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New Records of Freshwater Algae from the Jordanian Aquatic System

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ABSTRACT
Although planktonic algae play a significant role in freshwater ecosystems, our knowledge of their variety and species distributions is still rather limited in Jordan. Due to its diverse morphology, hydrology, and geographic variation (latitudinal, longitudinal, and altitudinal gradients), the Jordanian environment has a high capacity to host a diverse array of algae. Therefore, in this study, ten species were identified for the first time. The description of the freshwater algae's biodiversity in Jordan is essential for the creation of conservation initiatives. The identification of these species emphasizes the need for more research to advance the understanding of the richness and ecology of this hydrographic system. A total of 20 phytoplankton samples were collected from three sites in Jordan. Ten species representing new records for the freshwater algal flora of Jordan were identified.

INTRODUCTION

Phycology, the specialized study of algae, encompasses a vast array of simple microorganisms with autotrophic tendencies, varying from unicellular to multicellular forms, often characterized by low differentiation and nutrient absorption through their surface structures. The term "algae," derived from the Latin word for seaweed, now encompasses a diverse group of organisms, ranging in morphology and physiological traits **(Bellinger & Sigee 2010)**. While some algae species are microscopic and require the use of microscopes for observation, others, such as seaweeds, can grow to several meters in length, underscoring the breadth of algal diversity. Algae serve as foundational components of aquatic ecosystems. They serve as foundation of the food chain occupying a pivotal role as primary producers and forming the base of food webs- in the aquatic ecosystem. Their significance extends beyond primary productivity, as algae also contribute substantially to water filtration and quality assessment. These organisms exhibit sensitivity to

environmental changes within inland waters, thus serving as reliable indicators of ecosystem health and water quality. Consequently, algae are integral to monitoring programs aimed at assessing the condition of aquatic environments worldwide **(Sheath & Wehr, 2015; Al-Rawajfeh *et al.,* 2023)**. Certain algae species serve as bioindicators, reflecting the ecological condition and health of water bodies. Monitoring changes in algal community structure can provide early warnings of environmental stressors such as pollution, eutrophication, and climate change impacts, enabling effective management and conservation strategies. The prevalence and ecological importance of algae in aquatic ecosystems have led to their extensive utilization in water quality assessment and pollution monitoring efforts. However, despite their widespread occurrence and ecological significance, the taxonomy and distribution of algae within Jordanian aquatic systems remain largely unexplored. This knowledge gap presents a compelling rationale for investigating the diversity, distribution, and ecological roles of algae in Jordan.

Microalgae, a subset of algae, thrive in aquatic environments and utilize light and carbon dioxide for biomass production. Recent research has underscored the potential of microalgae as sources of energy, metabolites, and bioactive compounds, with applications ranging from clean energy production to pharmaceuticals. Studies, such as those conducted on *Chlorella vulgaris*, have revealed the intricate mechanisms at play, demonstrating the profound sensitivity of these organisms to environmental cues. For instance, the external mass of *Chlorella vulgaris* exhibits a highly sensitive glutaminase compound, facilitating the assimilation of carbon sources from the surrounding media. Furthermore, despite the presence of cellulose supporting the external structure, microalgae exhibit genotype-specific responses, leading to increased biomass accumulation in environments with elevated carbon dioxide concentrations **(Al-Hussieny *et al*., 2022)**.

Moreover, algae's rapid reproduction rates and short life cycles make them ideal indicators of short-term environmental changes, providing valuable insights into water quality dynamics. In addition to their role in biomass production, microalgae are reservoirs of lipids and fatty acids, serving as sources of energy, metabolites, storage products, and essential components of cellular membranes. The versatile nature of microalgae-derived lipids and fatty acids makes them valuable resources with diverse applications. These include serving as sources of food, contributing to clean energy production, and acting as natural plant fertilizers. Furthermore, owing to their photoautotrophic nature and the presence of bioactive, antibacterial, and antioxidant compounds, microalgae represent promising candidates for various biotechnological and pharmaceutical applications **(Sirakov *et al.,* 2015; Al-Hussieny *et al*., 2022b)**. Moreover, algae's rapid reproduction rates and short life cycles make them ideal indicators of short-term environmental changes, providing valuable insights into water quality dynamics. Algal characteristics are essential for identifying various types of water deterioration and can complement other environmental indicators **(Garrido-Cardenas *et al.,* 2018)**.

Despite the ecological significance of algae, assessments of water quality in Jordan have largely overlooked biological markers such as algae. By elucidating the diversity and distribution of algae species in Jordanian aquatic systems, this study aimed to fill this critical gap in knowledge. Understanding the taxonomy and ecological roles of algae will not only enhance our comprehension of aquatic ecosystems but also facilitate the detection of water quality issues, pollution, and potential risks to public health.

In light of these considerations, this paper endeavours to explore the diversity, distribution, taxonomy, and ecological significance of algae species in Jordanian aquatic environments. By shedding light on the presence and ecological roles of algae, the study provides svaluable insights into the health and dynamics of Jordan's freshwater resources, paving the way for informed conservation and management strategies.

MATERIALS AND METHODS

1. Study area

The study focused on three locations from different provinces in Jordan (Amman, Tafila and Ma'an) (Fig. 1), specifically from the water springs. Sampling sites were selected to encompass a range of ecological conditions and anthropogenic influences. The sampling sites were stratified based on geographical regions, habitat types, and known anthropogenic pressures.

Fig. 1. Map showing study area and sampling stations (The map created by Datawrapper)

Location 1: Samples were collected from a water spring located in the capital city of Amman, at coordinates (32°03'06.9"N, 35°54'04.0"E) (Fig. 2A). The study area is situated in a valley, with surrounding peaks reaching elevations of 908.3 meters. August experiences the highest temperatures, averaging 25.5°C (77.9°F), while January has the coldest temperatures, with an average low of 7.4°C (45.4°F). Additionally, the area receives 271mm (10.7 in) of rainfall annually, or 22.6mm (0.9 in) per month.

Location 2: The second sampling site is located in the Tafila Governorate of Jordan. The water spring at this site serves as a source of drinking water and for watering sheep, with coordinates (30°40'49.3"N, 35°37'35.3"E) (Fig. 2B). The elevation of this study area is 1565.7 meters. The hottest month of the year in Aţ Ţafilah is July, with an average high of 30°C (86°F) and a low of 17°C (62°F). The cool season lasts for 3.1 months, from December 3 to March 7, with an average daily high temperature below 15°C (59°F). The coldest month in Aţ Ţafilah is January, with an average low of 2°C (36°F) and a high of 11°C (52°F). On average, Tafila receives approximately 5.72mm (0.23 in) of rainfall annually, spreading over 11.1 rainy days, accounting for 3.04% of the year.

Location 3: Qurain, located in the Ma'an Governorate of Jordan, is a residential area within the Al-Marigha district, which comprises 11 districts. The water spring in this area is used by residents for drinking and watering livestock, at coordinates (30.098335, 35.468842) (Fig. 2C). The elevation of this location is 1510.0 meters. The hot season lasts for 4.1 months, from May 24 to September 29, with an average daily high temperature above 29°C (84°F). The hottest month of the year in Ma'an is August, with an average high of 33°C (91°F) and a low of 18°C (65°F). The cool season lasts for 3.2 months, from November 30 to March 4, with an average daily high temperature below 17°C (62°F). The coldest month in Ma'an is January, with an average low of 2°C (36°F) and a high of 13°C (55°F). The month with the most rain in Ma'an is January, with an average rainfall of 0.5 inches. The month with the least rain is June, with an average rainfall of 0.0 inches.

Fig. 2. The sample sites

2. Sample collection

Samples were collected during the summer and spring of 2022 using sterile one-litre glass bottles. The samples were taken from a depth of approximately 30cm, as most phytoplankton are found within this range due to their need to capture sunlight in surface waters. The collected samples were immediately sent to the laboratory for analysis.

Algal samples were preserved in appropriate fixatives for subsequent taxonomic identification, which was conducted as soon as possible using morphological techniques. Microscopic examination of the algal specimens was performed to identify taxa to the genus or species level. A light microscope with magnifications of 10X, 40X, and 100X was used to identify distinct algal forms while preserving their taxonomic characteristics. Both live and preserved algal samples were examined using a compound light microscope to observe characteristics such as color, shape, length, width, filamentous structure, surface texture, size, layering of cell walls, and chloroplast shape and quantity.

A precise mercury thermometer (0-60°C), calibrated to 0.1°C, was used for immediate field measurements of water temperature. Taxonomic identification was conducted following the methodology outlined by **Al-Hussieny (2018)**.

3. Data analysis

a- Water temperature; measured locally by graduated mercuric thermometer from 0-100°C.

b- pH; measured by pH meter after calibration pH meter with buffer solutions 4, 7 and 9.

c- Electrical conductivity; measured by conductivity meter, results reached with units ìS/cm.

d- Nitrate NO_3; limitation of NO_3 depends on the method of **APHA (1998)**. 50ml of water sample after filtration was taken for removing suspended materials, then 1ml of HCl (1 normal) was added and mixed well, then the concentration was measured by spectrophotometer on wavelength 220nm. Results were expressed by unit of mg/l.

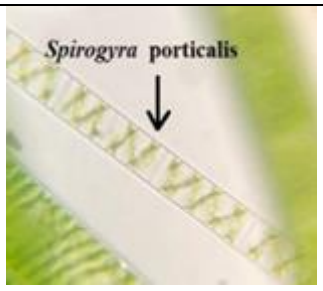
e- Nitrite NO_2; The determination of nitrite was carried out following the method described by **APHA (1998)**. A 10ml aliquot of the filtered sample was diluted to 50ml with distilled water. Then, 1ml of sulphanilamide reagent was added and the solution was gently shaken. After two minutes, N-(1-naphthyl) ethylenediamine dihydrochloride was added. The sample was left to stand for 5 minutes, after which the absorbance of the resulting pink color was measured using a spectrophotometer at a wavelength of 543nm. Results were expressed in mg/L.

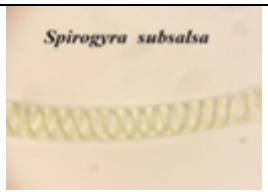
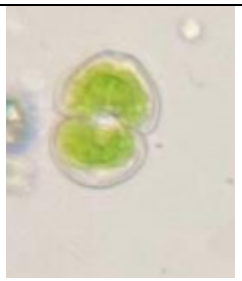
f- Phosphate PO_4; Phosphate concentration was determined according to the **APHA (1998)** method. A volume of 8ml of the combined reagent—prepared from ammonium paramolybdate $[(NH_4)_6Mo_7O_{24}\cdot 4H_2O]$, potassium antimony tartrate $(K(SbO)C_4H_4O_6)$, and ascorbic acid $(C_6H_8O_6)$—was added to 50ml of the filtered water sample, producing a blue-colored complex. The absorbance was measured

using a spectrophotometer at a wavelength of 860nm, and results were expressed in mg/L.

RESULTS

Ten species of algae were identified for the first time in the Jordan aquatic environment. They were found within three different sites:

Domain: Eukaryota **Kingdom**: Plantae **Phylum**: Chlorophyta **Class**: Zygnematophyceae **Order**: Zygnematales **Family**: Zygnemataceae **Genus**: *Spirogyra* **Species**: *fuellebornii*	
Domain: Eukaryota **Kingdom**: Plantae **Phylum**: Chlorophyta **Class**: Zygnematophyceae **Order**: Zygnematales **Family**: Zygnemataceae **Genus**: *Spirogyra* **Species**: *crassa*	
Domain: Eukaryota **Kingdom**: Plantae **Phylum**: Chlorophyta **Class**: Zygnematophyceae **Order**: Zygnematales **Family**: Zygnemataceae **Genus**: *Spirogyra* **Species**: *porticalis*	

Domain: Eukaryota **Kingdom**: Plantae **Phylum**: Chlorophyta **Class**: Zygnematophyceae **Order**: Zygnematales **Family**: Zygnemataceae **Genus**: *Spirogyra* **Species**: *subsalsa*	
Domain: Eukaryota **Kingdom**: Plantae **Phylum**: Chlorophyta **Class**: Zygnematophyceae **Order**: Zygnematales **Family**: Zygnemataceae **Genus**: *Mougeotia* **Species**: *robusta*	
Domain: Eukaryota **Kingdom**: Plantae **Phylum**: Chlorophyta **Class**: Ulvophyceae **Order**: Cladophorales **Family**: Cladophoraceae **Genus**: *Cladophora* **Species**: *fuellebornii*	
Domain: Eukaryota **Kingdom**: Plantae **Phylum**: Chlorophyta **Class**: Zygnematophyceae **Order**: Desmidiales **Family**: Desmidiaceae **Genus**: *Cosmarium* **Species**: *trilobula*	

Domain: Eukaryota **Kingdom**: Protista **Phylum**: Euglenozoa **Class**: Euglenophyceae **Order**: Euglenales **Family**: Euglenaceae **Genus**: *Trachelomonas* **Species**: *horrida*	
Domain: Eukaryota **Kingdom**: Plantae **Phylum**: Bacillariophyta **Class**: Bacillariophyceae **Order**: Naviculales **Family**: Naviculaceae **Genus**: *Navicula* **Species**: *trivialis*	
Domain: Eukaryota **Kingdom**: Plantae **Phylum**: Bacillariophyta **Class**: Bacillariophyceae **Order**: Fragilariales **Family**: Fragilariaceae **Genus**: *Diatoma* **Species**: *hiemale*	

DISCUSSION

The discovery of newly identified and lesser-known cyanobacterial and algal species in this study underscores the limited extent of our understanding of Jordanian algal biodiversity. This finding aligns with the prevailing notion that the algal flora of the Middle East remains largely unexplored, necessitating further comprehensive phycological investigations. Despite some studies in Jordan focusing on algae applications, such as biodiesel production, our knowledge of algal diversity in the region is still inadequate.

Given Jordan's diverse habitats and unique geographic position, which harbor numerous intriguing and uncommon cyanobacterial and algal species, we posit that Jordan's algal biodiversity holds global significance. Many innovative and fascinating

species, including diatoms, remain undiscovered, emphasizing the need for more extensive research using novel categorization approaches.

Additionally, it is important to assess the distributional patterns of diatom species using molecular, morpho-taxonomic, and ecological data to better understand species conspecific and distinguish putatively cosmopolitan morphospecies. Therefore, concerted efforts are required to elucidate the distribution and taxonomy of algal species, particularly diatoms, to advance our understanding of their ecological roles and evolutionary relationships

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