# Floristic Study on Aquatic and Terrestrial Algal Community in the Natural Ponds of the Shibkouh District (Hormozgan, Iran); Occurrence of a Green Bloom of *Dunaliella salina*

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#### Abstract

Natural ponds are renowned for harboring a unique microalgal community, encompassing a wide diversity of common and rare species, including diatoms, cyanobacteria, green microalgae. and Yet, no comprehensive floristic study has ever been conducted on the natural ponds within the Shibkouh District. In this study, water and soil samples were collected from three distinct sites within the Shibkouh District (water bodies, wetland, and soil) to investigate the algal diversity present in these habitats. To cultivate the collected samples for further analysis, two different culture media were employed - BG11 and BG11: 1.5 M NaCl. Notably, an intriguing occurrence observed during this study was the bloom of Dunaliella salina in its green phase specifically in the semipermanent pond. Our findings revealed a total of 26 taxa comprising 12 genera of cyanobacteria, four genera of diatoms, and two genera of Chlorophyta. Remarkable variations were observed in both species richness and taxonomic composition between aquatic and terrestrial ecosystems. Furthermore, notable differences were observed when comparing samples cultured in different types of culture media. Diatoms were also identified as the dominant microorganisms adapted to extreme conditions typically found within wetland habitats (Site 2). This Site exhibited higher taxa richness compared to other sampling locations. As a result, these natural ponds have a vital role in preserving algal biodiversity. They serve as habitats not only for typical freshwater microalgae but also for halotolerant taxa such as diatoms that can thrive under difficult environmental conditions.

**Keywords**: Algal diversity, Aquatic ecosystems, Bloom, *Dunaliella salina*, Hormozgan Province, Salinity

### Introduction

Natural ponds are highly regarded as valuable environments that support a diverse array of microalgae and cyanobacteria species, often considered unique to these

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habitats. The presence and composition of algal species in such ecosystems can serve as reliable indicators of specific water and soil characteristics within the respective ecosystem (Irisarri, et al., 2001; Arguelles and Monsalud 2017). There are different types of natural ponds, including ephemeral or vernal ponds; spring-fed ponds; kettle ponds; mountain ponds, and meadowstream ponds. Natural ponds are considered as an overlooked freshwater habitat that received less scientific attention than other freshwater bodies, however, they are usually the most species-rich aquatic ecosystems (Boix et al., 2012; Meland et al., 2020).

Microalgae are a diverse group of microorganisms that exhibit a wide range of colors, sizes, and shapes, including unicellular forms, filamentous structures, and colonies. These remarkable microorganisms possess unique biochemical properties that enable them to thrive in diverse habitats spanning from marine environments to hot springs, deserts, and even snow-covered or icy lands (Guschina and Harwood 2006; Rajkumar and Yaakob 2013). Their ability to adapt to such varied conditions is attributed to their fascinating physiological adaptations and metabolic capabilities.

Extremophile microalgae belong to a group of microorganisms capable of thriving in extreme and harsh environmental conditions, such as water bodies characterized by high temperatures or high salt concentrations. An exemplary taxon within this category is *Dunaliella salina*, known for its ability to tolerate environments with up to 5 M NaCl concentration (Borowitzka and Siva, 2007). Iran has been the subject of several studies focusing on extremophile microalgae, and the present study aims to contribute towards enhancing our understanding in this field. Previous research has covered various aspects related to these organisms, including taxonomy studies concerning their capacity for heavy metal absorption (Riahi et al., 2015), as well as investigations into terrestrial and aquatic strains of the halotolerant microalga Dunaliella in extreme habitats (Salehipour-Bavarsad et al., 2021). By building upon existing knowledge, this study will further enrich our understanding of extremophile microalgae in Iranian ecosystems.

The Shibkouh District, situated in Hormozgan Province in southern Iran, is characterized by the presence of several natural ponds. This region experiences a hot desert climate according to the Köppen classification (BWh), with scorching summers and mild winters. The area receives extremely low levels of precipitation, resulting in virtually no rainfall throughout the year. Despite the ecological significance of this region, limited research has been conducted on algal flora within Hormozgan Province. Therefore, the primary objective of this study was to evaluate and document the diversity of cyanobacteria and microalgae across three specific sites within natural ponds located in the Shibkouh District.

# Material and methods

Sampling was conducted in an area

within the Shibkouh District, situated to the west of Hormozgan Province (Fig. 1), in July 2022. Furthermore, a subsequent visit to this ecosystem took place in April 2023 when there was a notable decrease in water level accompanied by a distinct change in color. This specific location is positioned at an elevation of approximately 60 meters above sea level and comprises both a spring and a small pool within its boundaries.

Stewart and Kantrud (1971) established the primary classifications for natural ponds. Accordingly, random sampling was conducted at three distinct sites within the natural ponds of the Shibkouh District: (Site 1) referred to as "permanent ponds," (Site 2) designated as "semipermanent ponds," and (Site 3) identified as "ephemeral ponds": the wetland-low prairie zone predominantly occupied the deepest section of the pond basin, where surface water could potentially disappear (Fig. 2).

The collected samples consisted of both water and soil specimens. Soil samples were obtained from the dry floor surrounding the spring, while water samples were gathered from both the pool and spring, which were then placed in plastic bottles. All collected samples were subsequently transported to the laboratory for further analysis. In order to cultivate these samples, glass containers were utilized along with two different culture mediums: BG11 and BG11 supplemented with a concentration of 1.5 M NaCl according to Andersen's method (2005). The cultures were incubated under controlled conditions at a temperature of 25  $\pm$  2 °C with a photoperiod consisting of 16 hours of light followed by an 8-hour dark period, provided by cool white fluorescent lamps emitting approximately 52.84 µmol photons m<sup>-2</sup> s<sup>-1</sup>.

The semipermanent slides of colonies were prepared and a morphometric study was performed by light microscopy (Olympus, Model BH-2) based on Desikachary (1959), Prescott (1970), Komárek and Anagnostidis (1986,2005), Komárek (2014), etc. The morphometric study encompassed various parameters including the color and shape

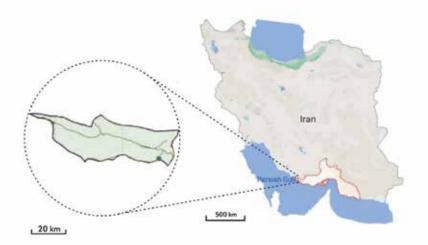
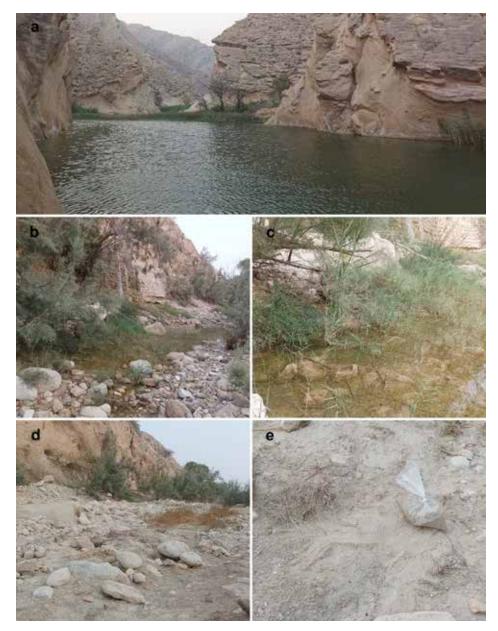


Fig. 1. Sampling location of Shibkouh District in Hormozgan Province (26.898463" N- 53.693137" E)



**Fig. 2.** Sample collection habitats showing three different locations of natural ponds in Shibkouh District; (a) Site 1: a permanent pond (b & c) Site 2: a semipermanent pond, (d & e) Site 3: an ephemeral pond

of colonies, as well as characteristics such as cell size, shape, and color. Additionally, features such as apical cell shape, presence or absence of akinetes, heterocysts, sheaths, and granular contents were considered during the analysis process.

### Results

The study focused on investigating the

natural ponds within the Shibkouh District, which is geographically classified as a tropical region characterized by low annual rainfall. The highest average monthly precipitation recorded was 3.9 mm (Fig. 3). Additionally, this area experiences high temperatures, with an annual mean temperature of 35 °C and a lowest mean monthly temperature averaging at 18.9 °C

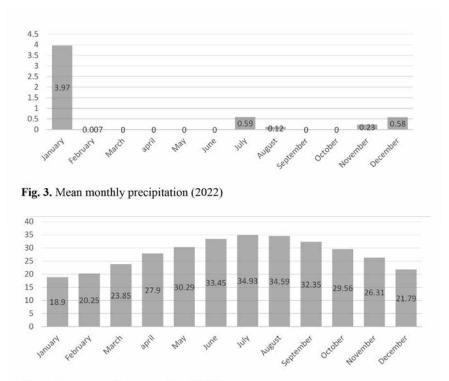


Fig. 4. Mean monthly temperature (2022)

(Fig. 4). These climatic conditions likely contribute to the limited vegetation observed in the area; Each sampling site displayed distinct vegetation characteristics: Site 1 was dominated by Gramineae species growing along the water's edge, while Site 2 featured a combination of Gramineae and *Tamarix* plants. Site 3 exhibited sparse shrubs and overall, very poor vegetative cover.

A total of 36 microalgal taxa were identified in this study (Table 1), and light micrographs of some taxa are presented in Figure 5. These microalgae included 26 taxa from cyanobacteria, eight diatoms from four genera belonging to four families and three orders, as well as two species from Chlorophyta belonging to two genera within two families and two orders. Among these taxa, *Pseudanabaena* exhibited the highest number of species with a contribution rate of approximately 19%, encompassing seven distinct species; following closely behind was the genus *Navicula* with an occurrence rate of around 14%, comprising five different species (Fig. 6).

There were notable dissimilarities between soil and water samples in terms of the number and diversity of species identified. Specifically, the soil samples yielded a total of 31 identified species, whereas the water samples only yielded nine identified species. Furthermore, alpha diversity demonstrated considerable variations among soil samples collected from Site 2 when cultivated using BG11 or BG11:1.5 M NaCl media types. These findings highlight significant differences between terrestrial and aquatic environments and also within specific sampling locations based on cultivation conditions.

In the aquatic ecosystems of both Site 1 and Site 2, the green algal taxon *Franceia* 

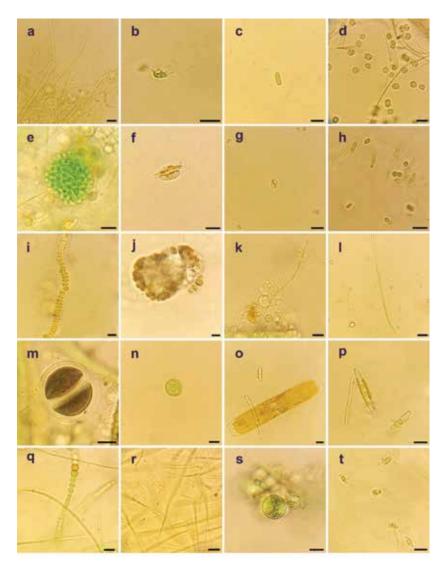


Fig. 5. Light micrographs of algal diversity in natural ponds of Shibkouh District; (a-d) aquatic taxa of Site 1 including (a) *Pseudanabaena* sp., (b) *Franceia* sp., (c) *Synechococcus mundulus*, (d) *Microcystis aeruginosa*; (e-h) aquatic taxa of Site 2 including (e) *Aphanocapsa* sp. (f) *Navicula* sp. (g) *Cyanobacterium minervae* (h) *Synechococcus elongatus*; (i-p) Terrestrial taxa of Site 2 including (i) *Johannesbaptistia* sp. (j) *Gomphosphaeria* sp. (k) *Pseudanabaena galeata* (l) *Pseudanabaena* sp. (m) *Chroococcus turgidus* (n) *Cyanobacterium crassiusculum* (o) *Pinnularia* sp. (p) *Navicula* sp.; (q-t) aquatic taxa of Site 3 including (q) *Trichormus* sp. (r) *Pseudanabaena amphigranulata* (s) *Gloeothece* sp. (t) *Navicula* sp. Scale bars 10 μm

sp. was observed. It exhibited robust growth when cultivated in BG11 medium without salinity, regardless of the sampling location. However, its growth was notably weaker when cultured in BG11:1.5 M NaCl medium, with successful cultivation observed only from samples collected at Site 2. Interestingly, an intriguing observation was made during another sampling event

where a green bloom of *Dunaliella salina* was discovered in the semipermanent pond of Site 2 (Fig. 7).

It should be noted that only soil samples from Site 2 contained diatom species, including *Navicula*, *Nitzschia*, *Pinnularia*, and *Melosira*. Moreover, a diatom species from the genus *Navicula* was identified in the soil sample collected from Site 3.

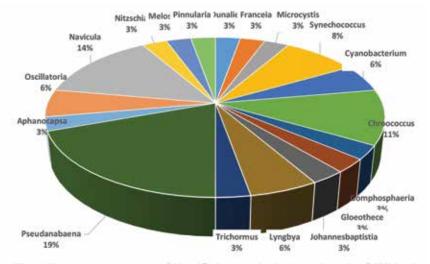


Fig. 6. Frequency percentage of identified genera in the natural ponds of Shibkouh District



Fig. 7. Sampling details of the semipermanent pond (Site 2) during a green bloom in April 2023; (a) a green bloom has occurred by changes in water level and salinity, (b) *Dunaliella salina* in orange phase, (c) red cyst of *Dunaliella salina* surrounded by the green cells of *Synechococcus elongatus* 

Table 1. Microalgal diversity in different sites of Shibkouh region of Hormozgan Province. All water and soil samples were cultivated in BG11 medium culture (\* represents BG11: 1.5 M NaCl medium culture)

Habitat	Site 1 Permanent pond			Site 2 Semipermanent pond		Site 3 Ephemera pond		meral
Taxa	Water	Water*	Water	Water*	Soil	Soil*	Soil	Soil*
Chlorophyta								
Franceia sp.	•		•	•				
Dunaliella salina				•				
Cyanobacteria								
Microcystis aeruginosa	•		•					
Synechococcus elongatus		•		•				
Synechococcus mundulus	•							
Synechococcus sp.					•			
Cyanobacterium minervae			•					
Cyanobacterium crassiusculum					•			
Chroococcus turgidus					•	•		
Chroococcus sp.1					•			
Chroococcus sp.2					•			
Chroococcus sp.3					•			
Gomphosphaeria aponina					•			
Gloeothece sp.					•		•	
Johannesbaptistia sp.					•			
<i>Lyngbya</i> sp. <sub>1</sub>					•			
<i>Lyngbya</i> sp.2					•			
Trichormus sp.					•		•	
Pseudanabaena galeata					•			
Pseudanabaena amphigranulata							•	•
Pseudanabaena sp.1						•		
Pseudanabaena sp.2						•		
Pseudanabaena sp.3		•	•					
Pseudanabaena sp.4							•	
Pseudanabaena sp.5	•							
Aphanocapsa sp.			•		•			
Oscillatoria corallinae						•		
Oscillatoria limosa					•			
Bacillariophyta								
Navicula sp.1								•
Navicula sp.2				•		•		
Navicula sp.3						•		
Navicula sp.4						•		
Navicula sp.5						•		
Nitzschia sp.						•		
Melosira sp.						•		
Pinnularia sp.						•		

#### Discussion

The present study aimed to investigate the algal diversity in various aquatic and terrestrial ecosystems within the Shibkouh District of Hormozgan Province. Freshwaters are generally among the most diverse ecosystems (Dudgeon et al., 2006). In particular, freshwater ponds are proven to exhibit a high level of biodiversity that can be considered biodiversity hotspots (Davies et al., 2008; Thiere et al., 2009). Our findings revealed that Site 2 exhibited the highest diversity, with a total of 26 taxa identified. This included two taxa from Chlorophyta, eighteen taxa from Cyanobacteria, and seven taxa from Bacillariophyta. The remarkable algal diversity observed at Site 2 aligns with previous literature on semipermanent ponds, which have been shown to possess exceptionally high alpha and beta diversity (Lukács et al., 2013). Noteworthy, there were distinct differences in algal diversity between terrestrial and aquatic strains at Site 2. Five aquatic taxa and 25 terrestrials were identified at this site. Furthermore, specific taxa such as Synechococcus elongatus (Nägeli) Nägeli, Cyanobacterium minervae (Copeland) Komárek, and Franceia sp. were exclusively observed in water samples collected from Site 2 but not detected in soil samples taken from the same location.

In addition, significant differences were observed in the algal diversity of aquatic strains from Site 2 when cultivated in different media types; BG11 and BG11: 1.5 M NaCl mediums. For example, *Cyanobacterium minervae* only exhibited growth in the BG11 medium, while *Synechococcus elongatus* was exclusively observed in the BG11: 1.5 M NaCl medium. This indicates that *Synechococcus* can tolerate a wide range of salinity levels (Li et al. 2019). Previous studies have reported high growth rates for this taxon at salinity levels as high as 400 mg/L NaCl (Alsamhary, 2020), highlighting its adaptability to varying salt concentrations.

Stated as such, the genus Franceia exhibited robust growth when cultivated in BG11 medium without salinity, while its growth was notably weaker in BG11: 1.5 M NaCl medium. This can be attributed to the fact that Franceia is primarily a freshwater alga (Arguelles, 2019). Consistent with this characteristic, the taxon was exclusively identified in water samples collected from aquatic ecosystems at Site 1 and Site 2. Within the terrestrial strains of Site 2, a remarkable algal diversity was observed when cultivated in different media cultures. Interestingly, Chroococcus turgidus (Kützing) Nägeli was identified in both media types, whether with or without salinity. In particular, members of Bacillariophyta were predominantly observed in the terrestrial ecosystem of Site 2 when cultivated in BG11: 1.5 M NaCl medium. Conversely, no species from the Bacillariophyta group were reported in the BG11 medium without salinity (Table 1). Noticeably, among the identified taxa, the genus Chroococcus exhibited the highest number of species within soil samples cultivated using BG11 medium.

In addition to Site 2, algal diversity was also

observed in Site 1 and Site 3 when cultivated using different medium cultures. For instance, the taxon Microcystis aeruginosa was observed in the sample collected from Site 1 and cultivated in BG11 medium. On the other hand, the taxon Synechococcus elongatus was specifically identified in the sample from Site 1 that was cultivated in BG11: 1.5 M NaCl medium. Furthermore, *Gloeothece* sp. was observed in the sample collected from Site 3 when cultured using BG11 medium. Conversely, Navicula sp., a diatom taxon, was identified exclusively in the sample from Site 3 that underwent cultivation with BG11: 1.5 M NaCl medium. Diatom species were exclusively observed in the BG11: 1.5 M NaCl medium, which can be attributed to their low sensitivity to salinity effects (Araújo and Garcia, 2005). These microalgae were not detected when cultivated in the BG11 medium, potentially due to limitations imposed by other microalgae present in that particular medium. In addition, within the terrestrial habitats of Site 2 and Site 3, several diatoms were identified, including five species from the genus Navicula, one species from the genera Nitzschia, Pinnularia, and Melosira; by contrast, the genus Navicula was observed in both aquatic and terrestrial ecosystems at Site 2.

In April 2023, we observed a rare green bloom of *Dunaliella salina* in a semipermanent pond, which initially exhibited a green color in the field (Fig. 7a). However, when the water sample was transferred to the laboratory, a distinct change in color from green to orange was observed. (Fig. 7b). The color change can be attributed to the synthesis of a bright red pigment by *D. salina*, which indicates a shift in cellular pigmentation caused by specific environmental factors or stressors. It is well-documented that various species of *Dunaliella* possess the capability to produce carotenoid pigments such as  $\beta$ -carotene and astaxanthin, which are responsible for imparting colors ranging from yelloworange to reddish hues (Oren 2005).

Blooms of Dunaliella salina have been reported in various locations worldwide, including both marine and inland waters such as lakes, ponds, and saline systems. One notable example is the Great Salt Lake. Similar to our observation, the bloom of D. salina and the highest activity of this Salt Lake was recorded in March-April. In addition, short-lived blooms were also recorded in the spring of 1971 and 1973 (Stephens and Gillespie 1976; Post 1977; Oren 2014). When Dunaliella spp. find suitable climatic conditions and sufficient nutrients, they grow profusely (Richmond 2004). Thus, environmental factors play a critical role in promoting the bloom development of D. salina. High salinity levels (>150%), elevated temperatures (>25°C), abundant sunlight exposure, and sufficient nutrient availability (particularly nitrogen and phosphorus), are considered favorable conditions for D. salina proliferation. During April, the semipermanent pond of Site 2 underwent dramatic changes in water level and accordingly in salinity amount

(Oren 2014). The impacts of *Dunaliella* blooms on ecosystem dynamics and biodiversity are multifaceted. These blooms can lead to changes in water chemistry, including changes in salinity levels and nutrient ratios. These changes have the potential to impact the composition and abundance of other phytoplankton species present in the water.

As time progressed, it was observed that populations of *Dunaliella salina* gradually weakened and were predominantly replaced by Synechococcus elongatus (Fig. 7c). This shift in dominance might indicate favorable growth conditions for S. elongatus within the laboratory setting. S. elongatus, similar to D. salina, has wide salinity tolerances (Li et al., 2019). The exact mechanisms underlying this replacement remain unclear and require further investigation. Possible factors influencing this shift could include variations in nutrient availability, light intensity, or competition between different algal species. Understanding these dynamics would provide insights into ecological interactions within algal communities and their responses under controlled laboratory conditions. It is important to note that our observations are limited to a specific experimental setup and may not necessarily reflect natural conditions present in field environments. Further studies involving more comprehensive sampling strategies along with detailed physiological analyses are needed to elucidate the intricate dynamics between D. salina and S. elongatus populations during algal blooms. Overall, our findings highlight dynamic changes occurring within algal communities when subjected to laboratory conditions.

In conclusion, our study investigated the algal diversity in aquatic and terrestrial ecosystems of the Shibkouh District. The findings revealed Site 2 as having the highest diversity, with a rare green bloom of Dunaliella salina observed in a semipermanent pond at that location. This indicates favorable environmental conditions for its growth. Variations in algal diversity were also found when strains were cultivated in different media types, highlighting the influence of salinity on growth patterns. These results emphasize the significance of semipermanent ponds as valuable habitats that support diverse algae spp. including common freshwater microalgae and halotolerant taxa-like diatoms. By providing suitable conditions for a wide range of microalgae, these ponds play a vital role in maintaining and protecting algal biodiversity. Further research is needed to understand the dynamics between different algal populations during blooms under natural field conditions.

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