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Environmental study using bioremediation to evaluation of gene expression of algae living in wastewater

Ahmed F. Shihab[1], Suaad H. Ali[2], Sanaa T. Jawed[3]*

Abstract

Various water bodies in many countries, especially developing ones, suffer from the continuous discharge of sewage, which affects water quality and quantity. Therefore, the role of bioremediation using environmentally friendly organisms has become prominent. These organisms play a role in treating and removing pollutants, such as algae, which are known for their ability to remove numerous pollutants, including heavy metals (HMs). However, there are few studies worldwide that address the gene expression of algae. This study aimed to compare the effectiveness of two algae species: L. platensis (cyanobacteria) and Spirogyra sp. (green algae) in removing heavy metals from wastewater. The results showed that the lowest mercury levels were recorded before and after biological treatment, while the highest iron levels were recorded in L. platensis. Similarly, the lowest mercury levels before and after treatment were recorded in Spirogyra sp., along with the highest iron levels. This study also revealed that the expression of the abc transporter gene in L. platensis was more efficient at removing heavy metals compared to the sod gene in Spirogyra sp.

[1,2,3]Department of Biology, College of Education for Pure Science, University of Thi-Qar, 64001, Iraq
*Email: sanaatalib.bio@utq.edu.iq
Corresponding Author*: Sanaa T. Jawed, Department of Biology, College of Education for Pure Science, University of Thi-Qar, 64001, Iraq, Email: sanaatalib.bio@utq.edu.iq

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Introduction

Various algae possess striking plasticity in diverse environments, which has garnered attention as eco-friendly resources in various sectors (Das and Bhattarai, 2025). The environmental acclimation of algae and their striking capabilities with various environmental factors is the emphasis of much research in environmental, medical, and agricultural sectors (Nayaka et al., 2017; Al-Badri and Al-Ebady, 2019). Algae live in aquatic environments, whether saline or non-saline, even polluted ones such as heavy water, because their wide potential for sorbent nutrients and HMs, which has led them to be a marker in bioremediation studies (Faruque et al., 2024; Zgeahr and Jawed, 2025).

Bioremediation is a procedure with diverse mechanisms for removal of pollutants, including HMs. Because of the varying abilities of organisms like plants, animals, or microorganisms to treat pollutants via biotransformation and intracellular accumulation (Pande et al., 2022). These mechanisms arise via two pathways. The first includes physicochemical processes that result in the adsorption of heavy metal ions onto the outer surfaces of algal cells. The second pathway involves the absorption of pollutants by the cells themselves. A slower more complex and more regulated operation that includes the transport of HMs via cell membranes (Chugh et al., 2022 and Jawed et al., 2022).

Gene expression is a procedure that examines the ability of genes to role within cells, depending on whether active or inactive, by regulating the production of targeted proteins and enzymes, such as metallothioneins, the superoxide dismutase (Sod) gene and ion transporters (Hassanien et al.,2025). One of the genes exhibited by green algae like Spirogyra sp. is responsible for the synthesis of the Sod enzyme. This enzyme protects cells from constant oxidative stress and maintains the balance of functional redox processes within the cells (Shams et al., 2024). In cyanobacteria including L. platensis, several enzymes are present that play a role in pollutant processing. Among them are abc transporter genes also called ATP-binding cassettes. These proteins are found in the plasma membrane that use the energy of adenosine triphosphate (ATP) molecules to transport various substances via the cell membrane including HMs (Ritter et al., 2014).

This research goals to examine the role of the algae Spirogyra sp. and L. platensis in algal bioremediation, and to assess the gene expression of both in HMs removal.

Methodology

The study area includes wastewater collection from a wastewater collection station located near Holland Bridge in Nasiriyah, Thi Qar Governorate, Iraq.

L. platensis was isolated from ALMASSAB ALAAM canal streaming through Nasiriyah city between the month of December 2024 and July 2025, at a depth of approximately 15 cm below the water surface from nearby wastewater collection stations. Clear 5-liter plastic bottles were used to collect the water samples in triplicate for physical and chemical testing and to determine heavy metal concentrations (Anitha and Sugirtha, 2013).

Physical and Chemical Properties and Heavy Metal Analysis

Dissolved oxygen and pH were measured using a multimeter model pH 03/618/k13 after calibration with standard solutions, also for the BOD5 value, it was measured after incubating the water sample for five days at a temperature of 20°C (APHA, 2023). Chemical oxygen demand measurement was performed according to the method adopted in (APHA, 2017), a quantity of the contaminated water sample to be tested was added to a 20 ml tube, followed by a quantity of $K_2Cr_2O_7$. According to the method described in (Wood et al.,1979) and explained by (Parsons et al.,1984). The effective nitrate measurement was used by using a cadmium column to reduce the nitrate to nitrite, then it was measured by spectrophotometer at a specific wavelength of 543 nm. According to the method described in (APHA, 1998), the active phosphates were measured using the ascorbic acid method

Collection of Algae

L. platensis was isolated from ALMASSAB ALAAM canal flowing through Nasiriyah between December 2024 and July 2025. According study (Weidman et al., 1984) The sample was washed several times with distilled water to ensure its purity from any microbial contamination and then centrifuged at 3000 rpm for 1.5 minutes. This procedure was repeated 12 times. Spirogyra sp. algae were also manually collected as algal clumps directly from the river, placed in plastic bags, and then washed with sterile distilled water. Microscopic examination was subsequently performed to confirm the presence of the two species identified in this study.

In the laboratory, the algal samples were placed in triple-copied glass tubes. Concentrated perchloric acid and nitric acid were added to each tube in a 3:1 ratio, and the tubes were then placed in a water bath for 30 minutes to accelerate digestion. The samples were then transferred to clean plastic bottles for analysis using inductively coupled plasma (ICP) spectroscopy, and the results were expressed in mg/L.

Preparation and sterilization of culture media

The culture zarrouk medium prepared by (Zarrouk, 1966) as stock solutions as a culture medium for L. platensis, as shown in table 1. In table 2 the modified culture medium Chu-10 prepared by (Al-Aarajy, 1996) is used as a culture medium for Spirogyra sp. as stock solutions were prepared using distilled water without sterilization until use. The prepared culture media was then stored in a refrigerator at 4°C in the dark. Equal amounts (1 ml) of

each of the stock solutions were mixed, and the volume was brought up to 1 liter with distilled water. Both culture media were then sterilized in an autoclave at 121°C and 15 psi for 20 minutes and allowed to cool to laboratory temperature. Finally, phosphate salts were added after sterilization by filtration using filter paper with a specified mesh size (0.45) microns to prevent phosphate precipitation on the walls of the glass bottle during sterilization.

Table 1. The chemical composition of the zarrouk culture medium

The Compound	**g/L**	**The Compound**	**g/L**
$NaHCO_3$	**16.8**	H3BO3	2.86
K_2HPO_4	0.5	MnCl2.4H2O	1.81
$NaNO_3$	2.5	ZnSO4.7H2O	0.22
K_2SO_4	1.0	CuSO4.5H2O	0.08
NaCl	1.0	MoO3	0.01
$MgSO_4.\ _7H_2O$	0.20	CoCl2.6H2O	0.01
$CaCl_2$	0.04		
$FeSO_{4.\ 7}H_2O$	0.01		
Na_2-EDTA	0.08		

Table 2. The chemical composition of the Chu-10 culture medium

The compound	**g/L**	**The compound**	**g/L**
NaNO3	53.3	NaHCO3	25
K2HPO4	10	MnCl2. 4H2O	0.045
MgSO4. 7H2O	25	(NH4)6Mo7O24. 4H2O	0.007
CaCl2. 2H2O	40	ZnSO4.7H2O	0.056
FeCl3. 6H2O	1.46	CuSO4. 6H2O	0.02
Na2SiO3. 9H2O	6.2	H3BO3	0.72
Na2EDTA	31.8	CoCl2. 6H2O	0.01

Cultivation and propagation of algae

10 ml from the species of algae in this study and pre identified for the purpose of culturing on the liquid culture medium were transferred using a sterile pipette to a number of sterile glass flasks with a volume of (100) ml containing (70) ml of sterile Chu10 culture medium for Spirogyra sp., also 70 ml of Zarrouk culture medium for L. platensis The mouths of the flasks were closed with clean cotton and incubated at a temperature of (2 ± 25) °C for Spirogyra and (2 ± 32) for L. platensis with provide light of (260) microeinsteine /m2/s in the growth chamber and on a (8:16) hour dark : light system, taking into account the continuous shaking of the samples until the required growth is obtained, according to the method described in (Tomaselli et al., 1981).

Adding Algal Cultures to Wastewater

To carry out the biological treatment of wastewater, 500 ml of algal culture of both types was added independently to 2500 ml of sterilized wastewater in sterilized plastic containers, and their opening were covered with suitable pieces of gauze under sterile conditions. The algal cultures were left for two days to acclimatize to their environment and incubated at a temperature of (25 ± 2) °C for Spirogyra sp. and (32 ± 2) °C for L. platensis, with a dark-light cycle of 8:16 hours, providing light of (260) microeinsteine /m2/s in the growth chamber (Tomaselli et al., 1981). After thirty days, the algal mass was harvested to determine the concentrations of accumulated heavy metals in the algal cells. After Phytoremediation, according to the method described in (Stein, 1973), to obtain unialgal culture, the dilution and streaking methods were used to obtain pure isolates for gene expression studies using qPCR.

Gene expression

Gene expression was performed on both L. platensis and Spirogyra sp. RNA was initially extracted according to the instructions in the Geneaid RNA Extraction Kit. The RNA was then converted to complement DNA (cDNA) using reverse transcriptase, where 1 µg of RNA was used to synthesize the cDNA. The reaction was performed

using random hexamer primers to ensure uniform reverse transcription of all RNA transcripts. The required qPCR reaction mix was prepared using the SYBR Green master mix (which contains DNA polymerase, dNTPs, $MgCl_2$, buffer, and SYBR Green dye), gene-specific primers, cDNA template, and nuclease-free water, with a final volume of 20 µL.

To determine the gene expression of both the abc transporter gene for L. platensis and the sod gene for Spyrogyra. The following primers were used: forward primer AGGTGACTTGGCTTTCCTCG and reverse primer TTCGATGGCAGGATAGTCGC for the abc transporter gene, a primer was designed according to the (22). Additionally, a primer was designed for the Sod gene for Spyrogyra sp., using the following: forward primer TTCGATGGCAGGATAGTCGC and reverse primer CCTTGGATGTGGTAGCCGTT .

Primers were also designed in this study as a guide and housekeeping gene for comparing gene expression and differentiating between algae and their functional genes. For example, 16S rRNA was used for L. platensis, with the forward primer AAGCCTGACGGAGCAAGA and the reverse primer GGACGCTTTACGCCCAAT. Additionally, a primer was designed for an 18S rRNA gene for Spyrogyra sp., using the forward primer TTCGATGGCAGGATAGTCGC and the reverse primer CCTTGGATGTGGTAGCCGTT. The qPCR amplification was performed under optimized thermal cycling conditions involving of an initial denaturation (95°C for10 minutes) followed by 40-45 cycles of denaturation (95 °C for 30 seconds), annealing (58 °Cfor 30 seconds) , and extension (72 °C for 30 second**s)** with fluorescence obtained at each cycle.

Data Analysis

Cycle threshold (Ct) values were analyzed using the comparative Ct ($2^{-}\Delta\Delta Ct$) method (Giulietti et al., 2001) to determine relative expression levels of any gene normalized to a reference gene.

Statistical Analysis

The results and variables of the current studied were evaluated statistically using the Statistical Package for Social Sciences program (SPSS) version 26 (2019), One and two way anova at a probability of ($P \leq 0.05$) as a significant level.

Result

Physical and Chemical properties

The results showed that there was a difference in the studied values between the summer and winter seasons, as shown in Table 3, for all the indicators used in this study.

Identification of Algae in this study

Cultural and microscopically characteristics

L. platensis is a blue-green alga, ranging from light green to blue-green due to the presence of chlorophyll and phycocyanin pigments. Its small rod-shaped or cylindrical cells are connected by a spiral chain, giving it a characteristic spiral appearance under a microscope, as shown in figure 1, 2 and 3. Sometimes floats on the surface of the water because the cells contain internal gases that reduce their density (Habib et al., 2008 ; Santos et al., 2023 ; Sinetova et al., 2024).

Figure 1. illustrates the development of L. platensis

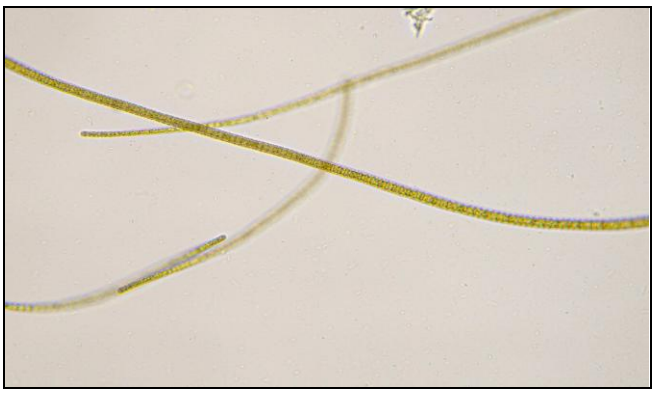

Figure 2. shows the L. platensis strand under a 40x microscope.

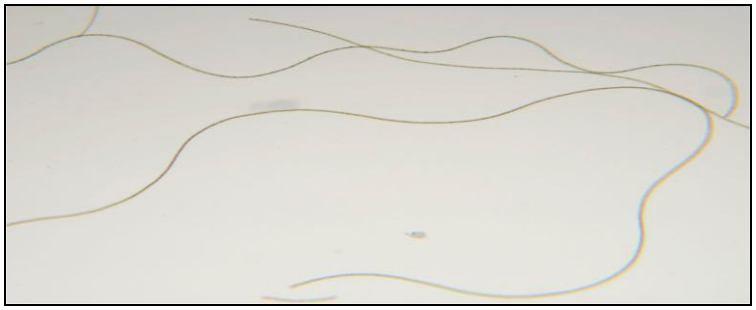

Figure 3. shows the L. platensis strand under a 10x microscope

As shown in figure 4 and 5 Spirogyra sp. A green filamentous algae characterized by its shiny resembling threads of hair. It is usually seen with the naked eye as green masses floating in still or slow-flowing freshwater. These algae are characterized by the presence of long, unbranched filaments composed of cylindrical cells connected at the ends to form a straight chain of cells surrounded by a transparent mucus layer. Each cell is surrounded by a thick cellulose cell wall and contains a large central vacuole and a spiral-shaped chloroplast extending the length of the cell (Wongsawad and Peerapornpisal, 2014).

Figure 4 illustrates the development of Spirugyra sp.

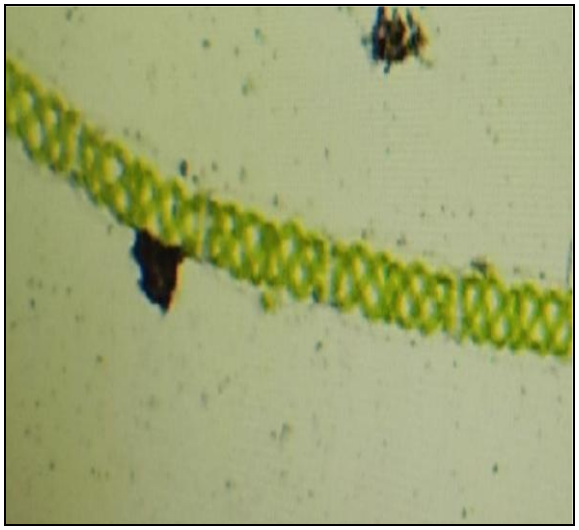

Figure 5. shows the Spirugyra sp. strand under a 40x microscope**.**

Heavy Metals

The results shown in Table 4 for L. platensis and Table 5 for Spirogyra sp. showed a difference in the values of all elements obtained before and after biotreatment. The lowest value was obtained before and after biotreatment for Hg, while the highest value was obtained before and after treatment for Fe in L. platensis algae. The lowest value was also obtained before and after treatment in Spirogyra sp. for Hg, and the highest value for Fe.

Table 3. Physio-chemical properties in wastewater by ppm

Physiochemical properties	Winter	Summer
pH	6.24 a	6.62 a
DO	0.59 a	0.51a
BOD5	29.60 b	33.00 a
COD	39.00 b	47.00 a
NO_3	8.90 b	10.20 a
PO4	0.90 b	1.40 a

Table 4. Heavy Metals concentration in Spirogyra sp. (dry weight mg/l) in summer

Heavy Metals	Before treatment	After treatment
Hg	0.001 b	0.005 a
AS	3.100 b	5.450 a
Se	2.900 b	4.940 a
Pb	1.530 b	2.421 a
Zn	29.32 b	51.47 a
Cd	1.4031 b	2.125 a
Ni	19.41 b	44.81 a
Mn	71.59 b	101.50 a
Fe	587.80 b	601.20 a

Gene Expression

Sod gene for Spirugyra sp. and abc transporter gene for L. platensis

The results of the gene expression measurement showed that there are differences between the two algae in this study in their ability to remove heavy metals, as the gene expression of the abc transporter gene in L. platensis showed higher efficiency in removing heavy metals at a significant level of $P \leq 0.05$ compared to the Sod gene in Spirogyra sp., with no significant differences at a significant level of $P \leq 0.05$. As shown in figure 6, 7, 8 and 9.

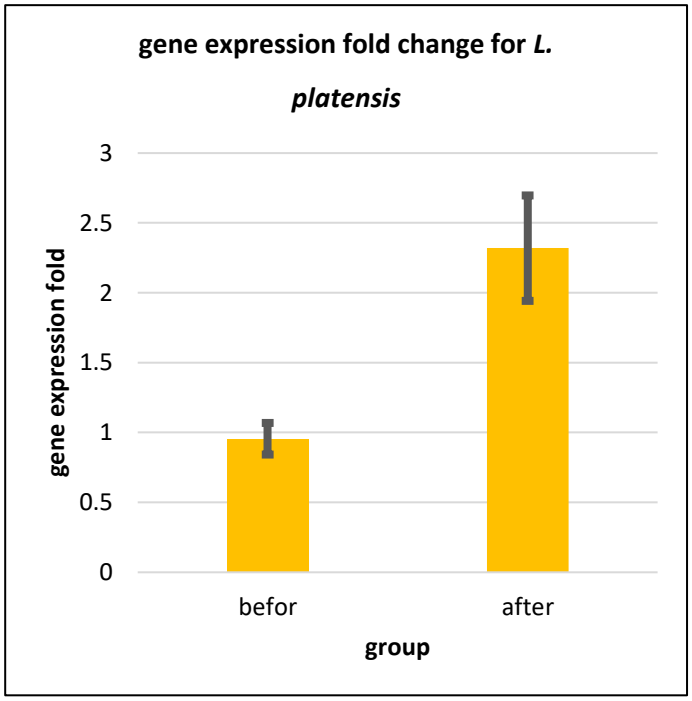

Figure 6. the effect of treatment with heavy metals on gene expression of *abc transporter* gene , represented as fold mean ± STD.

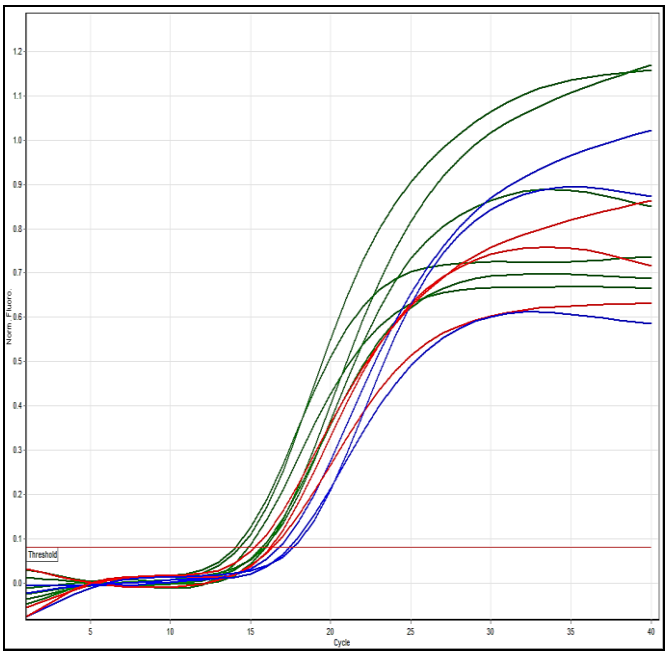

Figure 7. Show amplification curves and estimation of *abc transporter* gene expression by r t-qPCR for two *L. platensis* Groups , red and blue curve in before and after treatment group respectively, green curves for housekeeping gene.

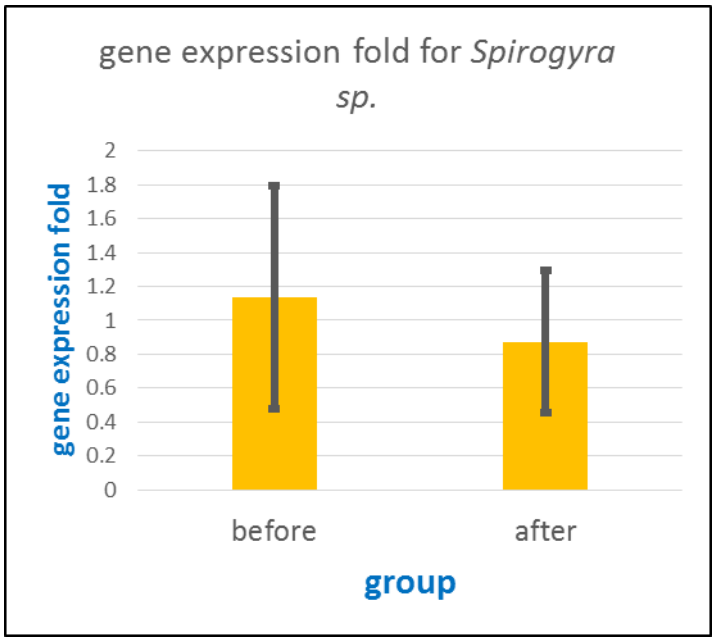

Figure 8. the effect of treatment with heavy metals on gene expression of *sod* gene , represented as fold mean ± STD.

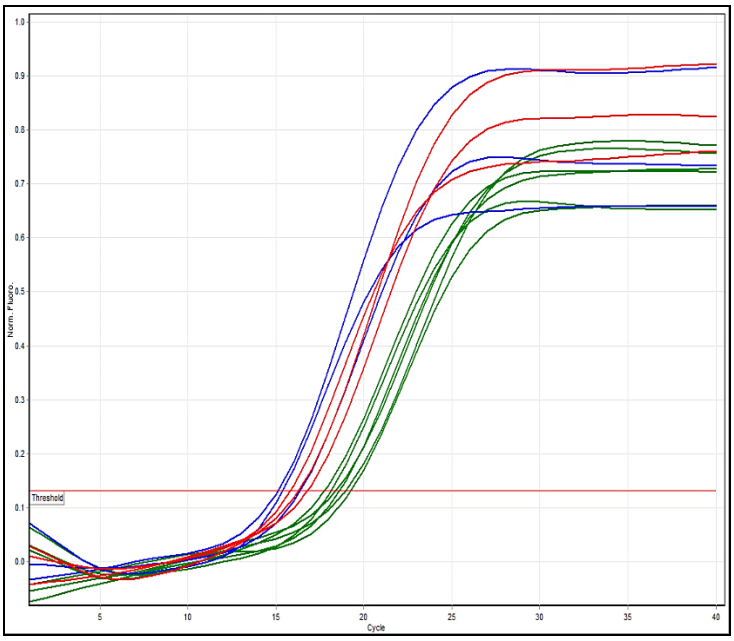

Figure 9. Amplification curves estimation of *Sod* gene expression by rt-qPCR for two *spirogyra sp.* Groups , red and blue curve for *sod* gene in before and after treatment group respectively , green curves for housekeeping gene .

Discussion

Physical and Chemical properties

There is a difference in the values of physical and chemical indicators between the summer and winter seasons for wastewater. The values for all indicators were higher in summer compared to winter, except for dissolved oxygen. For example, the higher dissolved oxygen value in winter is attributed to the lower rate of decomposition of organic matter at low temperatures and the reduced metabolic activity of aquatic organisms, as explained in (Sharma and Sharma, 2019). As for the remaining indicators, these indicators affect microbial activity and the presence of industrial waste in sewage water, with values differing between summer and winter, as this is consistent with (Alsulaili et al., 2020).

Heavy Metals

Tables 2 and 3 show differences in the heavy metal removal efficiency of the two studied algae compared to the before treatment versus after treatment stages, confirmed that algae achieve their heavy metal removal capacity through rapid and efficient absorption of heavy metals at the cell surface via physical absorption (Talib et al., 2022). These ions then slowly migrate into the cell cytoplasm to be utilized as an energy source in the process of chemical absorption, this aligns with the findings of (Chugh et al., 2022 and Behnke and LaRoche, 2020). However, a comparison between the two algae reveals that L. platensis is more efficient at removing heavy

metals than Spirogyra sp. This is attributed to L. platensis possessing a cell wall with diverse functional groups and mechanisms for detoxifying and sequestering metals, thus increasing its absorption capacity and tolerance to the physiological stress of metals on its cells. On the other hand, Spirogyra sp. relies primarily on surface biosorption via its cell wall, which is rich in cellulose, while its internal physiological capacities are limited, thereby reducing its efficiency in removing heavy metals and the sensitivity of its algal cells to high metal concentrations In agreement with (Mehta and Gaur, 2005 and Gupta and Rastogi, 2008).

Gene expression

Figures 6 and 7 illustrate the efficiency of the abc transporter gene in gene expression under exposure to various environmental stressors, particularly heavy metals. This study confirmed the presence of high gene expression (up regulation) of the abc transporter gene, consistent with (Wang et al., 2025) through the secretion of specific substances that enable it to cope with environmental stressors. It also came in agreement with (Hassanien et al., 2025) for the role of the abc transporter gene; the plasma membrane transporter was identified, indicating its important role in the bioremediation of heavy metals from wastewater. Furthermore, in table 8 and 9 the Sod gene expression results showed no response or activity compared to the period before wastewater treatment in this study. This is attributed to the fact that metal toxicity is linked to the production of reactive oxygen species (ROS), which causes imbalances in cellular oxidation-reduction processes. High toxicity causes cell damage because it exceeds the algae's tolerance level, while low or chronic toxicity allows algal cells to accumulate heavy metals, which are then transferred to other organisms within the food chain, this is consistent with (Pinto et al., 2003). Although this the Sod gene showed a response in another study, as indicated by (Kanematsu et al., 2010).

Conclusion

These pollutants impact water quality and change its physical properties. Therefore, these results indicate the requirement of treating wastewater before its discharge into surface waters. This can be realized through the operation of primary treatment stations, which are crucial in treating polluted water, in addition to using the role of various microorganisms and algae in the biological treatment of wastewater before it is released back into the river. Finally, future studies that should be conducted include analyzing the potential of other algal species in the biological treatment of various pollutants. In addition to focusing on molecular studies and gene expression.

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