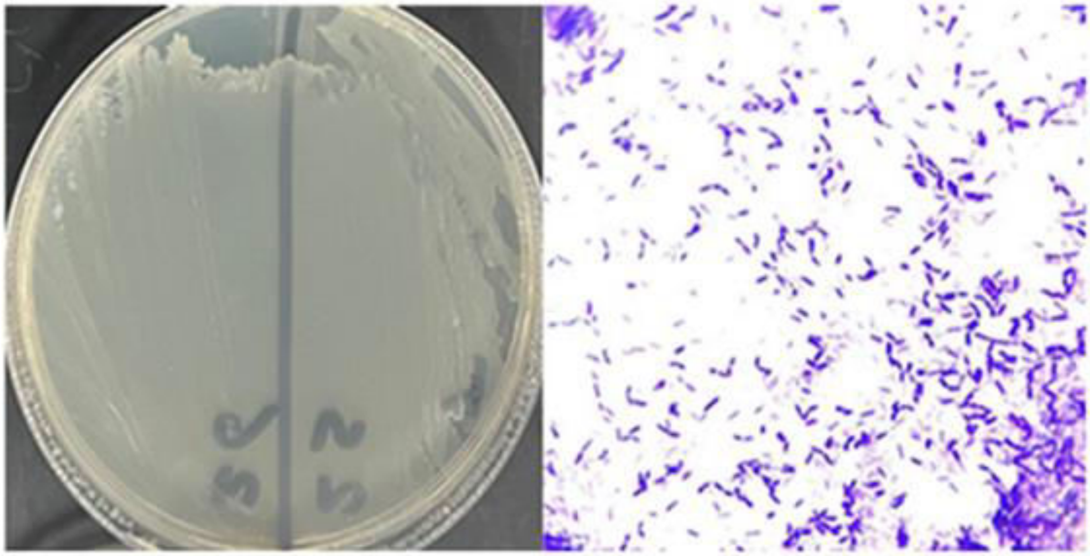
Growth at 37ºC into nutrient agar (NA), normal cell Gram-positive single bacilli and spore-forming

B



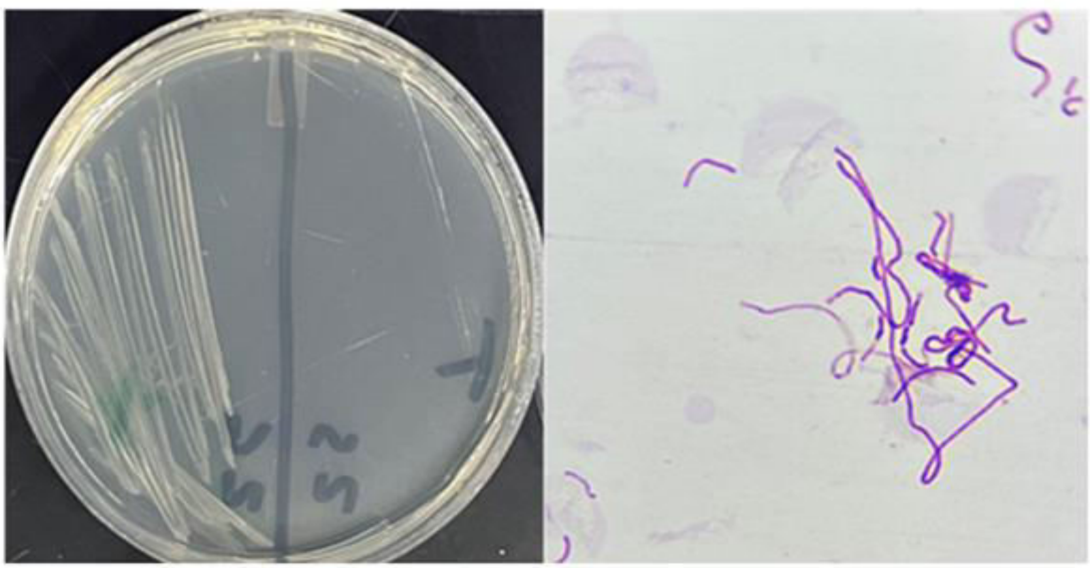
Growth at 37ºC into nutrient agar (NA) plus 10% NaCl, Gram-positive long and short bacilli with no endospores

C



Growth at 60° °C into nutrient agar (NA), Gram-positive bacilli, some bacilli not stained

D



Growth at 60°C into nutrient agar (NA) plus 10% NaCl, Gram-positive very long filament with no spores, stained with light blue because of the effect of that conditions on peptidoglycan

(A) Growth at 37ºC in nutrient agar (NA), normal cell Gram-positive single bacilli and spore forming
(B) Growth at 37ºC in nutrient agar (NA) plus 10% NaCl, Gram-positive long and short bacilli with no endospores
(C) Growth at 60ºC in nutrient agar (NA), Gram-positive bacilli, some bacilli not stained
(D) Growth at 60ºC in nutrient agar (NA) plus 10% NaCl, Gram-positive very long filament with no spores, stained with light blue because of the effect of conditions on cell wall peptidoglycan

Laçin *et al.,* (2015) found that microorganisms from different groups such as *B. cereus* (bacteria) and *Aspergillus versicolor* (fungi) were active in resisting and accumulating boron. However, the role of these microbes in the biosorption of boron should be studied and clarified as some *Bacillus* species could efficiently reduce some trace metals (such as Sr) in water and soils (Ghazvini *et al.,* 2007).

Notably, this conclusion is supported by Small *et al.,* (1999), who found that some bacteria (*Shewanella* spp.) have Sr-sorptive capacities in natural habitats. Other bacteria species from this sabkha can be isolated by using MacConkey media to isolate Gram-negative bacteria such as coliform and *Pseudomonas* bacteria and possibly others that are involved in biogeochemical cycles. Moreover, Cyanobacteria (such as *Anabaena* and *Nostoc*) are found in such sabkhas when soil is obtained and grown under more favorable of lab conditions (Al-Thani and Yasseen, 2017).

Roles of Bacillus species in sabkhas

Bacillus species might play various biological roles in sabkhas and saline patches that support wildlife and human activities. Their diverse metabolic capabilities and adaptations to extreme environmental conditions make them important contributors to the functioning and stability of these environments (Skariah *et al.,* 2023). One of their roles is the bioremediation of organic components of petroleum hydrocarbons from IWW and in saline habitats, as reported previously (Wang *et al.,* 2019). Furthermore, the salt and drought resistance of these microorganisms in such habitats might provide traits to native plants to help them withstand high levels of osmotic stress of salinity and desiccation (Al-Thani and Yasseen, 2018a).

Decomposition and nutrient cycling by these microorganisms might also provide essential nutrients to the environment, which become available to other organisms in the ecosystem (Saxena *et al.,* 2020). These species can participate in the cycling of carbon compounds and can degrade complex organic matter such as plant debris and algal biomass into simpler compounds. These compounds can be utilized by other organisms or recycled back into the environment. Such activities contribute to the overall carbon dynamic and energy flow within the ecosystem (Gupta *et al.,* 2016). Some bacillus bacteria within the genera *Bacillus*, *Paenibacillus*, and *Virgibacillus* could have the ability to fix atmospheric nitrogen into biologically usable forms, such as NH_3 and NO_3^-, which are vital nutrients for plants (Yousuf *et al.,* 2022). This is especially important when considering that nitrogen levels in Qatari lands are low (Ashore, 1991). *Bacillus* species also produce biofilms on various surfaces such as rocks, sediments, plants, and other organisms. These biofilms can offer many benefits for various organisms, such as protection from harsh environmental conditions (Qurashi and Sabri, 2011), nutrient acquisition, and promoting genetic exchange among different microorganisms and perhaps native plants as well (Al-Thani and Yasseen, 2018a).

Microorganisms also produce secondary metabolites that might have various antimicrobial, antifungal, and antiviral effects, which provide a means of defense against other microorganisms (Bode and Müller, 2003). Some *Bacillus* species possess the ability to mineralize salts by converting soluble salts into insoluble mineral forms (Liu *et al.,* 2021).

This process can contribute to stabilizing the soil structure and reducing salt toxicity in sabkhas and saline patches. Moreover, transformation of soluble salt to less harmful forms by these bacteria could improve soil quality and promote plant growth.

Some works in the last decade have shown the role of *Bacillus* bacteria in mineralization in Qatar. For example, they can form Mg-rich calcite, which is a precursor of dolomite, an important reservoir mineral of petroleum (Al Disi *et al.,* 2017). They also participate in other processes of carbonate and silicate biomineralization (Perri *et al.,* 2017) and produce carbon sources in sabkhas (Abdelsamad *et al.,* 2022). *Bacillus* sp. also have many symbiotic relationships, including mutualistic relationships with halophytic plants. These plants provide suitable habitat and nutrients for *Bacillus* species, while the bacteria contribute to plant growth and stress tolerance through nutrient acquisition, hormone production, or protection from pathogens (Yasseen and Al-Thani, 2013; Al-Thani and Yasseen, 2018a, b).

Problems facing humanity are increasing, and the challenges of pollution and climate change are mounting. With these challenges, there is increasing hope to find solutions to many of these dilemmas, and it seems clear that microorganisms may have a role.

Despite these problems, there is still determination to find creative solutions to stop the environmental deterioration in many countries, especially those that provide the world with energy sources. Biological approach has been developed recently as environmentally friendly, efficient, and sustainable, and if native plants and the associated microorganisms (such as endophytes) with their gene banks working together could make promising candidates and real hope for phytoremediation of polluted soils and waters in the Arabian Gulf region.

Modern approaches are being adopted to develop efficient soil-BSC-plants systems to remediate pollutants from soil and water contaminated with sea salts and petroleum hydrocarbons. Research centers, universities, scientists, and decision-makers are making progress in this regard. The role of new recognized species of *Bacillus* spp. at this area is also being investigated and discussed.

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Author Contributions

R. F. Al-Thani: Investigation, formal analysis, writing—original draft. B. T. Yasseen: Validation, methodology, writing—reviewing. P. Balakrishnan:—Formal analysis, writing—review and editing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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