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Bacteriological contamination status and phytochemical characteristics of Al-Chibayish marsh regional plants

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Abstract

This study aims to evaluate the status of Al-Chibayish marsh water and regional plants in regard to bacterial contamination and to investigate the phytochemical characteristics of the region's plants. For this purpose, water samples were collected from three different sites of Al-Chebaysh marshes located in Dhi Qar governorate, south of Iraq in 2017 to investigate water bacteriological contamination. Additionally, samples of five different plant species were collected from the same region to evaluate plant bacterial contamination status as well as their phytochemical characteristics. Water and plant samples were then examined by bacterial culture and powdered dry plant samples were analyzed using Fourier Transform Infrared (FTIR) spectroscopy. Bacteriological results showed that water was worryingly contaminated with clear bacterial contamination in the local flora. The major chemical compositions of the studied local plant were within the normal ranges under pollution conditions with clear signs of antibacterial potentials in *S. natans* and *Ceratophyllum* spp. Additionally, FTIR spectroscopy results showed that many of the regional plants contain various important phytochemical functional groups which increase their importance in the marsh ecosystem sustainability.

1. Introduction

The Mesopotamian marshes cover a total area of 7500 Km^2 and represent the largest wetland area in the Middle East and West Asia [1]. Additionally, these areas have a role in determining the course of Tigris and Euphrates rivers and their branches. Freshwater ecosystem of these important wetlands, especially at Al-Chibayish, Al-Hammar, and Al-Hawiezah marshes were heavily affected since the area was subjected to deliberate desiccation during the eighties of the last century [2]. Al-Chibayish marsh is a group of marshes to the east of Nasiriyah, which is considered an extension of the Abu Zark and Al Hammar marshes. It is fed by water from the Euphrates and the Tigris rivers, and considered the natural habitat for many types of birds such as mallard, the grey heron, and seagull; and wide varieties of fish such as Liza, carp, and Tigris asp in addition to a rich flora of reeds and papyrus.

Water is a crucial component in any environmental system, in addition to its importance for human direct uses, agriculture, industry, and energy production [3]. Water contamination is a major worldwide issue that requires continuous updates and progressing assessments at all levels as it has been suggested that water scarcity and pollution are among the main reasons for mortalities and sicknesses worldwide [4]. For that purpose, many classification strategies and analysis protocols are used at the physical, chemical and biological levels [5].

Biological methodology in water analysis is based on the utilization of plants and other living creatures, as well as microbial life which capacity, population, or status can serve as markers to screen the wellbeing of an ecosystem [6]. Coliform, which are not a genuine reason for infection, are generally utilized as a bacterial marker of water contamination; however, a wide spectrum of accompanying infectious microorganisms are also found in surface

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waters including *Burkholderia pseudomallei*, Salmonella, *Cryptosporidium parvum*, *Giardia lamblia*, in addition to parasitic worms including the Schistosoma type, and viruses such as norovirus [7]. Coliforms include different bacterial species such as *Escherichia coli* that is commonly found in soil, vegetation and in the gastrointestinal tract of creatures and thus can be transferred to freshwater supplies from the immediate pollution by wastes into streams or lakes, or from spillovers and leakages from pastures and water treatment facilities into streams or groundwater; therefore, its presence in water resources is conclusive evidence of pollution [8].

Various Plant varieties inhabit the Iraqi marshes ecosystem and have a substantial place in the food chain as a component of different animals and fish diets [9]. Furthermore, many of these plants have antibacterial and biofiltration potentials. Therefore, this study aims to evaluate the status of Al-Chibayish marsh water and regional plants in regard to bacterial contamination and to investigate the phytochemical characteristics of the region's plants.

2. Materials and Methods

2.1. Water and plant samples

The present study was conducted on three water samples sites and various types of plants from Al-Chibayish marsh, south Iraq (Fig. 1). Samples were collected in February 2017. The water and plant samples were collected using sterilized disposable plastic containers, then labeled and transported to the laboratories of the Ministry of Science and Technology for examination.

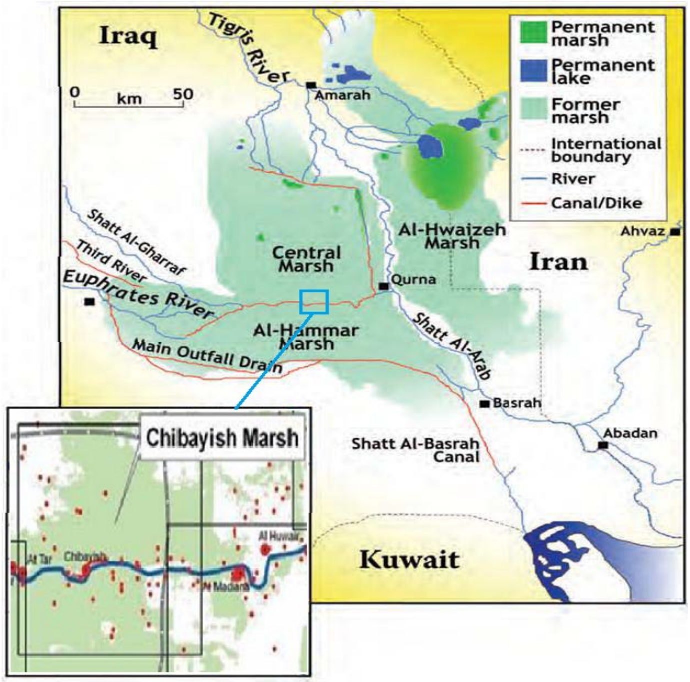

Figure 1. Al-Chibayish marsh geographic location in Iraq (Source: UNEP 2010 modified by researcher)

2.2. Laboratory tests

Water and plant root samples were analyzed for coliform concentration using a 5-tube decimal dilution multi-tube flow system (MTF) and then, the results were interpreted according to the table of most probable number (MPN) for FDAs bacterial analytical manual [10]. *Pseudomonas aeruginosa* determination was carried out according to [11]. Two types of presumptive and confirmatory media were used and evaluated for the presence of *P. aeruginosa*: asparagine enriched broth and a cetrimide agar. Culture media were incubated at 37 °C and 42 °C respectively and then evaluated using ultra-violet fluorescent [11].

Plant samples of various regional plants (*Chara vulgaris*, *Salvinia natans*, *Tamarix Arabica, Ceratophyllum* spp., *Schoenoplectus litoralis*, and *Typha australis*) were analyzed for their carbohydrate, protein, fiber, ash, and oil content using the methods proposed in [12]. Additionally, the functional phytochemical groups were analyzed using Fourier Transform Infrared (FTIR) Spectroscopy. An amount of dried powdered plant sample was analyzed independently in the presence of potassium bromide (KBr). Pellet was set up with the assistance of pellet creator and this pellet was set in IR chamber and investigated using Bruker Alpha-T FTIR Spectrometer (Burker – USA) [13].

2.3. Statistical analysis

All tests and analyses were carried out in triplicates. Data were analyzed using IBM SPSS software, using Tukey's test at a probability level of *P< 0.05*.

3. Results and Discussion

The results of bacteriological test (MPN test) of all three water samples demonstrated a higher presence for that fecal Streptococcus and Enterococcus groups in sample sites 1 and 2 when compared to other studied forms; while total coliform showed higher frequency in sample site 3 as shown in (Table 1). Overall, Total Coliform MPN.100 ml^{-1} ranged between 17-420, while fecal Streptococcus and Enterococcus groups MPN.100 ml^{-1} ranged between 22-230. On the other hand, *Pseudomonas aeruginosa* MPN count per 100 ml ranged from 6.8-70. Total Coliform was also investigated in all plants and sediment samples as shown in (Table 2). The bacteriological study illustrated that all plant samples were contaminated with bacterial species found in the sediments; however, some plant species exhibited moderate to high contamination resistance compared to other types such as *Salvinia natans* with 100, 10, 0, and 200 for the total count, *Staphylococcus aureus*, fecal coliform, and total coliform.

Table 1. Bacterial analysis of three water sampling sites of Al-Chebaysh marsh

Sample site (1)	MPN 100 ml^{-1}	95% Confidence range	
Total coliform	64	17-180 (Confidence limits)	
		Low	high
Fecal Streptococcus And Enterococcus groups	70	22	170
Pseudomonas aeruginosa	30	10	70
Sample site (2)	MPN 100 ml^{-1}	95% Confidence range	
Total coliform	75	17-200 (Confidence limits)	
		Low	high
Fecal Streptococcus And Enterococcus groups	94	34	230
Pseudomonas aeruginosa	21	6.8	42
Sample site (3)	MPN 100 ml^{-1}	95% Confidence range	
Total coliform	120	37-420 (Confidence limits)	
		Low	high
Fecal Streptococcus And Enterococcus groups	79	22	70
Pseudomonas aeruginosa	26	9.8	70

Table 2. Bacteriological analysis of sediments and various plants samples from Al-Chebaysh marsh

Sample name	Total count	*Staphylococcus aureus*	Fecal coliform	Total coliform
Sediment samples	$4.8x10^8$	5000	$1.2x10^4$	$1.5x10^6$
Ceratophyllum spp.	$2.0x10^5$	100	80	100
Typha australia	$3.0x10^8$	800	$8.0x10^5$	$1.0x10^6$
Salvinia natans	100	10	-	200
Tamarix arabica	$1.2x10^5$	500	10	$1.0x10^4$

All samples of water were contaminated with bacterial species, especially fecal *Streptococcus* and *Enterococcus* groups followed by total coliform. These results are in agreement with several previous studies regarding the same issue, all of which demonstrated high bacterial contamination cases in Iraqi marshes and illustrated both point and/or non-point sources of fecal pollution [14-19]. However, the high pollution rates in the current study are mostly due to consolidated sewers systems which may release untreated sewage during precipitation storms during the time of the study [20].

The presence of *P. aeruginosa* in all water samples is concerning as it can cause various infections whether the polluted water was consumed directly or by contact with contaminated water [21]. Therefore, [22] previously recommended the absence of *P. aeruginosa* in 100 ml of drinking water while [23] recommended its absence in 250 ml of bottled water. On the other hand, [24] recommended strict regulations regarding *P. aeruginosa* presence in water resources for human uses. Plant samples also demonstrated high bacterial contamination rates which were in some cases almost identical to those of sediments such as *Typha australia* (Table 2); however, the low bacterial counts observed in *Salvinia natans* are interestingly promising as the in vitro antimicrobial effects of this plant's extract was previously reported against *E. coli*, *Vibrio sp*. and *S. aureus* [25].

Chemical composition of the collected plant samples was evaluated using Fourier Transform Infrared (FTIR) Spectroscopy and plants' content of oil, protein, carbohydrate, fiber, minerals, and water was determined as seen in (Fig. 2). All components were within normal values previously reported in [26][27].

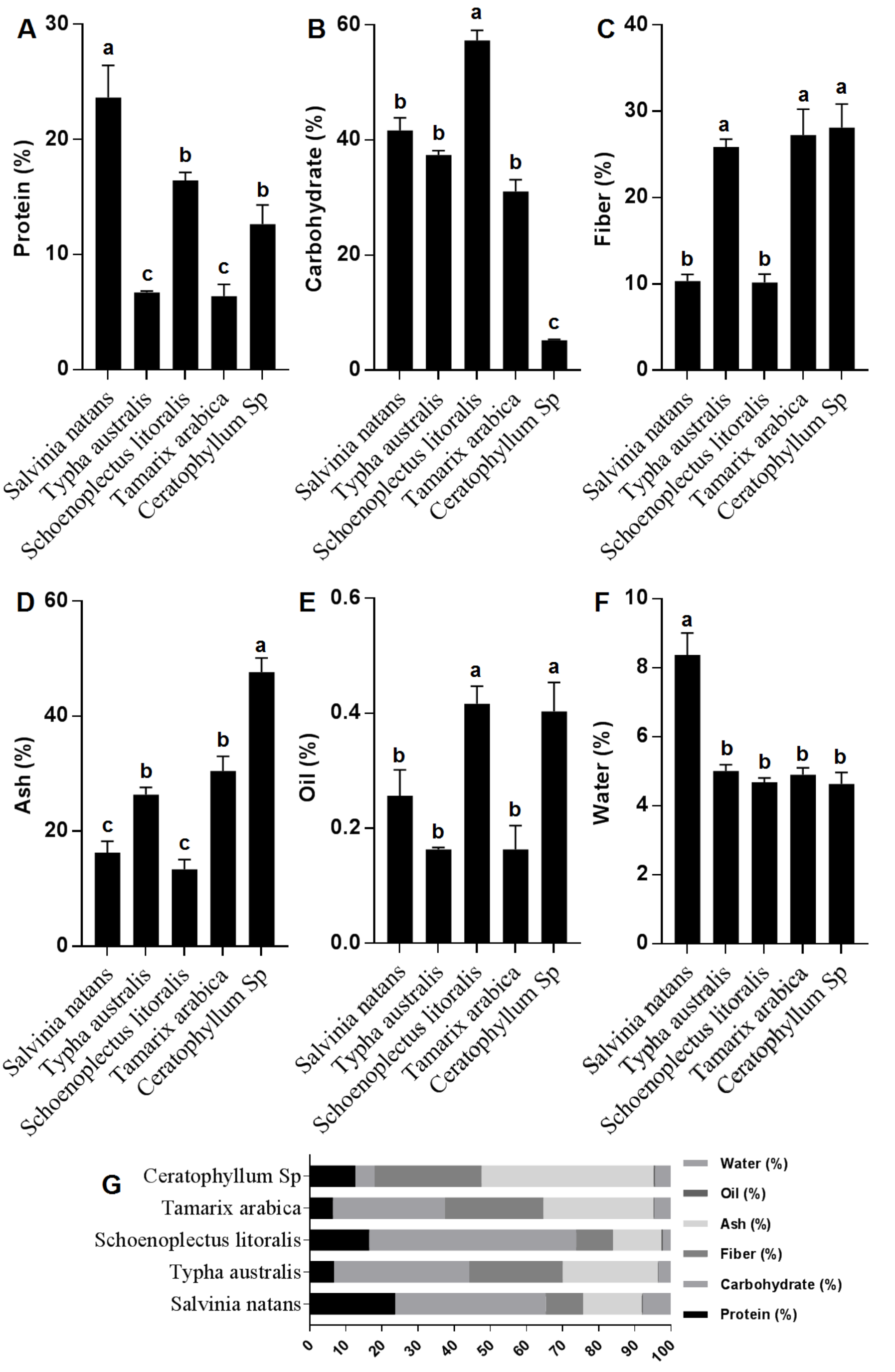

Figure 2: Chemical composition of various plant species inhabit Al-Chibayish marsh based on the air-dried weight. (A-F) represent protein, carbohydrate, fiber, ash, oil, and water percentages in the analyzed samples respectively introduced as (mean ± SD) compared using Tukey's test at a probability level of *P< 0.05*; (G) represents the accumulated percentages in different plant types

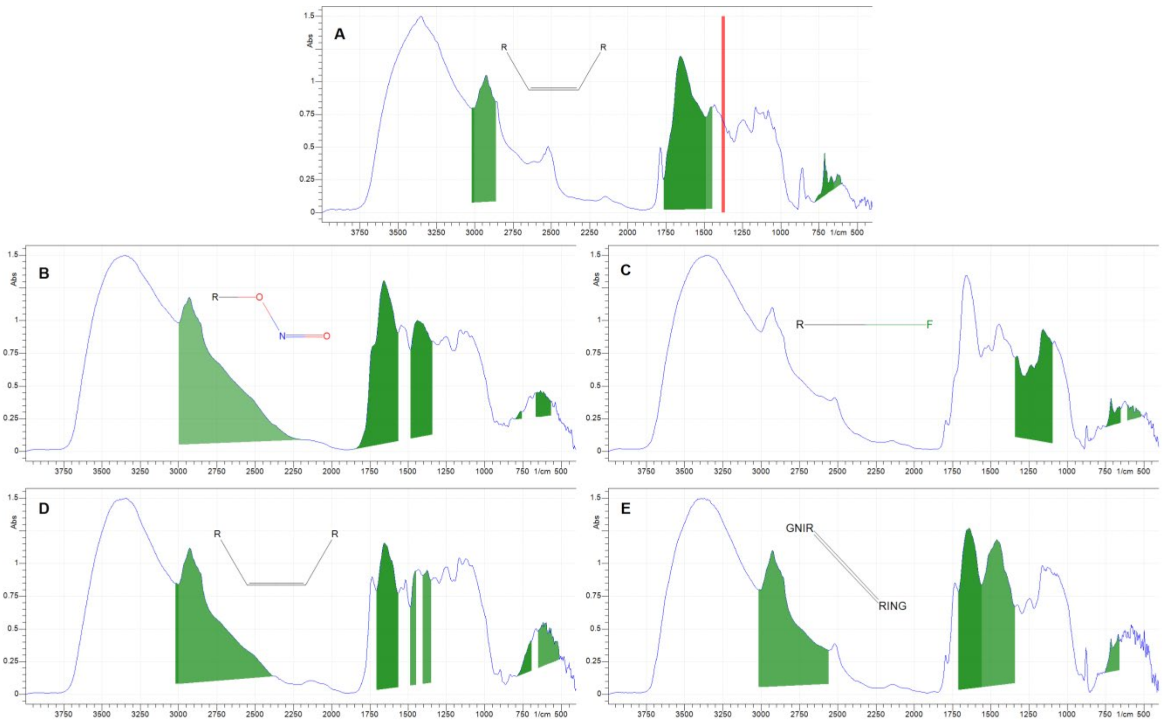

Figure 3. FTIR spectrum analysis of air-dried *Chara vulgaris* **(A)**, *Salvinia natans* **(B),** *Ceratophyllum* spp. **(C)**, *Schoenoplectus litoralis* **(D)**, and *Typha australis* **(E)** samples collected from Al-Chibayish marsh

FTIR spectroscopic analysis for some of Al-Chibayish marshes plants (*Chara vulgaris*, *Salvinia natans*, *Ceratophyllum*, *Schoenoplectus litoralis*, and *Typha australis*) was carried out. The functional groups were separated based on their bonding positions and the resulting FTIR analysis peaks confirm the presence of a wide range of bioactive compounds functional groups [28].

The results of the FTIR spectrum for *C. vulgaris* (Fig. 3 A) confirmed the presence of Cis Alkene at the peak 2925.41 cm^{-1} [29]; additionally, the peak at 2856.66 cm^{-1} corresponded to the HC=CH stretching, which means that some alkene compounds are phenols and alcohols while the peak at 1750-1480 cm^{-1} corresponded to C=O and/or C=C, which refers to the presence of celluloses and hemicelluloses [30]. The high alkenes and carboxylic compounds content in addition to the moderate amounts of phenolic compounds present in *C. vulgaris* refer to the antioxidant potentials of this plant [27][31][32].

The peaks at the values 3000-2350 cm^{-1}, 1648.78 cm^{-1}, 1546.16 cm^{-1}, confirm the presence of primary amines, carboxylic acids [33], and nitro compounds [34] respectively in *S. natans* (Fig. 3 B). The high levels of aliphatic nitro-oxy compounds most probably contributed to protein and carbohydrate content as seen in (Fig. 2 A and B) which might render this plant as an important component in animals and fish diets [34][40].

FTIR analysis results of *Ceratophyllum* spp. demonstrated a presence of aromatic amines as the corresponding peak between 1321.96-1100 cm^{-1} was noticed [35] (Fig. 3 C). This submerged macrophyte has a high vegetative propagation capacity and biomass production even under modest nutritional conditions [36-41]. Furthermore, *Ceratophyllum* species are known for their heavy metals filtration and mineral accumulation potentials [37-40] which can be noticed from the significantly higher ash content in this plant in comparison to other studied plants (Fig. 2 D) and antibacterial potential [38] which was also observed in this study against *S. aureus*, fecal coliform, and total coliform when compared to sediment samples (Table 2). As for *S. litoralis* FTIR analysis revealed two major peaks at 2923-2483 cm^{-1} and 1650-1440 cm^{-1} that corresponded to HC=CH, and Cis Alkene respectively (Fig. 3 D). *T. australis* analysis results demonstrate two distinctive peaks, the first at 3030-2700 cm^{-1} was assigned a-glucose and the second at 1710-1460 cm^{-1} which was assigned to C=C alkane (Fig. 3 E).

4. Conclusions

The microbial contamination status of Al-Chibayish march water is worrying as the polluted areas spread across the region with clear bacterial contamination in the local flora. However, the regional plants have managed to retain their major chemical composition within the normal ranges under pollution conditions and some types introduced clear signs of antibacterial potentials such as *S. natans* and *Ceratophyllum* spp. Furthermore, FTIR spectroscopy results showed that many of the regional plants contain various important phytochemical functional groups which increase their importance in the marsh ecosystem sustainability. More studies should be carried out to evaluate the chemical pollution in the region to provide a better understanding of the situation and to introduce effective remediation solutions. Additionally, more expanded studies regarding the phytochemical potentials of marsh flora under polluted conditions should be carried out.

Conflict of interest statement

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Data availability statement

The authors declared that all related data are included in the article.

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