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*Original Research Article*

# Evaluation of Heavy Metal Contamination in *Chlorella vulgaris* in the Southern City of Iraq Using Scanning Electron Microscopy and the Risk Assessment

Abdul Wahab R. Ayal*, Suaad H. Ali, Shrrog H. Hlail

*Department of Biology, College of Education for Pure Science, University of Thi-Qar, Thi-Qar, 64001, Iraq.*

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ABSTRACT

Heavy metals pose a significant threat to aquatic environments due to their widespread industrial use and untreated disposal into water. Studies have demonstrated that algae can effectively absorb and accumulate heavy metals from polluted areas. The present study investigated the ability of the green alga, *Chlorella vulgaris* to accumulate some heavy metals. *Chlorella vulgaris* was isolated and identified from water samples collected from the surface water of the Euphrates River. The alga was treated with different concentrations (10, 15, 20, and, 25 mg/L) of heavy metals (copper, lead, cadmium, and cobalt) for 14 days. Scanning electron microscopy (SEM) was used to examine the surface morphology of algae. The results showed that *C. vulgaris* can accumulate heavy metals. Cobalt was the highest accumulated metal followed by low accumulated metals of copper, lead, and cadmium. Heavy metal concentrations of cobalt, copper, lead, and cadmium were accumulated in *C. vulgaris* with concentrations of 0.57, 0.12, 0.05, and 0.02% dry weight, respectively. Cadmium was the most toxic metal to cell shape and contents, followed by cobalt. However, copper and lead showed no effect. The findings of the study suggest that *C. vulgaris* can be used in bioremediation to remove heavy metal contamination from water.

***Keywords*:** Bioaccumulation, Heavy metals, Scanning electron microscopy, *Chlorella vulgaris*

## Introduction

Heavy metals are among the most dangerous pollutants in the aquatic environment, because of their widespread industrial use, and their disposal into the water without any treatment. Therefore, the phytochemical analysis of macroalgae revealed the presence of secondary metabolites, such as alkaloids, flavonoids, tannins, saponins, steroids, triterpenoids, and phenolics.[1] They cause dangerous water pollution, with elements, such as cadmium, copper, lead, and cobalt. These elements are among the most common pollutants in the aquatic environment. Their harmful effect is due to their direct impact on the growth and presence of aquatic organisms, when they accumulate within the tissues of aquatic organisms. They directly affect the overall vital activities carried out by living organisms.[2]

Some types of algae can resist different concentrations of heavy metals. Many researchers report that heavy metal contamination of the aquatic environment is an indicator of pollution, based on its short life cycle.[3] The physical and chemical factors of the environment are a direct factor in the sites where algae live. [4] Algae mechanisms enabled them to withstand high concentrations of heavy elements, including the process of bioaccumulation. This phenomenon in algae has been investigated by many researchers because of its important applications in phytoaccumulation.[5] These organisms can be invested and used to extract heavy elements from soil and water. Numerous studies have shown that algae can absorb and accumulate heavy metals from polluted areas.

*Corresponding author. E mail: abdalwahabayal.bio@utq.edu.iq
Tel: +9647808455927

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On the other hand, the genetic factor of algal plays an important role in tolerating toxic concentrations of some elements, such as lead, cadmium, copper, and cobalt.[6]

Therefore, this study was conducted to evaluate heavy metal contamination in the green alga, *Chlorella vulgaris* using scanning electron microscopy and its risk assessment.

## Materials and Methods

*Source of chemicals*

$Na_2NO_3$, $K_2HPO_4$, $MgSO_4.7H_2O$, $CaCl_2.2H_2O$, $FeCl_3.6H_2O$, $Na_2SiO_3.9H_2O$, $Na_2.EDTA$, $NaHCO_3$, $MnCl_2.4H_2O$, $(NH4)_6Mo_7O_{24}.4H_2O$, $ZnSO_4.7H_2O$, $CuSO_4.5H_2O$, $CoCl_2.6H_2O$, $H_3BO_3$ were obtained from Merk (Darmstadt, Germany).

*Sample collection*

Samples were collected from the surface water of the Euphrates River (*Al-sharif Regin which is located northwest of the city of Nasiriya and its coordinates* $N=31^0 3' 36.46''$ *and* $E=46^0 7' 42.45''$, *respectively*) at a depth of 30 cm from different areas of the river in the city of Nassiriyah, Thi-Qar Governorate using plastic bottles of 500 mL and were brought to the laboratory for analysis. The period of sample collection in this study was from March to June 2023. The Chu-10 culture medium was used to prepare algal isolates (Table 1). The culture medium was prepared in the form of standard stock solutions and kept at refrigerator temperature. The culture medium was prepared using a specific amount of distilled water spawn within a pH between 7.4-7.0 for the purpose of growing algae. Then, drops of sodium hydroxide solution were added at a concentration of 0.2 N. Then, the culture media were sterilized using an autoclave (HV-110 Hirayama, Japan) at a temperature of $121^0C$ and a pressure of 1.5 pounds/ Hang for 20 minutes.[7]

*Isolation and identification of alga*

Water samples were collected from different areas of the river to investigate the types of algae, which were placed in tightly closed plastic bottles containing a sterile Chu-10 culture medium. They were

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then brought to the laboratory to isolate the algal species. Two methods of dilution were used to obtain a single culture. [8] The study adopted the following sources to identify the isolated algae.[7] Purification of unialgal cultures was carried out as described. [9]

*Preparation of concentrations of heavy metals*

Heavy metals were prepared in different concentrations (10, 15, 20, and 25 mg/L) of cadmium, cobalt, copper and lead. Then, different concentrations of the elements were added to the algae on the first day of the experiment, across a period of two weeks, as previously described.[10]

*Scanning electron microscopy*

The surface morphology of algae was investigated by using scanning electron microscopy (SEM) (Zeiss SUPRA 55-VP, Germany), according to the method previously described.[11]

**Table 1:** Components of the Chu-10 culture medium

| Chemical compound | Concentration (g/L) | Chemical compound | Concentration (g/L) |
|---|---|---|---|
| $Na_2NO_3$ | 53.3 | $MnCl_2.4H_2O$ | 0.045 |
| $K_2HPO_4$ | 10 | $(NH4)_6Mo_7O_{24}.4H_2O$ | 0.007 |
| $MgSO_4.7H_2O$ | 25 | $ZnSO_4.7H_2O$ | 0.056 |
| $CaCl_2.2H_2O$ | 40 | $CuSO_4.5H_2O$ | 0.02 |
| $FeCl_3.6H_2O$ | 1.46 | $CoCl_2.6H_2O$ | 0.01 |
| $Na_2SiO_3.9H_2O$ | 6.2 | $H_3BO_3$ | 0.72 |
| $Na_2$.EDTA | 31.8 | pH | 7.3 |
| $NaHCO_3$ | 25 | | |

## Results and Discussion

As observed in (Figure 1), algal dells are spherical or semi-spherical. They are green in color, with a diameter ranging between 2-10 micrometers. They lack flagella, and contained chloroplasts. The results of the scanning electron microscopy of the control sample showed that the algae are spherical. The cell wall is clear, and the plasma membrane and the nuclear region occupying almost the center of the cell can be distinguished(Figure 2). As for the absorption spectrum, it is clear that algae contain elements of carbon, oxygen, nitrogen, silicon, sodium, chlorine, calcium, magnesium, silver, fluorine and *aluminum*, with different absorption peaks (Figure 3). Comparing this result with the treatment (20 mg/L of copper), it was found that the cell retained its spherical shape, and the wall surrounding it could be seen. The nuclear region appeared in the form of a black spot in the center of the cell, and the distribution of copper ions appeared in the form of bright points of low density and concentration. That is 0.12% of the dry weigh as displayed in (Figure 4). The results (Figure 5) of the absorption spectrum showed that the copper ions were at a voltage of 0.9 KV. Also, it was observed that the algae contain calcium, oxygen, magnesium and silicon ions with different absorption values.

The effect of treating the moss with the same concentration of lead indicated that the moss still retained its spherical shape. The cell wall was also visible with its sites showing contact between each other. The cytoplasm was observed, as well. It was found that the highest accumulation of lead ion was around the outer cell wall, when compared to its concentration inside the cell. The amount equaled 0.05% of the dry weight (Figure 6). The absorbance results showed that the lead ion was found at a voltage of 2.3 KV. It was also noted that algae contained carbon, oxygen, fluorine, sodium, magnesium, and silicon ions with different absorbance values as presented in (Figure 7). The results of cadmium examination revealed that it has a definite effect, as it was observed that the cell lost its prominent spherical appearance, as well as difficulty in defining the boundaries of the cell wall. The internal structures could not be distinguished from nuclear material or cytoplasm. It was found that the cadmium ion accumulated in all parts of the cell inside and outside, with a clear density and concentration, which accounted for 0.02% of the dry weight (Figure 8). The absorption spectrum revealed that the element cadmium had its highest concentration at a voltage of 3.1 KV, and that ions of calcium, oxygen, fluorine, sodium, and magnesium had varying absorptive values ranging from 3.4-0.1 KV to another peak at a voltage of 0.1 KV, as observed in algae (Figure 9). The results (Figure 10) of the cobalt ion demonstrated that the cell wall was the site of its first observed effect. Although the cell borders could be distinguished, there were indentations and ripples in the cell wall, as well as the nuclear region and the cytoplasm. The highest accumulation of the cobalt ion was in the intracellular structures at a concentration of 0.57% by dry weight, compared to its extracellular accumulation. It was noted from the results of the absorption spectrum that the cobalt ion recorded the highest concentration at a voltage of 0.7 KV while different absorption peaks appeared for each of calcium, oxygen, iron, sodium, aluminum, sulfur, rubidium, magnesium and silicon (Figure. 11).

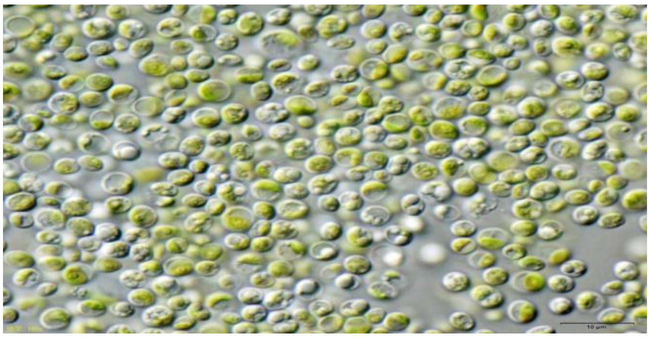

**Figure 1:** The green alga, *Chlorella vulgaris*.
(40x magnification)

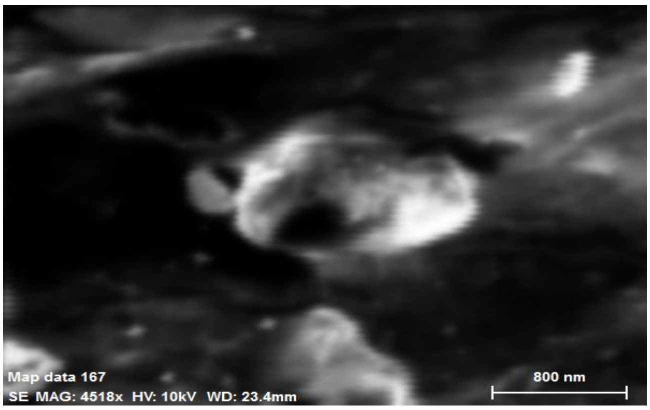


**Figure 2:** Control sample of *Chlorella vulgaris*.

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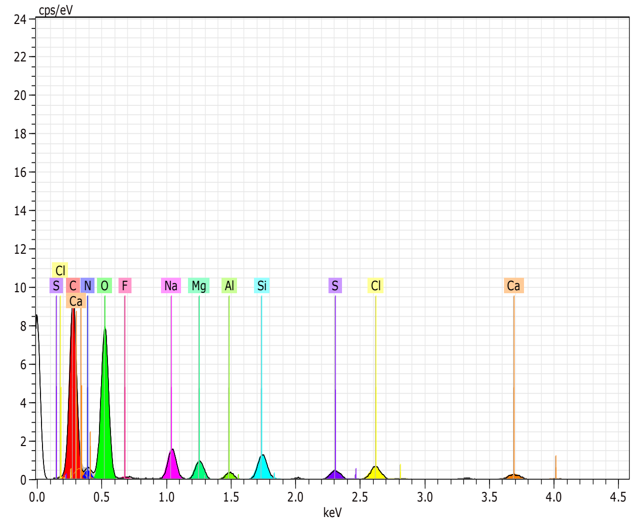


**Figure 3:** Absorption spectrum of a control sample of *Chlorella vulgaris.*

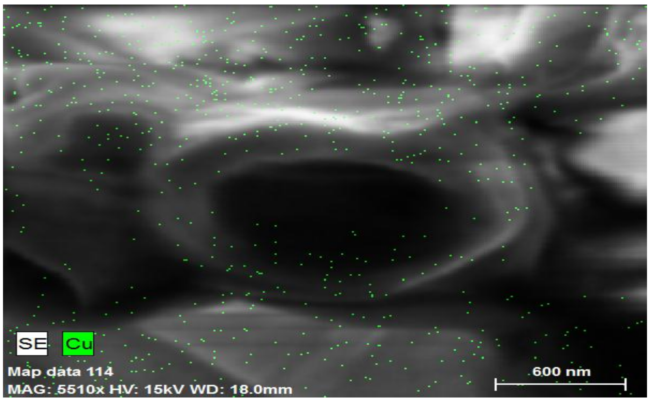


**Figure 4:** Effect of copper at a concentration of 20 mg/L on *Chlorella vulgaris.*

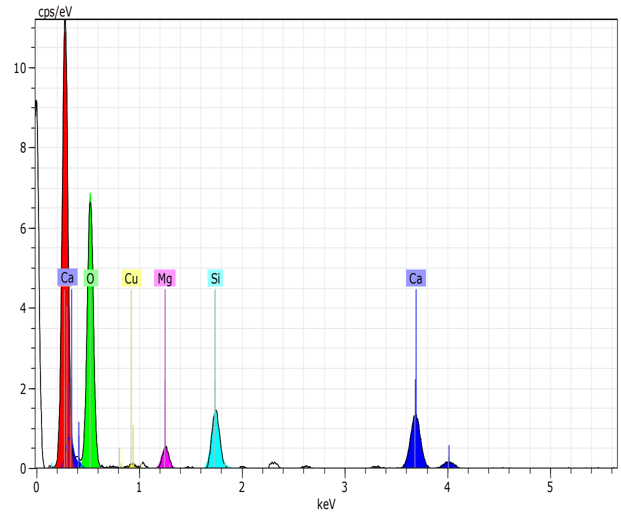


**Figure 5:** Absorption spectrum of copper at a concentration of 20 mg/L of *Chlorella vulgaris.*

The results of the study showed that the algae did not suffer from any obvious changes in its external shape after being treated with copper at a concentration of 20 mg/L. This indicates that the algae resisted the toxicity of the element resulting from the increase in its concentration. The appearance of the cell wall and the internal structures of nuclear material and cytoplasm were clear without showing signs of damage, in addition to its ability to accumulate this element inside and outside the cell. This was done on purpose to take advantage of the high heavy element concentrations to the permissible limit without going above the toxic concentration. Alternatively, reduction of copper can be achieved by increasing the production of enzymes, including the copper element that enters its composition, or through algae that regulate the absorption of copper by increasing the number of transporters that accumulate inside the algae's body. These results are consistent with those previously reported.[12] However, in regards to the effect of lead, it was less on algae, as evidenced by the clear cell shape and internal structures of the algae as well as their cell wall. The accumulation of the lead element outside the algal cell was more than that inside it. The reason for this observation may be due to the mechanism used in the absorption, which is the restriction of the element with effective aggregates present on its outer wall, thus reducing the toxic effect.[13] As for the cadmium element, it was the least element accumulated by the alga when observing the results. This showed that the highest accumulation of the individual elements was for the cobalt element, followed by the copper element, then the lead element, and finally the cadmium element, due to its severe toxicity that caused damage to the cell structure.[14] A change in the shape of the algal cells was observed, due to the age of cadmium. This led to damage to the cell wall and internal structures, thus affecting the external shape as a result of the high toxicity of cadmium compared to copper, lead and cobalt. These results are consistent with the findings of the researchers.[15] The reason, perhaps is due to the effect of cadmium on the respiratory organs of algae through its effect on phospholipids present in the composition of plastids and mitochondrial membranes.[16-17] As for the cobalt element. the algae cells showed resistance to it despite the occurrence of dents in the cell wall, which affected the external appearance of the cell. It appeared that the cell accumulated this element inside more than outside. It was found that the effect of heavy metal ions differs from one type to another depending on the different composition of the cell wall and the exchange sites of positive and negative ions in the wall and membranes.[18] Another reason for this difference could be due to the ability of cobalt to affect the formation of proteins inside and outside the cell.[19-20]

## Conclusion

The findings of the present study reveal that the levels of heavy metals in the algae examined in three sites of the Euphrates River in Nassiriyah, Thi Qar Governorate were higher than acceptable limits. The accumulations of heavy metals were very high, indicating that the pollution of those sites was due to the increased discharge of waste materials and pollutants. The use of modern technologies to identify the genes responsible for the bioaccumulation of heavy metals in the algal cell is therefore recommended.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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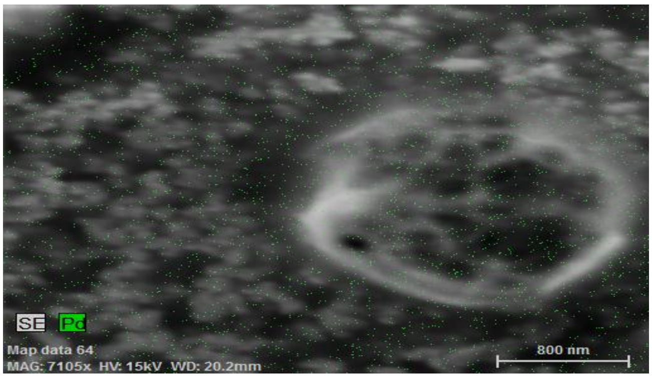


**Figure 6:** Effect of lead at a concentration of 20 mg/L on *Chlorella vulgaris.*

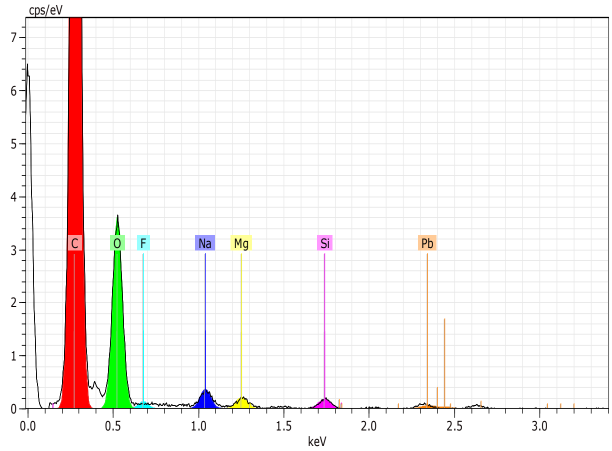


**Figure 7:** Absorption spectrum of lead at a concentration of 20 mg/L of *Chlorella vulgaris.*

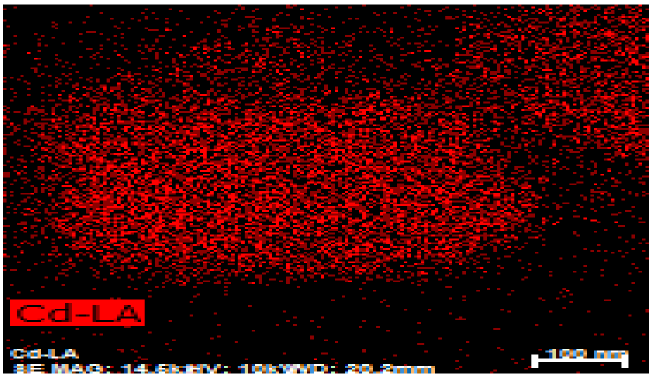


**Figure 8:** Effect of cadmium at a concentration of 20 mg/L on *Chlorella vulgaris.*

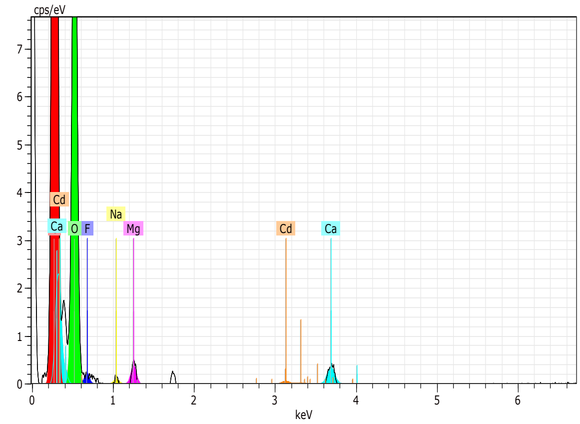


**Figure 9:** Absorption spectrum of cadmium at a concentration of 20 mg/L of *Chlorella vulgaris*.

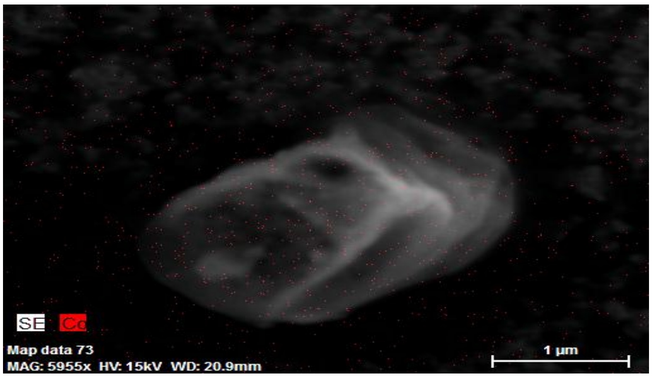


**Figure 10:** Effect of cobalt at a concentration of 20 mg/L on *Chlorella vulgaris*.

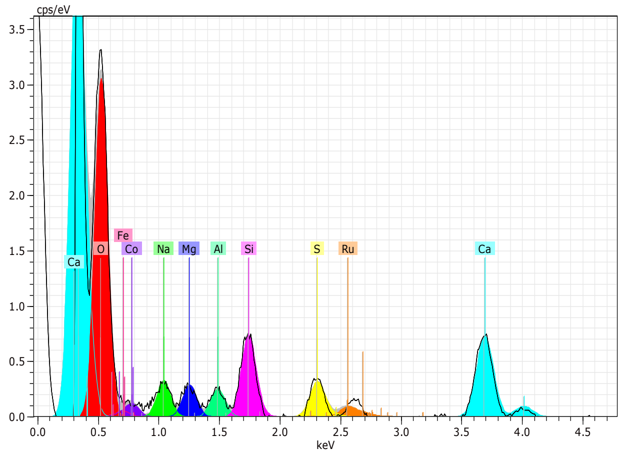


**Figure 11:** Absorption spectrum of cobalt at a concentration of 20 mg/L of *Chlorella vulgaris*.

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