

The bioremediation of some heavy metals by using of some blue green algae isolated from the Al-Gharraf River in the Al-Shatra City

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ABSTRACT

For this study, two blue-green algae species (*Spirulina platensis* and *Anabaena* sp.) were isolated from the Al-Gharraf River in Al-Shatra city of the Iraq in 2025, and were exposed to two types of heavy metals chromium (Cr) and lead (pb) at several concentrations 1.0, 2.0, 0.25, 0.50, and 0.10 mg/l for two weeks in order to determine their capacity to accumulate. The study results indicated that the algal *Spirulina platensis* collected the highest amount 1.726 µg/gm dry weight for chromium element at the same treatment 2 mg/l whereas the algal *Anabaena* sp collected the highest amount 1.535 µg/gm dry weight for chromium element at the same treatment 2 mg/l. In contrast, the algal *Spirulina platensis* accumulated 0.59 µg/gm of dry *Anabaena* sp weight of the same element at the same treatment 2 mg/l while the algal accumulated more of the element 1.28 µg/gm of dry weight. The statistical analysis showed some significant differences between the two types of algae with respect to concentration of chromium and lead elements for the different treatments. There was also a gradient in the concentrations of the elements added between the treatments; The higher the concentration added the higher was the concentration of the heavy metals on the algal.

Keywords: Al-Gharraf River, blue green algae, bioremediation, heavy metals.

INTRODUCTION

Heavy metals are one of the most harmful pollutants that enter the freshwater for mining and agricultural activities, i.e., via the usage of pesticides and fertilisers, which of these elements, considering that they are traps for various elements (Khan et al., 2018; Ateshan and Misnan, 2025b). Most researchers agree on defining environmental pollution as the unsuitable transformation of our entire or most of our environment as a result of human and natural activities through their direct and indirect effects on energy flow,

radiation levels, physical and chemical composition, and the abundance of living organisms (Dai et al., 2013; Ateshan and Saxena 2015; Jha et al., 2024). Heavy metal pollution is considered one of the most dangerous types of water pollution because it consists of transition elements that can form stable, complex compounds with most organic and inorganic compounds found in the bodies of living organisms. This leads to their accumulation within these living ecosystems. Furthermore, they are characterized by their inability to decompose, their stable nature, and their toxicity (Pagar et al., 2024; Ateshan and Misnan,

2025a; Kazmi et al., 2026). Heavy metals have a direct impact on living organisms, including algae. The concentration of heavy metals in aquatic organisms depends on several factors, some related to the environmental conditions surrounding the organism and others related to the organism's biological characteristics (Anastasiadi and Beemelmans 2023; Ateshan, 2025a; Hanusch et al., 2026). As a result of the increasing discharge of industrial, agricultural, and domestic waste into the aquatic environment, coupled with the inefficiency of traditional methods for detecting the chemical pollutants contained in this waste, researchers have had to use other methods for detection, including bioindicators. Many living organisms, including algae, have been used as bioindicators for this purpose (Martínez-Burgos et al., 2024; Haider, 2025a). Algae are important components of various ecosystems, and their importance lies in being primary producers upon which other living organisms depend for their food. They are the first link in the food chain, and through photosynthesis, they provide the medium in which they grow with large quantities of oxygen, which is essential for the respiration of other organisms (Dyksma and Pester, 2023; Misnan et al., 2026). It has attracted considerable global attention in recent years for the natural products it produces, its ability to treat wastewater and heavy water, as well as its usefulness as an energy product (Al Sailawi et al., 2019; Dhokpande et al., 2024). Its significant role in reducing environmental pollution is due to its suitability for the bioremediation process (Bala et al., 2022; Al-Janabi, 2026). It has the ability to take up many heavy elements present in the aquatic environment and accumulate them within its cells. The accumulated quantities depend on the type of alga, its physiological state, its resistance mechanism, the type and concentration of the element, and the presence or absence of other elements in the growth medium (Azemi et al., 2021; Yang et al., 2026). Given the importance of algae, they can be used in biological treatment, including wastewater treatment, by consuming nutrients such as carbon, nitrogen, and other elements resulting from the decomposition of industrial, agricultural, and domestic waste. Thus, algae can be considered an important biological filter for recycling various types of waste (Jasim et al., 2021; Yana et al., 2026). Therefore, the study aimed to use bioremediation in rehabilitating the ecosystem using the algae *Spirulina*

platensis and *Anabaena* sp. due to their high efficiency in removing heavy metal elements from the aquatic environment.

METHODOLOGY

Study locations

Four sites were selected for the current study on the Al-Gharraf River, a branch of the Tigris River near the city of Al-Shatra. The first site is located northwest of Al-Shatra, at the river's entry into the city in the Al-Gharraf area, 10 km from the sewage treatment plant. The second site is located on the river in the sewage discharge area from the Al-Shatra treatment plant. The third site is the most polluted with organic pollutants, as it receives large quantities of untreated sewage and contains organic waste. It is also located on the river in the sewage discharge area. Finally, the fourth site is located southeast of the city on the Al-Gharraf River, approximately 10 km from the third site.

The collection of algae samples

Algae samples were collected from the study sites on a monthly basis for a full year, from January 2025 to December of the same year. Water samples were brought to the laboratory using clean 500 ml plastic bottles. The samples were fixed using 4% formalin for microscopic examination, while the remaining portion was left unfixed for culture.

Agricultural medium

The modified culture medium (10-Chu) developed by (Touloupakis et al., 2023) was used to cultivate the algae *Spirulina platensis* and *Anabaena* sp. The medium was prepared as a stock solution and then sterilized using an autoclave (type 2, manufactured by Hirayama Manufacturing Corporation, Japan) at a temperature of 121 °C and a pressure of 5.1 psi for 20 minutes.

Isolation and diagnosis of algae

The algal samples were concentrated after collection using a centrifuge at 3000 rpm. The filtrate was discarded, and the sediment was examined using a Novel Xsz107BN light microscope at 40x magnification by preparing microscope

slides. The algae were then cultured on solid culture media using the streaking method with a sterile loop inoculator. For liquid media, the dilution method was used, as described by (Verma et al., 2021) to obtain monoalgal culture isolates. These were then transferred to a prepared algal culture chamber where environmental factors were controlled to achieve optimal growth. These factors included a light intensity of 130–150 $\mu\text{S}/\text{m}^2/\text{s}$ and a light duration of 16 light cycles. At a temperature of 20–25 °C the algae were then identified based on the source (Ambati, 2015).

Algae purification

After obtaining the single algal isolates from the previous section, they were purified of bacteria and fungi using the method described by Dabban et al. 2024 in order to obtain pure axenic culture isolates. Subsequently, the pure algal isolates were cultured using the 10-Chu medium. Each type was transferred from the liquid medium using a sterile pipette and from the solid medium using a sterile inoculant. The isolates were then transferred from both media into several sterile glass flasks. The flask openings were then sealed with sterile cotton, and the flasks were transferred to the algal culture chamber.

Propagation of algal isolates

Dry, clean glass flasks with a capacity of (1000) cm^3 were used. 700 cm^3 of sterile culture medium was added to each flask, and each flask was inoculated with 70 cm^3 of the storage culture to obtain sufficient quantities of algal cultures. Following the method of (Eshtiwi and Ibrahim, 2025) and under the previously mentioned inoculation conditions, sufficient cultures were obtained for subsequent laboratory experiments.

Preparation of heavy metal ion concentrations

Standard solutions of 1000 mg/L of the heavy metal ions chromium (Cr) and lead (Pb) were prepared by dissolving Cr and Pb respectively, in de-ionized distilled water. Concentrations of 1.0, 2.0, 0.25, 0.50, and 0.10 mg/L for each element were prepared. After preparing these concentrations of the heavy metal ions, 250 ml glass flasks were prepared, each containing 150 ml of sterile liquid culture medium. Each flask was inoculated with

15 ml of the pure culture and left to stand for two days to allow the two algal species to acclimatize. The heavy metal concentrations were then added to the cultures and incubated. A shaking incubator was used at a temperature of 2–20 °C with a light-to-dark cycle of 16 hours for two weeks. Afterward, the concentrations of heavy metals in both the algae and their culture medium were determined at the end of the experiment. Millipore filter paper with a pore diameter of 0.45 μm and a known weight was used to separate the algae from the filtrate (culture medium) into clean plastic bottles for measuring the concentrations of heavy metal ions. The concentrations of the remaining heavy metals in the culture medium (filtrate) were then measured using a Phoenix 986 flame atomic absorption spectrophotometer. Specific cathode lamps were used for each element. For measuring the concentrations of heavy metals accumulated in the algae, the filter papers containing the algae were collected after filtration. It was dried using a freeze dryer, and then the leaf was weighed to determine the weight of the dried algae, and the concentrations of heavy metal elements accumulated in the algae were determined according to the method described in (Singh et al., 2016).

Statistical analysis

The results were statistically analyzed using a two-factor, three-replication factorial experiment design. The first factor represented the two algal species, *Spirulina platensis* and *Anabaena* sp. The second factor represented the concentrations at five levels: 1.0, 2.0, 0.25, 0.50, and 0.10 mg/L. The treatments were randomly distributed. The statistical software SPSS software version 23 was used to calculate and record data, and the least significant difference (LSD) test was used to analyze the variance at a probability level of $p > 0.05$.

RESULTS

Table 1 shows the concentrations of chromium accumulated in the alga *Spirulina platensis* and *Anabaena* sp. its culture medium, the concentrations of the element in the algae were (0.061, 0.152, 0.327, 0.816, and 1.726) for the dry weight treatments (g/ μg) and (0.10, 0.25, 0.50, 1.0 and 2.0 mg/L, respectively) the chromium concentrations in the culture medium were (0.033, 0.096, 0.168, 0.181, and 0.272 mg/L respectively for

the same treatments. It was observed that there was a gradual increase in the concentration of chromium in the algae with a steady increase in the concentration of the element in the treatments used. Regarding the concentrations of chromium removed from the algae, the results of the statistical analysis proved the existence of significant differences in concentration at the treatment (2.0 mg/L) compared to the treatments (0.10, 0.25, and 0.50 mg/L). While no significant difference was observed between the two treatments (1.0 and 2.0 mg/L), no such differences were noted with regard to the element concentrations in the culture medium across all treatments used. Table 2 shows the accumulated concentrations of chromium.

In the alga *Anabaena* sp. and its culture medium, the concentrations of the element in the alga were: (1.535, 0.711, 0.302, 0.149, and 0.056 dry malt weight g/μg) (2.0, 1.0, 0.25, 0.50 and 0.10 mg/L) respectively, while the concentrations of the element in the culture medium were (0.440, 0.270, 0.189, 0.095 and 0.025)mg/L) respectively for the treatments mentioned above. It was observed that there was a gradual increase in the chromium concentration in the algae with increasing chromium concentration in the treatments used. Regarding the concentrations of chromium removed from the algae, the results of

the statistical analysis showed significant differences between the concentrations in the 0.2 mg/L treatment and the treatments of 0.10, 0.25 and 0.50 mg/L. While no significant difference was observed between the two treatments (1.0 and 2.0 mg/L), no significant differences were observed with regard to the concentrations of the element in the culture medium with regard to all the treatments used.

Table 3 shows the concentrations of lead accumulated in the alga *Spirulina platensis* and its culture medium, where the concentrations of the element in the alga were (0.05, 0.09, 0.25, 0.50 and 0.59 μg/g) dry weight respectively, while the concentrations of lead in the culture medium were 0.03, 0.10, 0.21, 0.47, and 0.78 mg/L respectively for the aforementioned treatments. It was observed that there was a gradual increase in the concentration of lead in the algae with increasing concentration of the element in the treatments used. Regarding the concentrations of lead removed from the algae, the results of the statistical analysis proved that there were no significant differences between the concentrations and across all treatments used, regarding the concentrations of the element in the culture medium, significant differences were observed between the concentrations in the two treatments (0.10 and 2.0 mg/L),

Table 1. Chromium element concentrations μg/g dry weight (in *Spirulina platensis* alga and its culture medium)

Chromium content (mg/L)	Concentration of the element in the moss μg/g dry weight	Concentration of the element in the culture medium mg/L
0.10	0.061 ^a	0.033 ^a
0.25	0.125 ^b	0.096 ^a
0.50	0.327 ^b	0.168 ^a
1.00	0.816 ^{ab}	0.181 ^a
2.00	1.726 ^a	0.272 ^a
Average	0.616	0.150

Note: L.S.D for algae ($p < 0.05$) = 0.971, L.S.D for the growing medium ($p < 0.05$) = 0.353. Numbers that have the same letters in the alphabet have no significant differences at the probability level ($p < 0.05$).

Table 2. Chromium concentrations (μg/g dry weight) in *Anabaena* sp. algae and its culture medium

Lead content (mg/L)	Concentration of the element in the moss μg/g dry weight/g dry weight	Concentration of the element in the culture medium mg/L
0.10	0.05 ^a	0.03 ^b
0.25	0.09 ^a	0.10 ^b
0.50	0.25 ^a	0.21 ^{ab}
1.00	0.50 ^a	0.47 ^{ab}
2.00	0.59 ^a	0.78 ^a
Average	0.296	0.318

Note: For the medium (L.S.D.) ($p < 0.05$) = 0.471, for the algae (L.S.D.) ($p < 0.05$) = 0.912.

Table 3. Concentrations of lead element (micrograms/g dry weight) in *Spirulina platensis* algae and its culture medium

Chromium content (mg/L)	Concentration of the element in the moss µg/g dry weight	Concentration of the element in the culture medium mg/L
0.10	0.056 ^b	0.025 ^a
0.25	0.149 ^b	0.095 ^a
0.50	0.302 ^b	0.189 ^a
1.00	0.711 ^{ab}	0.270 ^a
2.00	1.535 ^a	0.440 ^a
Average	0.550	0.203

Note: For the medium (L.S.D.) ($p < 0.05$) = 0.561, for the algae (L.S.D.) ($p < 0.05$) = 0.576.

Table 4. Concentrations of lead element (micrograms/g and dry weight) in *Anabaena* sp. algae and its culture medium

Lead content (mg/L)	Concentration of the element in the moss µg/g dry weight/g dry weight	Concentration of the element in the culture medium mg/L
0.10	0.03 ^a	0.06 ^a
0.25	0.06 ^a	0.16 ^a
0.50	0.17 ^a	0.29 ^a
1.00	0.35 ^{ab}	0.58 ^a
2.00	1.08 ^a	0.91 ^a
Average	0.338	0.40

Note: For the medium (L.S.D.) ($p < 0.05$) = 0.690, for the algae (L.S.D.) ($p < 0.05$) = 0.77.

while no such differences were observed in the treatments (1.0, 0.50, 0.25, and 0.10 mg/L).

Table 4 shows the concentrations of lead accumulated in the alga *Anabaena* sp. and its culture medium. The concentrations of the element in the algae were 0.04, 0.11, 0.31, 0.56, and 1.28 µg/g dry weight for the treatments: 2.0, 1.0, 0.50, 0.25, and 0.10 mg/L. The lead concentration in the culture medium was consistently (0.05, 0.09, 0.14, 0.36 and 0.59 mg/L) for the aforementioned treatments. A gradual increase in lead concentration in the algae was observed with increasing lead concentration in each treatment. Regarding the lead concentrations removed from the algae, statistical analysis revealed significant differences between the concentrations in the 2.0 mg/L treatment and those in the 0.10, 0.25 and 0.50 mg/L treatments. No such differences were observed in the 1.0 and 2.0 mg/L treatments. As for the lead concentrations in the culture medium, no significant differences were found in any of the treatments.

DISCUSSION

These metals, namely lead (Pb) and chromium (Cr), were chosen for the current study because

chromium is a micronutrient involved in cell metabolism but can become toxic when added in high concentrations to the culture medium, it shuts down the Krebs cycle and cellular respiration (Ali et al., 2023; Patra et al., 2024), lead, however, is a non-essential element for the body of a living organism and is considered toxic to all living systems. It interferes with the metabolic processes of chromium and lead, which are essential elements in the body of a living organism (Koty and Iskra, 2025; Habib et al., 2026). Blue-green algae are among the organisms most frequently exposed to heavy metal pollution due to the discharge of industrial, domestic, and agricultural waste into waterways. The growth of these organisms in polluted environments demonstrates their remarkable ability to tolerate high concentrations of toxic elements (Ateshan and Talib, 2025; Das et al., 2025).

The study results showed the ability of the algae *Spirulina platensis* and *Anabaena* sp. to accumulate elements in their bodies at higher concentrations than those found in the culture medium (Kaamouch et al., 2022; Diaconu et al., 2023). It has become increasingly apparent that phytoplankton, especially cultivated green algae, have a high capacity for accumulating elements in their bodies (Ateshan, 2025b; Detoni et al., 2026).

Blue-green algae are the most sensitive to heavy metals, followed by green algae and then diatoms (Rathore et al., 2026). Regarding the element chromium, the study results showed that the two algae have a high capacity to accumulate chromium within their bodies; however, each algae differs in its ability to concentrate the element from the other (Ghaed et al., 2025). Reed and Gadd, 2024 stated that algae vary in their ability to accumulate heavy metals depending on the type and concentration of the element they are exposed to. Some algae exhibit a high affinity for absorbing a particular element ion, while others do not (Haider, 2025b; Xi et al., 2026).

However, in general, there was an increase in the concentration of the element within each alga, corresponding to a decrease in the concentrations in the culture medium, the study results showed that the alga *Spirulina platensis* has a high capacity for accumulating chromium, attributed to its large surface area resulting from its filamentous structure and numerous branches (Al Sailawi et al., 2020; Singh and Rao, 2025). Or perhaps it is due to the fact that the difference in the degree of algal response to the effect of heavy metals depends on the amount of positive ions entering the cell through the cell membranes (Ateshan et al., 2020; Jomova et al., 2025). Or it may be due to the selective mechanism of its absorption of various heavy elements present in the aquatic environment, as well as to the element in *Anabaena* sp. algae, in comparison with the physicochemical properties (Ahammed et al. 2023). The results are consistent with those of several researchers, including Ammar et al. 2022 on the algae *Rivularia* sp* and *Spirulina platensis* (Sanju and Thakur, 2025) on the algae *Lyngbya tylosii* and *Oscillatoria angustissima*. Murali et al. 2014 on the alga *Platnsis* sp. As for the element lead, the results also showed a variation in the effect of the element on the two algae. The effect of heavy metal ions varies from one type to another depending on the composition of the cell wall and the sites of positive and negative ion exchange in the cell wall and cell membrane (Patil et al., 2025). It was also observed that the algae *Anabaena* sp. was more effective at concentrating lead than the other alga. This may be attributed to its content of polyphosphate body polysaccharides, which play an important role in detoxifying elements, in addition to storing phosphorus (Liu et al., 2023), or perhaps it is due to increased biomass tolerance, an increased surface area ratio,

and a longer incubation period (Shalaby et al., 2023). This may be due to genetic differences between the two algae. These findings are consistent with those of several researchers, including Kozlova et al., 2017 on the algae *Scenedesmus quadricauda* and Patil et al., 2025 on the algae *Ulot cylindricum*, and Soeprbowati and Hariyati (2014) on the algae *Spirulina platensis*.

CONCLUSIONS

The study results showed the ability of the algae *Spirulina platensis* and *Anabaena* sp. to accumulate elements in their bodies at higher concentrations than those found in the culture medium. Blue-green algae are the most sensitive to heavy metals, followed by green algae and then diatoms, regarding the element chromium, the study results showed that the two algae have a high capacity to accumulate chromium within their bodies; however, each alga differs in its ability to concentrate the element from the other. The concentration of the element within each alga, corresponding to a decrease in the concentrations in the culture medium, the study results showed that the alga *Spirulina platensis* has a high capacity for accumulating chromium, attributed to its large surface area resulting from its filamentous structure and numerous branches, Based on the results of the statistical analysis, there were a few significant variations in the concentrations of chromium and lead elements on the two species of algae between the treatments used. The concentration of the heavy metals was gradually increasing on the algal with the increase of the concentration on the treatment.

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