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The role of *Spirogyra grevilleana* in purifying the well of Fatima Al-Zahra neighborhood in Al-Bayda city

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ABSTRACT

Water pollution refers to any changes in water's physical, chemical, or biological properties that affect its suitability for various uses. Surface water pollution, including rivers, lakes, and springs, occurs when untreated effluents are discharged into them. However, water pollution is not limited to surface water alone; groundwater and well pollution is also a prevalent issue, primarily due to the increased use of chemical fertilizers and pesticides in agricultural areas and the discharge of sewage and industrial waste into unregulated areas. Following the discovery of *E. coli* contamination in a well in Al Bayda, Libya, after Hurricane Daniel in January 2024, an experiment was conducted to purify the polluted water using algae. Specifically, the algae *Spirogyra grevilleana* collected from a stagnant pond in the Al Faydiya area was washed, dried, and examined under a microscope. The algae were used as a purifying agent for the polluted water. Subsequent tests showed complete elimination of coliform bacteria, as well as significant reductions in total dissolved solids, pH, calcium, magnesium, iron, lead, nitrate, phosphate, and biological oxygen demand values. Hence, we would like to report the significant activity of algae as a natural resource and green chemistry in purifying polluted water.

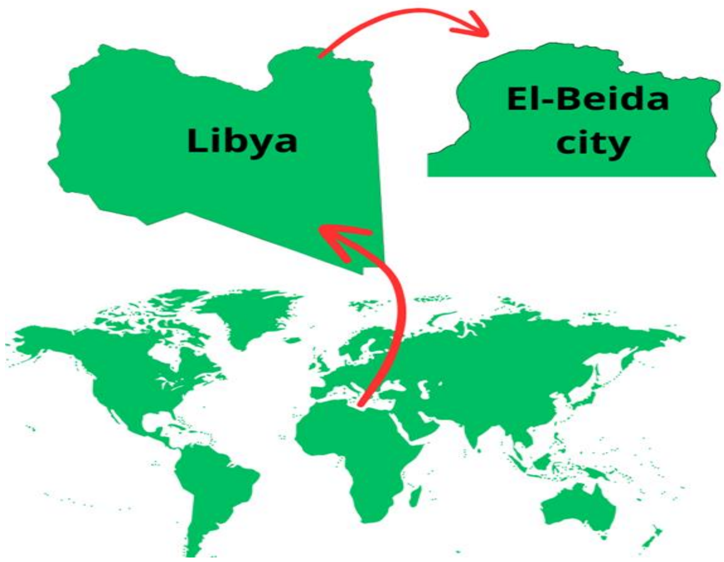
Introduction

Improving the environmental status of water resources is a growing concern for many developed and developing countries, particularly by reducing microbial damage in wastewater [1]. Water pollution has become a global problem now and is ongoing[2]. There is a need to evaluate water resources policy to address this problem. Deaths and diseases occur worldwide due to water pollution, and approximately 14,000 people die every day due to water pollution [3]. Both developed and developing countries face the problem of water pollution, and water quality is affected by several factors such as rainfall, climate, soil type, vegetation, geology, groundwater conditions, and human activities [4, 5]. In recent years, microalgae have received increasing attention in their implementation as part of wastewater treatment. This is based on their ability to utilize organic carbon, as well as inorganic nitrogen (N) and phosphorus (P) in wastewater for their growth, with the desired results of reducing the concentration of these substances in the water [6]. Research has shown that water treated with microalgae has demonstrated rapid reductions in metals, nitrates, and phosphates. This indicates the potential for microalgae to be marketed as a wastewater treatment solution. Before beginning any type of treatment. Large-scale water pollution is a significant global issue. Natural water bodies are becoming increasingly contaminated with pathogenic bacteria and organic dyes, posing serious health risks to humans and aquatic life. The demand for safe drinking water is growing worldwide, with approximately 2 billion people still lacking access to clean water. Every day, around 2 million tons of waste from industrial, agricultural, and human activities are released into rivers, carrying harmful substances such as synthetic dyes and pathogenic bacteria [7]. The freshwater algae *S. grevilleana* was utilized in a pilot biofiltration system to decrease *E. coli*, nitrate, and phosphate levels. Water from a 2.32-hectare lake in Atlanta, Georgia, USA was continuously pumped through algal filters with low and high concentrations of *S. grevilleana*. The wastewater was regularly tested for *E. coli*, nitrate, phosphate, dissolved oxygen, and pH levels. Both concentrations of *Spirogyra grevilleana* reduced *E. coli* by 100% and significantly decreased nitrate (30% ± 13%) and phosphate (23% ± 5%) concentrations while maintaining normal levels of dissolved oxygen and pH. Using *Spirogyra grevilleana* in an algal filter system could offer a sustainable, adaptable, and cost-effective method for reducing *E. coli* in freshwater lakes worldwide. Early results indicate that using *Spirogyra grevilleana* with an algae filter system may produce drinkable water [8]. *Spirogyra*, known as "water silk," is a filamentous green alga that thrives in slow-flowing and shallow water bodies. The intracellular metabolites of phytochemicals and antimicrobial activity were studied. An aqueous extract containing *E. coli* was found to be bactericidal against *E. coli* [9]. The filamentous algae genus *Spirogyra* gets its name from the distinctive spiral shape of the chloroplasts found in its members. Sometimes referred to as "water silk," "mermaid tufts," or "pond scum," the high presence of unbranched algae often indicates nutrient enrichment in freshwater bodies.

Materials and Methods

Study location

The well is situated to the southeast of Al Bayda city and provides water to the surrounding residential areas (**Scheme 1**). It was previously contaminated, but after Hurricane Daniel and subsequent flooding, residents became concerned, leading to a decrease in water withdrawal (see Figure 1). This caused the well to overflow into the streets, resulting in a depletion of its water level. Before the overflow, the amoeba was observed in the water, and a sewage smell was emitted from the well. The residents now rely on the second well for their daily water needs (see **Figure 1**).



Scheme 1 :The city of Al Bayda is located in eastern Libya. Its geographical coordinates are 32.756417 north and 21.7376178 east.

Tools and materials:

- 6-liter bottles - Oxygen pumps- Light source (neon lamp) - Barley water for algae feeding
- Microscope slides 7101 for examination- Leica microscope DM750

(a)

(b)

(c) (d)

Figure (1): (a) Water cut off from the first well. (b) Contaminated waste on the first well. (c) Collect samples from the contaminated well. (d) Algae gathering in Ain Manza in Al Faidiyah area

In January 2024, samples of well water contaminated with sewage were collected. The samples were stored in 10-liter plastic bottles, refrigerated, and kept in the dark until returned to the laboratory. Additional contaminated well water samples were collected, and a few drops of strong nitric acid were added to measure heavy metal levels. The sampling was conducted following specific protocols (American Public Health Association) [10, 11]. Upon return, the pH of the contaminated water was measured using a pH meter (Orion 290A) and found to be between 7.8 and 7.6, with a temperature ranging between 18°C and 20°C. Freshwater macroalgae were collected from Ain Manza (refer to **Figure 1**) in the Faydiya area. The macroalgae were washed with distilled water, and the green filamentous species were manually separated and transferred into three 6-liter bottles filled with mineral water. Oxygen pumps were added to each bottle, and the bottles were illuminated. The algae were fed with alcohol-free barley water daily, 5 ml per bottle (refer to **Figure 2**).

(a) (b)

Figure (2): (a) Creating the optimal conditions for algae to grow**. (b)** The algae were fed with alcohol-free barley water daily, 5 ml per bottle.

X-Gluc medium for detection of *Escherichia coli*

X-Gluc Dicyclohexylamine is a dye-based reagent used to detect the presence of β-glucuronidase, an enzyme produced by *E. coli.* X-Gluc Sodium is used to detect *E. coli* contamination in food, water, and urinary tract. Furthermore, X-Gluc Sodium is widely used in molecular biology experiments for labelling and detecting the expression of target genes. 20 mg of X-Gluc Dicyclohexylamine was dissolved in 1 ml of dimethylformamide (DMF) to prepare the X-Gluc master mix. The prepared X-Gluc solution was added to agar plates at a final concentration of 50 µg/ml without sterilization. The plates were allowed to air dry. The plates were incubated at 35 °C and observed for 16–24 h after inoculation [12].

RESULTS

Water pollutants can include pollutants, microbes, and other suspended or dissolved solids in wastewater and nutrients. Discharging wastewater into water bodies is a serious environmental problem that can cause health issues for humans, animals, and plants. Various methods have been used to remove nutrients such as phosphorus and nitrogen from wastewater and wells, including chemical treatments and conventional biological methods. However, their use is limited by their high cost and increased sludge production. As a result, researchers are now focusing on microalgae as a cost-effective and low-sludge alternative for removing nutrients from wastewater, wells, and rivers. The physical and chemical composition of the collected wastewater used as a culture medium is detailed in **Table 1**.

Table (1). Colour, pH, nitrogen, phosphorus, BOD (ppm) and reduction rate before and after treatment

	Colour	pH	Nitrogen	Reduction%	Phosphor	Reduction%	BOD	Reduction%
Before	pale yellow	8.1	1.3	76.92	0.12	83.33	497	73.64
After	unicolour	7.6	0.3		0.02		131	

The wastewater and post-purification water were analyzed for total nitrogen (TN), total phosphate (TP), and heavy metals. The results showed that these substances were present in higher amounts in the wastewater than in the water after purification with *S. grevilleana*. A high BOD indicates an excess of organic carbon. Adding wastes with high BOD values to aquatic ecosystems causes oxygen depletion. The higher the BOD of a waste source, the more potent its pollutant is. The study results, presented in **Tables 1**, demonstrate that cultivating *S. grevilleana* in contaminated well water for 10 days led to significant removal of BOD, with a rate of 73.64% [13]. The growth of algae populations facilitates the decomposition of organic matter by producing oxygen, which in turn replenishes the oxygen required by heterotrophic bacteria. Over time, oxidation ponds tend to fill up due to the sedimentation of bacterial cells and algae formed during the decomposition of contaminated water[14]. In May and June 2024, the freshwater alga *S. grevilleana* was isolated and used in an experimental biofiltration system to reduce *E. coli*, nitrate, and phosphate levels. The phosphorus concentration decreased to 83.33% in the water contaminated with *S. grevillea* (see Table 1). Phosphorus is essential for growth and many biological processes, including energy transfer and DNA production. Its deposition and uptake into biomass, as well as intracellular polyphosphate dynamics, have been proposed as mechanisms for phosphorus removal, especially at high pH levels. It is hypothesized that the primary mechanism for TN and TP removal is biomass uptake. Thus, contaminated water with a higher growth rate results in higher TN and TP removal. Two samples were taken from the first well and the second well, and *E. coli* analysis was performed on both samples. The results showed that the second well was free of *E. coli*, while *E. coli* appeared in the first well (Figure 4), with the number of colonies exceeding one hundred. According to Libyan water quality standards, this water is considered microbially contaminated. *S. grevilleana* algae was introduced into the contaminated well sample for treatment. It was provided with pumps and permanent lighting. Worms were visible when the sample was examined under a microscope (see Figure 5). These worms typically feed on sewage sludge and thrive in anaerobic conditions [15], in addition to harmful protozoa. The examination lasted for about six days. On December 5, 2024, the first cilia appeared, indicating that the purification process was on the right track. *Vorticella* (see Figure 5) is a genus of protozoa with cilia shaped like inverted bells. Sometimes, *Vorticella* are held in groups by a single stalk and can break away from the group when environmental conditions are unfavourable [16]. They are multicellular, swimming or crawling organisms, 40-500 µm long, characterized by a ciliated or funnel-shaped region at the anterior end that may resemble spinning wheels. Vorticella or vorticella are commonly found in the settled activated sludge environment of wastewater treatment plants and are often used as an indicator of the performance of a wastewater treatment system. In addition to their function as predators of bacteria and suspended biomass particles, they can also secrete a type of mucous glue as a means of protection. Thus, vorticella and vortices have a significant effect in reducing waste and turbidity [17]. Harmful worms and protozoa disappeared, *paramecium* appeared, and the green colour of the algae increased (see Figure 6) due to the increased biomass and activity of the algae. After two weeks, the algae began to decrease and the ciliates and paramecium disappeared, indicating the completion of their mission. The sample was examined and confirmed to be free of harmful and beneficial protozoa. Subsequent tests were conducted to detect *E. coli*, and the results showed that *E. coli* had completely disappeared (Figure 4). Thus, the main objective of the experiment was achieved, which was to raise awareness of the importance of algae as a renewable natural resource and its role in the purification process without cost or negative impact on the environment. The algae were then used as a natural fertilizer for plants near the College of Natural Resources and Environmental Sciences building.

Table (1). Concentration of chemical elements (ppm) before and after treatment

	Na	K	Ca	Mg	Pb	Cd
Before	26.3	9.55	25.37	4.7	0.966	0.076
After	18.5	8.71	11.69	2.6	0.585	0.069

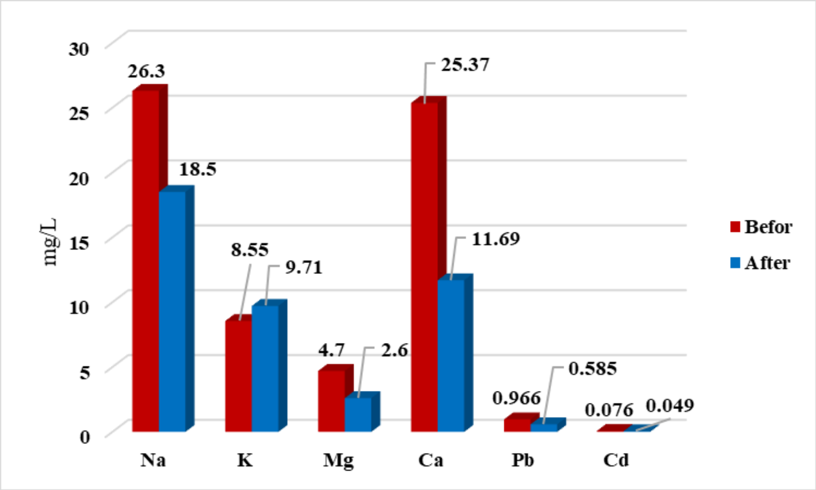
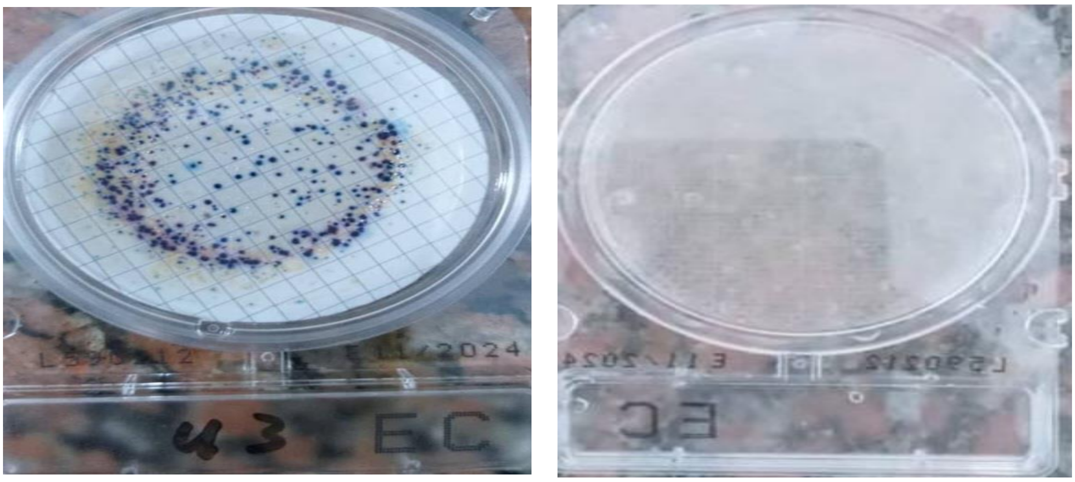


Figure 3 The concentration of chemical elements (ppm) before and after treatment



(a) (b)

Figure 4 (a) *E. coli* colonies appear in the contaminated well (b) *E. coli* disappears after treatment with algae

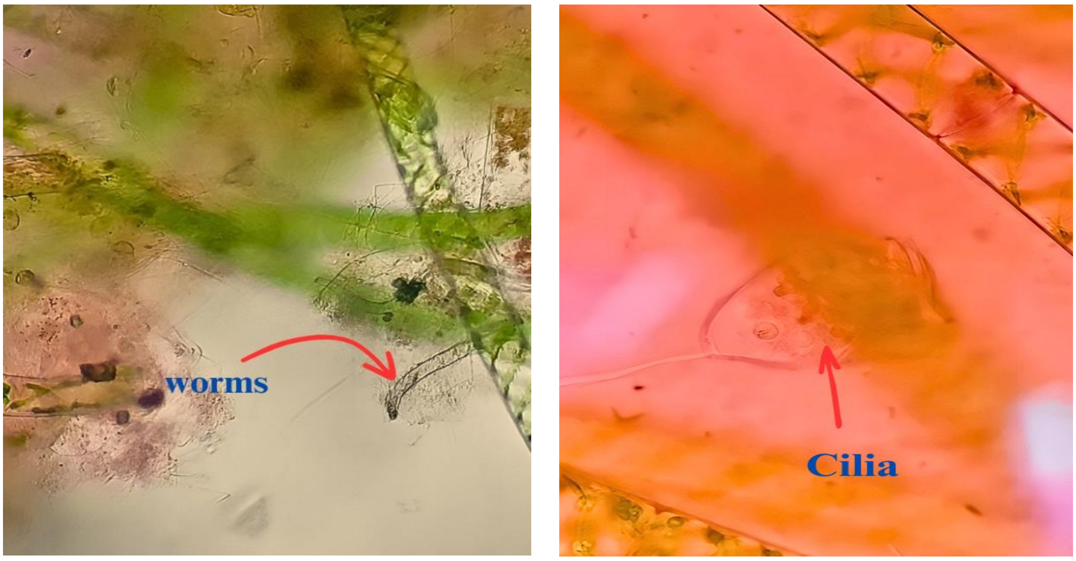


Figure 5 (a) Worms appear when testing contaminated water. **(b)** The presence of cilia (*Vorticella*) indicates that the purification process is progressing well.

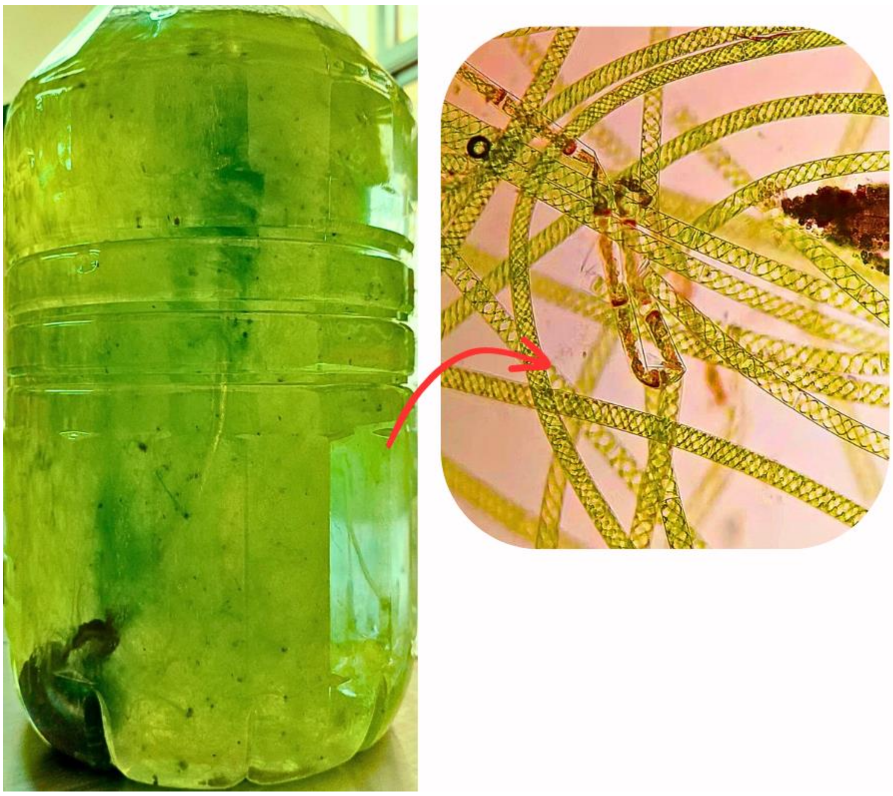


Figure 6 Increased biomass and activity of Spirogyra algae

Recommendations

- Build systems that prevent rainwater from being wasted in streets and elsewhere.
- Reduce the use of plastic waste that causes water pollution.

- Replace chemical detergents that pollute the water system with other organic detergents.
- Contribute to water conservation to preserve water suitable for consumption.
- Do not dispose of medicines by draining them into waterways.
- Do not throw waste into water and dispose of waste properly.
- Adopt a water-saving approach as much as possible.
- Recycle and reuse used water.
- Do not use pesticides and herbicides.
- Use earthen (grass) surfaces instead of cement surfaces and protect the soil from erosion factors.
- Continue to clean waterways.
- Conduct routine inspection of home sewage system lines every 3-5 years.
- Cleaning water bodies and disposing of accumulated waste.

Conclusion

Spirogyra algae, extracted from freshwater, can remove nutrients and heavy metals. They also act as an antibacterial agent and natural fertilizer. This dual process offers several advantages, including low costs, minimal energy input, and low greenhouse gas emissions. The use of microalgae as a biological tool for monitoring and evaluating environmental toxicology has been successfully demonstrated. Research results show that *Spirogyra* algae can effectively clean wastewater from nutrients and heavy metals. The levels of the studied metals in the contaminated well water was lower, except cadmium. Moreover, the contamination of the well water with *E. coli* bacteria was reduced by over 100%. Further research on phytochemicals is necessary to identify the components responsible for the antibacterial activity of these extracts against bacteria.

Author contributions: S.H. developed the experimental design, performed the laboratory analyses, and contributed to data interpretation.
R.S. supervised the project, reviewed the results, and contributed to the final version of the manuscript. Both authors discussed the results and approved the final manuscript.

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