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Influence of Zinc, Aluminum and Chromium on Morphology and Cell Contents of Alga *Chlamydomonas* sp.

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ABSTRACT

The current study was conducted to determine the ability of the green alga *Chlamydonas* to accumulate some heavy elements, namely zinc, aluminum and chromium, in its cells after exposing it to these elements for a period of 14 days. Moreover, to observe the changes that appear on the alga as a result of its growth in media containing different concentrations of these heavy elements, which are 10, 15, 20, 25mg.L^{-1} individually, the scanning electron microscope technology was used. The results showed that the algae has the ability to accumulate these elements, and the chromium element was the most accumulated, followed by the zinc element and finally the aluminum element, as the amount of concentrations reached 0.71, 0.15, and 0.30% of the dry weight for the three elements, respectively. In terms of effect, the chromium element was the most harmful to the shape of the cell and its contents, followed by the aluminum element, while the effect of the zinc element was less severe when added individually.

INTRODUCTION

Algae play an important role in reducing environmental pollution as a result of the process of biological accumulation and removal, as most algae that live in water containing heavy elements tend to have a concentration of these elements higher than what is found in the environment in which they are located **(Murugesan *et al.*, 2008)**. Microalgae are known for their ability to accumulate heavy elements, such as copper, lead, and zinc. These algae have developed natural ways to respond to elements such as copper, lead, and cadmium through passive accumulation inside their cells and through the outer surface of the cells, linked to different functional aggregates **(David *et al.*, 2006)**. The accumulated amounts of heavy elements depend on the type of algae, its physiological condition, the mechanism of its resistance to the toxicity of those elements, the type and concentrations of the elements, as well as the presence or absence of other elements in the growth medium **(Becker, 1983)**. Most aquatic organisms have the ability to accumulate heavy elements within their bodies to concentrations several times higher than what is found in the environment in which they live **(Abaychi & Douabul, 1985)**. There are many factors that affect the treatment and bioaccumulation of heavy elements,

such as salinity, pH, temperature, nutrients, and oxygen concentration, which are influential in the process of bioaccumulation of elements through their effect on the interaction between the elements and cell walls and on the metabolic rates of the alga **(Favero *et al.*, 1996)**. Heavy elements enter the algae's body in three ways: phagocytosis, absorption through cell membranes exposed to the surrounding water, and adsorption, as the elements accumulate on the surfaces of areas exposed to them **(Cobbett & Goldsbrough, 2002)**. There are several mechanisms followed by algae to get rid of the toxicity of these elements, including formation of plant chelating compounds, which are peptide or protein compounds rich in the amino acid cysteine. These compounds are able to bind element ions to their structure, producing complexes of elements, and these compounds are built by the enzyme Phytochellating Synthetase **(Philips, 2006)**. Moreover, some algae have the ability to form organic compounds inside cells such as carbohydrates, which in turn tend to bind to elements and then reserve and bind them **(Macfie & Welbourn, 2000)**, and other resistance mechanisms in algae. It is the formation of polyphosphate bodies inside cells, through which elements are removed from active sites in the cells **(Jensen & Corre, 1993)**. Algae follow these mechanisms to resist the toxicity of heavy elements by restricting the elements within polyphosphate bodies **(Jensen, 1994)**. There are also many studies that indicated that the carboxyl group present in algae cells is responsible for the binding of different ions **(Gardea *et al.*, 2014)**. Algae are characterized by their ability to withdraw many trace elements and to accumulate them within its body, either because of their abundance in the environment or because of their need for them in biological processes **(Becker, 1983)**. The aim of this study was to evaluate the ability of the green alga *Chlamydonas* to accumulate some trace elements.

MATERIALS AND METHODS

1-Source of chemicals

The sources of chemicals included: Na2NO3, K2HPO4, MgSO4.7H2O, CaCl2.2H2O, FeCl3.6H2O, Na2SiO3.9H2O, Na2.EDTA, NaHCO3, MnCl2.4H2O, (NH4)6Mo7O24.4H2O, ZnSO4.7H2O, CuSO4.5H2O, CoCl2.6H2O, H3BO3.

2-Alga collection

The alga was obtained from the surface layer of Euphrates River water (the filming area is located north of the city of Nassiriyah in southern Iraq) and at a depth of 25cm in different locations of the river. This method requires taking tests and returning them to the laboratory only for the study, which is valid up until Aigust 2024, provided that the study is taken from a reputable city. There are several media used to grow algae, including BG11 and Ch12, but in the current study, Ch12 was used (Table 1). This medium was prepared in the form of a standard solution, and then stored at a temperature of 4°C in the refrigerator without sterilization for the time of use. 1ml of each of them

was mixed during preparation, and then it was supplemented with distilled water according to the required volume. The pH was raised to 7.4 during moss cultivation by adding a few drops of 0.2 M sodium hydroxide solution. The culture medium was then sterilized using a HANNA autoclave under conditions of temperature (121^0C) while the pressure was 1.5 pounds/ang for a period of 20 minutes. In the final stage, phosphorus was added after sterilization for the purpose of preventing its precipitation during the sterilization process **(Stein, 1973)**.

3- Alga isolation and identification

For the purpose of obtaining unialgal cultures, the methods of dilution and streaking were used **(Stein, 1973)**.

3-1 Dilution method

Ten test tubes were prepared, each containing a volume of 9ml of the liquid culture medium. One ml of the aqueous sample was taken and transferred to tube No. 1, then a volume of 1ml was taken from it and transferred to tube No. 2, and so on to tube No. 10. Each time the test was done, it was then incubated. Test tubes were placed in the growth chamber at a temperature of 2±25°C, with lighting ranging between 130-150 microns/m^2/s, for a period of 8:16 hours light:dark until unialgal cultures isolate was obtained.

3-2 Streaking method

One or two drops of the filtrate of the sample, from which the isolate and purified algae were obtained, were placed on the surface of a Petri dish containing the solid culture medium. The dishes were spread and incubated for a period ranging between 4-8 days in the growth cabin and under the growth conditions mentioned above. After the algae grew on the solid medium, they were isolated using a sterile loop, transferred to another solid medium, and this process was repeated until the unialgal culture was obtained **(Rescott, 1984)**. After that, the unialgal culture was purified according to **Weidman *et al*., (1984)**.

4-Preparing concentrations of trace element ions

Concentrations were prepared (10, 15, 20 and 25mg.L^{-1}) and were then added to the algae farm on the first day of the study and were announced for 14 days according to what was outlined by **Tomaselli *et al*. (1981)**.

5- Microscopic study

The general external appearance of the vegetative form of the alga was conducted using a scanning electron microscope produced by the company Zeiss SUPRA 55-VP, Germany.

12 culture medium **Table 1.** Chemical composition of the CH

Macronutrients ml.L^{-1}	**Concentration gm.L^{-1}**	**Macronutrients ml.L^{-1}**	**Concentration gm.L^{-1}**
$MnCl_2.4H_2O$	0.045	$NaNO_3$	53.3
$(NH4)_6Mo_7O_{24}.4H_2O$	0.007	K_2HPO_4	10
$ZnSO_4.7H_2O$	0.056	$MgSO_4.7H_2O$	25
$CuSO_4.5H_2O$	0.02	$CaCl_2.2H_2O$	40
$CoCl_2.6H_2O$	0.01	$FeCl_3.6H_2O$	1.46
H_3BO_3	0.72	$Na_2SiO_3.9H_2O$	6.2
pH	7.3	Na_2.EDTA	31.8

RESULTS AND DISCUSSION

The electron microscope's results for the control sample demonstrated that the alga's shape is spherical and the cell wall is transparent. The plasma membrane is easily distinguishable from the nuclear region, which is approximately in the middle of the cell (Fig. 1). The absorption spectrum demonstrated that the algal is primarily composed of ions of carbon and oxygen (Table 2). When contrasted with the treatment (25mg.L^{-1}) of zinc, the cell's spherical shape was maintained, the cell's wall around it was observed in greater detail, and the nuclear region appeared as a dark spot in the middle of the cell. It turns out that the distribution of zinc looks like brilliant spots with low concentration and density. It is 0.30% of dry weight (Fig. 2). Regarding the absorption spectrum's results, copper had a voltage of 0.9 kilovolts. The results also showed that it contains elements, oxygen, magnesium, silicon and calcium elements with different sensitivities (Table 3). As for treating the alga with the same concentration of aluminum, it was found that it maintained its spherical shape, while the cell wall was visible and the locations of the cells communicating with each other became clear, as well as the cytoplasm. It was discovered that the greatest concentration of aluminum ions was located around the outer cell wall instead of inside the cell; this reached a maximum of 0.15% of the dry weight (Fig. 3), and when scrutinizing the absorption results, lead element was discovered which has a voltage of 2.3 kilovolts. Additionally, ions of carbon, oxygen, fluorine, sodium, silicon and magnesium voltage levels, were observed. The effect of the chromium ion was evident and perceptible (Fig. 4). The results also showed that the algal cell had suffered a change in its spherical shape, in addition to the inability to distinguish its wall, while the internal structures could not be differentiated from the nuclear material and cytoplasm. It turned out that chromium accumulated in all parts of the cell in a dense form (0.71%) of the dry weight of the moss. The absorption spectrum had the highest value at 3.1 kilovolts, while the elements oxygen, calcium, fluorine, magnesium, and sodium showed voltage values that ranged between 0.1 - 3.4 kilovolts (Table 5).

The results showed that the algal cell appeared to have no obvious changes in its external appearance following the treatment of 25mg.L^{-1} of zinc. This indicates that the algae is resistant to the toxic effects of zinc as demonstrated by the results that the algae maintained its spherical shape. The results showed that the cell wall and internal structures of the nucleus and cytoplasm did not undergo any change, in addition to the capacity to store this component both inside and outside of the cell. The cause of this may be attributed to zinc being one of the most important components that algae need, as a result, the algae can take advantage of the high concentrations of this element while avoiding its toxicity; the first way is by increasing the activity of the enzyme that reduces zinc to a state that is less toxic; the second is by increasing the number of transporters that are associated with the algae's body in order to increase the amount of zinc that is accumulated within the algae's body **(Ayal *et al*., 2023)**. The influence of aluminum on the algae was minimal, as algae appeared to be in good health and have a clear internal structure, besides their cell wall. It has been demonstrated that the amount of aluminum outside the algal's cell is greater than the amount inside it. The cause of this is likely to the mechanism used to absorb the element, which confined the element to the active groups on its external surface. This reduced the toxic effect that accumulated inside the algae's body **(Khadija *et al*., 2020)**. Ultimately, the component chromium had a severe toxicity that adversely affected the cell's structure. It was observed that the shape of the algae cells changed as a result of the element's toxicity toward the cell wall and internal structures. This led to an external appearance that was different from the two other elements, namely, zinc and aluminum **(Malik & Abdul wahab, 2020; Suaad & Abdul-Wahab, 2020)**.

Fig. 1. Control sample of *Chlamydomonas* sp.

Table 2. Concentrations of elements in the control sample of *Chlamydomonas* alga, estimated in dry weight%

Element	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (3 Sigma) [wt.%]
Carbon	K-series	55.39	48.64	59.49	18.86
Oxygen	K-series	28.96	24.59	24.26	8.88
Nitrogen	K-series	17.05	15.23	13.45	7.07
Chlorine	K-series	4.02	3.84	1.18	0.47
Sodium	K-series	3.81	2.66	1.54	0.55
Magnesium	K-series	1.83	1.79	1.03	0.37
Silicon	K-series	1.56	1.56	0.71	0.32
Sulfur	K-series	1.45	1.36	0.66	0.29
Potassium	K-series	0.87	0.850	0.25	0.17
Iridium	M-series	0.92	0.87	0.06	0.21

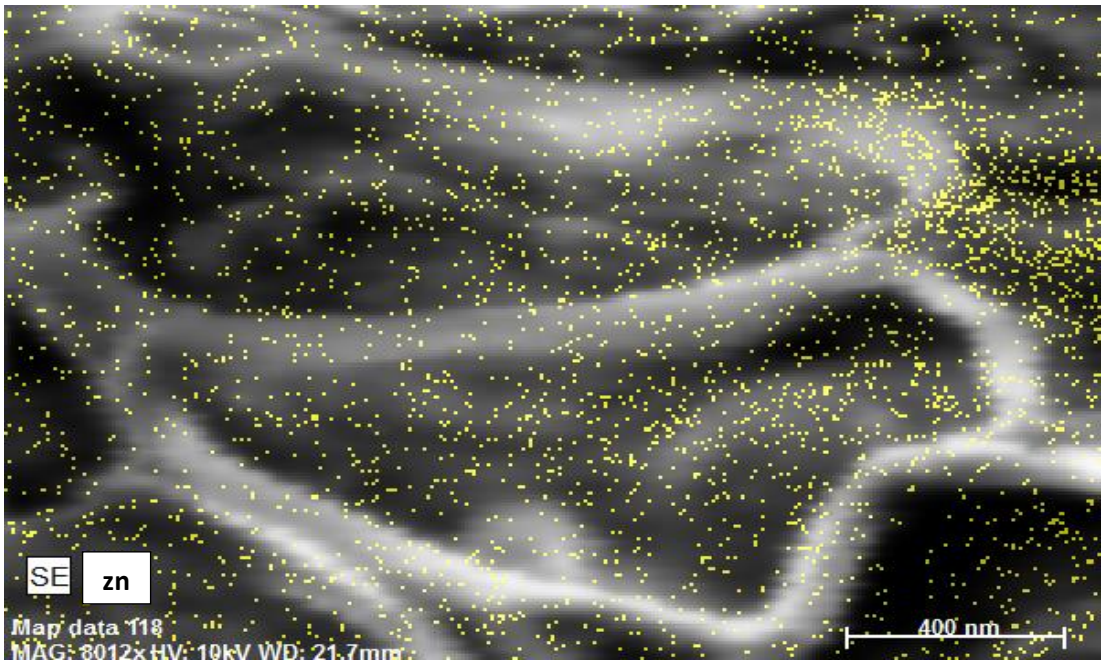

Fig. 2. Effect of zinc at a concentration of 25mg.L^{-1} on *Chlamydomonas*

Table 3. Zinc ion concentration in *Chlamydomonas* alga, estimated in dry weight%

Element	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (3 Sigma) [wt.%]
Carbon	K-series	55.39	48.64	59.49	18.86
Oxygen	K-series	28.96	24.59	24.26	8.88
Nitrogen	K-series	17.05	15.23	13.45	7.07
Chlorine	K-series	4.02	3.84	1.18	0.47
Sodium	K-series	3.81	2.66	1.54	0.55
Magnesium	K-series	1.83	1.79	1.03	0.37
Silicon	K-series	1.56	1.56	0.71	0.32
Sulfur	K-series	1.45	1.36	0.66	0.29
Potassium	K-series	0.87	0.850	0.25	0.17
Iridium	M-series	0.92	0.87	0.06	0.21

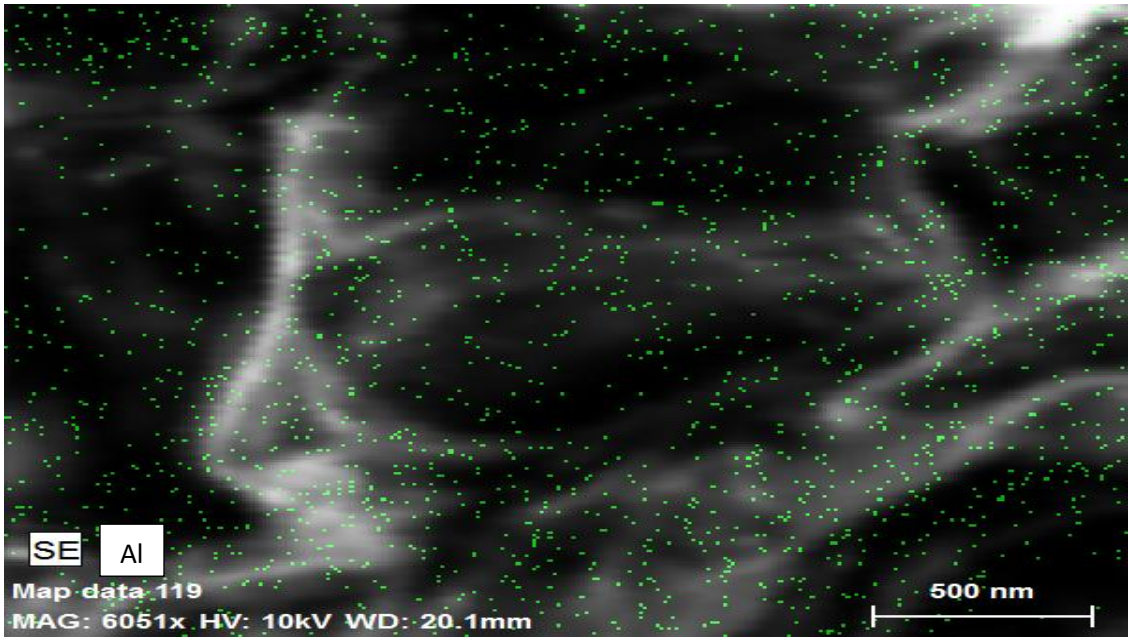

Fig. 3. Effect of aluminium at a concentration of 25mg.L^{-1} on *Chlamydomonas*

Table 4. Aluminium ion concentration in Chlamydomonas alga, estimated in dryweight%

Element	Series	unn. C [wt.%]	norm. C [wt.%]	Atom. C [at.%]	Error (3 Sigma) [wt.%]
Carbon	K-series	54.81	54.81	61.03	18.47
Oxygen	K-series	22.18	22.18	18.54	8.50
Nitrogen	K-series	20.02	20.02	19.12	8.44
Aluminium	K-series	2.62	2.62	0.30	0.15
Lead	M-series	0.37	0.37	0.02	0.10

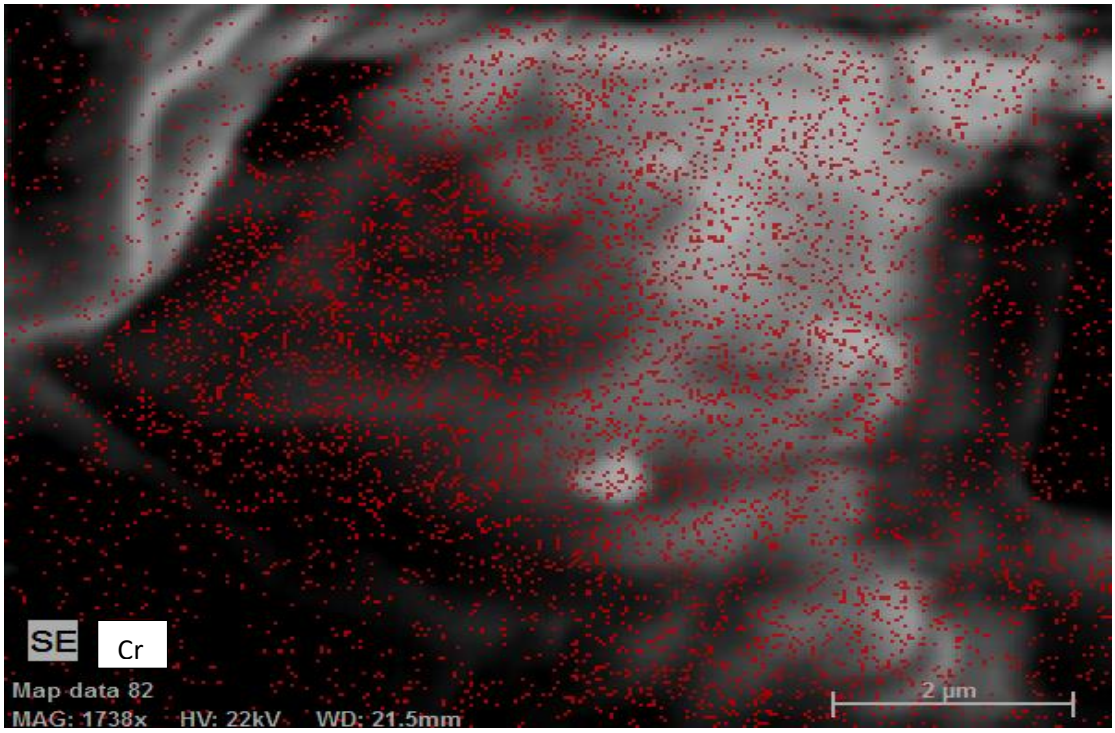

Fig. 4. Effect of chromium at a concentration of 25mg.L^{-1} on *Chlamydomonas*

Table 5. Chromium ion concentration in *Chlamydomonas* alga, estimated in dry weight%

Element	Series	unn. C [wt.%]	norm.C [wt.%]	Atom. C [at.%]	Error (3 Sigma) [wt.%]
Carbon	K-series	49.39	42.15	53.16	17.20
Oxygen	K-series	46.46	39.65	37.54	16.29
Silicon	K-series	6.04	5.15	2.78	0.84
Chromium	K-series	0.69	0.59	0.28	0.71
Fluorine	K-series	2.52	2.15	1.72	1.38
Cadmium	L-series	2.49	2.12	0.29	0.38
Aluminium	K-series	2.27	1.94	1.09	0.40
Sodium	K-series	2.15	1.83	1.21	0.48

Magnesium	K-series	2.11	1.80	1.12	0.41
Calcium	K-series	1.53	1.31	0.49	0.28
Iridium	M-series	0.77	0.66	0.05	0.20
Chlorine	K-series	0.75	0.64	0.27	0.18

CONCLUSION

The results showed that the values of trace elements in the algae under study were higher than the permissible limits, and this indicates that the pollution at the studied sites is the result of the abundance of pollutants and waste in all their forms and types at those sites. Therefore, this study recommends the use of advanced techniques to detect and identify the genetic genes responsible for the phenomenon of bioaccumulation of these elements within the algal cell body.

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