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Bioadsorption of heavy metals using microalgae isolated from Mosul city wastewater

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Abstract. This study examined the ability of two genera of microalgae chlorosarcinoopsis and Dietyosphoerium Sp. The presence of heavy metals in wastewater as hazardous environmental pollutants has led to the consideration of bioremediation as a means to mitigate this risk. Accordingly, microalgae isolated from wastewater belonging to the Chlorophyta division (green algae) and morphologically identified as Dictyosphaerium sp. and Chlorosarcinopsis eremi. These isolates demonstrated the ability to adsorb the heavy metals examined in this research (Cr, Ni, Cu). It was observed that Dictyosphaerium sp. exhibited an optimal capacity for nickel adsorption at 0.03599, whereas Chlorosarcinopsis eremi achieved the highest adsorption percentage for chromium at 0.3282. The biosorption mechanism of such pollutants is based on the functional groups' binding sites found within the cell wall of the microalgae. In this work, Dictyosphaerium sp. cell wall contained amino, alkane, carboxyl, amidic, and sulfonate groups as well as alcohol and phenol groups. The same functional groups were detected in the cell wall of Chlorosarcinopsis eremi namely amino, alkene, carboxylic acid, aldehyde, sulfonate, alcohol, and phenol groups. There was a variation in the number of these chemical functional groups prior to and subsequent to the heavy metal treatment of the isolates, and they were analyzed using Fourier Transform Infrared (FTIR) spectroscopy.
Keyword: Heavy metals, Mosul city, Microalgae, Chlorosarcinoopsis, Dietyosphoerium Sp

1. INTRODUCTION

Population growth worldwide has coincided with agricultural development, industrialization, and urbanization and has led to a sharp increase in waste generation and environmental pollution (Abdel-Ghani and EL-choghaby, 2014). Among numerous issues of contemporary society, one is to treat urban wastewater efficiently and sustainably since untreated sewage can enrich aquatic habitats and become a source of hazard to water bodies (Ljahadali and Alhassan, 2020; Bulska and Ruszczyuika, 2017). The sanitary discharge of wastewater from industrial and urban areas ushers in environmental issues via the leaching of potential metallic elements into diverse ecosystems. Bioaccumulation of the elements within living organisms, alongside their repetitive accumulation in food chains, necessitates effective solutions (Yilmaz et al., 2023). Also, the excessive content of heavy metals in wastewater effluent from industrial and urban processes is a threat to groundwater and river systems with potentially fatal consequences. These negative impacts include loss of biodiversity, ecosystem deterioration, and hazards to human health via heavy metal toxicity (Rambabu et al., 2020, Al-shearaefy etal, 2023).

As a measure to prevent environmental degradation and attain sustainability, biotreatment using microorganisms—among them microalgae—has been an alternative due to the simplicity, low cost, and ease of handling of microalgae, as well as their effectiveness in heavy metal removal from solid waste, which is also a secondary waste stream. Microalgae-based biotreatment has drawn significant attention from researchers because of the low costs of process design and development, the resilience of microalgae to harsh conditions, and the possibility of metal recovery from the biomass. Not only is this biotreatment enhancing the circular economy, but it is also the primary environmentally friendly alternative in this field. It has become economically possible to extract heavy metals from microalgae, following the industrial resource recovery principle and in line with the circular economy theory, embarking on a new age of sustainability. (MIcaela et al., 2024).

At the same time, microalgae are multi-functional in biotreatment because they can convert pollutants into less toxic products that can be recycled or disposed of safely, thus allowing for the reuse of treated water or safe disposal. (Silva, 2023; Azarpour et al, 2022).

For executing scale-up measures towards biotreatment processes, microalgal dead biomass is used since it avoids jeopardizing the exposure of the environment towards risks of algal blooms. Secondly, less additional nutritional material has to be supplemented while using non-vital biomass for further development. Thus, more beneficial economic factors result. Microalgae have been ranked among the most promising forms of biomass for future biotechnological applications due to the fact that they can thrive under conditions that have chemical wastes contaminating them. Their physiological traits and genetic acclimatizations—induced by natural selection through mutations—are the reasons they can survive in such contaminants (Ahmed et al., 2021; calijuri etal, 2022).

The biotreatment microalgae cells possess various mechanisms to regulate metal ion concentration within their tolerance range, which include ion exchange, biotransformation, and chelation, both intracellularly and extracellularly (Cortes et al., 2018). Moreover, physical and chemical environmental factors directly impact the resistance of microalgae cells against heavy metals in ecological systems by affecting the binding of metals to the cell surface (flocculation).

Spherical green microalgae form one of the most diverse sets of microalgae, i.e., green algae that is spherical in shape, one of the most critical types of phytoplankton. Dictyosphaerium is a spherical green alga that occurs in the form of irregular colonies formed by 4 to 64 cells. The cells are contained in a sheath of gelatin and it has a size of 1-10 μ m. Each cell contains a single nucleus and a plastid with a pyrenoid. (Krenilz et al., 2011; contas etal, 2022).

This species was recently isolated in Iraq as a phytoplankton species, which is largely present in both freshwater and brackish environments in some Iraqi cities (Merza and Abul-Doonej, 2020). Dictyosphaerium was used as a biosensor in contaminated ecosystems due to its ability to survive at high hexavalent chromium concentrations even when exposed to photosynthetic inhibition and morphological change (Do'rs et al., 2010). Moreover, the biomass of Dictyosphaerium has been employed in biotreatment to reduce the chemical and biological risks that exist in animal farm wastewater that contains high levels of ammonia and pathogenic microorganisms (Xinjie et al., 2019). Moreover, (Gentili and Fick, 2017; Daneshvars et al 2022) utilized this genus efficiently to eliminate pharmaceutical contaminants partially or completely in urban areas.

The green microalga species Chlorosarcinoperemi belongs to the order Chlamydomonadales, which was isolated from terrestrial plants. Order members have been gaining increasing attention because of their biotechnological applications, particularly for the generation of β-carotene (Guiry and Guiry, 2018; Goswami etal 2022). The genus includes functional receptors that respond to stress environments such as high salinity, high illumination, and starvation of nutrients (nitrogen and carbon). When so, the colony shifts from green to reddish-orange or orange in hue due to enhanced synthesis of carotenoids, antioxidants utilized by cells for protection of them from harsh environment conditions (Wongsnanslip et al., 2007; Gomez. J etal, 2024).

Thus, the above two genera were selected in the current study as microalgae used for biotreatment of Mosul urban wastewater contaminated with heavy metals.

2. MATERIALS AND METHODS

2.1 Isolation Methods Under Study:

The Mosul wastewater outfalls (location of Hawi Al-Kanisah and Al-Maidan area) were selected for the isolation of under study microalgae.

2.2 Isolation Method:

The algal samples of were collected in sterile bottles, and forceps and droppers were used for isolation from riverbanks at wastewater points of discharge in the above-stated areas (Patteison et al., 1993). CHULO medium is used to isolate and grow algae (Bold and Wynne, 1985).

2.3 Morphological Identification:

Morphological identification was based on determining the genus and its corresponding algal division. Temporary slides were prepared from purified isolates and examined using an Olympus light microscope, following identification references (Wehr and Sheath, 2003; Bolol and Wynne, 1985; Prescott, 1968). The planning method used for insulation. (Patterson, et al., 1993)

2.4 Measuring the Ability of the Microalgal Isolates Under Study to Adsorb Heavy Metals:

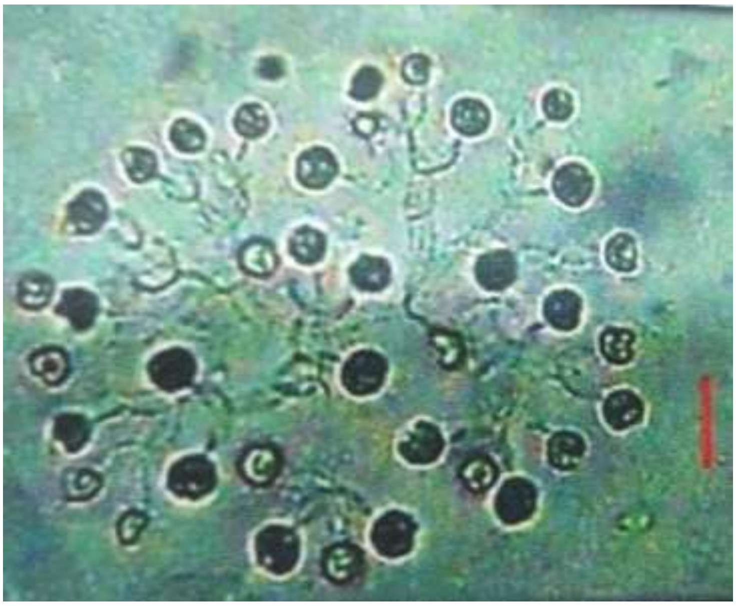
To determine the ability of the microalgae to adsorb the specific heavy metals under study, 100 ml of Chulo medium was prepared with the addition of 0.5 g of dried microalgae and 0.005 of heavy metal. An Atomic Absorption Spectrophotometer (AAS) (Germany), model 5YO7CS-10082/A04H, was used. The microalgae samples were digested using the wet digestion method, based on the source.

2.5 Study of the Effect of Heavy Metals on Functional Groups in Microalgae:

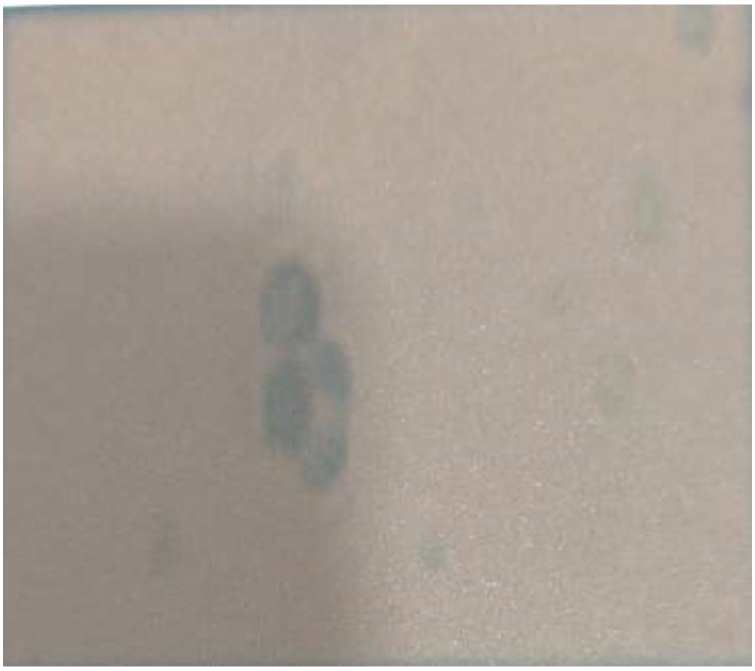
To study the effect of heavy metals on the chemical groups of the microalgae under study, Fourier Transform Infrared Spectroscopy (FTIR) was used. The infrared spectroscopy technique was used to detect the effect of heavy metals present in the plant kingdom. Measurements were taken using the FTIR device (Bruker Company, Alpha II model, UV-Visible/Fluorescence spectrometer category, United States of America) at the central laboratory of the Department of Chemistry, College of Science, University of Mosul.

3. RESULTS AND DISCUSSION

3.1. The microalgae isolated during the current study were part of the Chlorophyta (green algae, Class: Trebouxiophceae, Order: Chorellales, Family: Chlorellaceae). The first isolate appeared as colonies consisting of 4-64 cells, exhibiting the morphological transformation of Dictyosphaerium, with an outer mucous membrane. The cells were spherical to oval and connected by microscopic gelatinous threads Figure 1. In laboratory cultures, they were typically single-celled due to the loss of mucous filaments.



(a)



(b)

Figure 1: .(A) *Dictyosphaerium Sp.* *(B) Chlorosarcinopsis.eremi*

The second isolate, Chlorosarcinopsis eremi, (CL: chlorophylcave, Ol: chamydomonodales, F: chorosavinaceae) belonging to the Chlamydomonadales order and Chlorosacinaceae family, exhibited single spherical cells under electron microscopy. The cells displayed alternating pairs and quartets.

3.2 The Ability of Microalgae to Adsorb Heavy Metals

Microalgae that can grow in polluted areas possess a form of resistance to pollutants, including heavy metals such as copper, zinc, nickel, and cadmium (Stokes, 1983). Therefore, researchers have relied on the ability of microalgae to resist heavy metals by using them in the fields of biosorption and bioremediation.

The microalgae under study, Dictyosphaerium sp. and Chlorosarcinopsis eremi, were used for bioremediation and to investigate their biosorption capacity. The results of biosorption of heavy metals, based on an Atomic Absorption Spectrophotometer, showed that Dictyosphaerium sp. had the ability to absorb heavy metals. The concentration of these metals in the CH10 medium decreased from (0.0359, 0.0373, 0.0392) mg/L to (0.00476, 0.00131, 0.00373) mg/L for Cu, Ni, and Cr, respectively. It was observed that the highest percentage of adsorption was for Ni, at 0.03599. For Chlorosarcinopsis eremi, the biosorption efficiency for heavy metals was from (0.0342, 0.0345, 0.0323) mg/L to (0.00138, 0.00828, 0.00180) mg/L, indicating a significant decrease in the concentration of these metals in the treatment medium. The element most adsorbed by C. eremi was chromium, with an adsorption rate of 0.3282, as shown in Table. (1)

Table 1. Concentration of heavy metals under study in the food medium Ch10

moss Chlorosarcinopsis sp.		moss Dictyosphaeriums sp.		heavy element
After the transaction	Before the transaction	After the transaction	Before the transaction	
0.00180	0.0323	0.00373	0.0359	Cu
0.00828	0.0345	0.00131	0.0373	Ni
0.00138	0.0342	0.00476	0.0392	Cr

These results are consistent with those presented by (Shanab et al., 2012), regarding the biosorption capacity of microalgae Pseudochloroccum typicum and Scenedesmus sp. for heavy metals such as Cd, Pd, and Hg from aqueous solutions under laboratory conditions. In contrast, the ability of Chlorella vulgaris to absorb copper and cadmium was (0.17, 0.33) mg/g, respectively, compared to the capacity of Scenedesmus obliquus, which reached (9.12, 10.02) mg/g for copper and cadmium, as reported by (Hockoday et al., 2022).

The use of live biomass of Botryococcus sp. for the removal of chromium reached 99% from wastewater when using 15 cells/mL (Onn, 2023).

The strains D. salina and N. salinicola showed promising efficiency in removing heavy metals such as lead and chromium. Based on the mechanisms of ionic metal removal and bioaccumulation inside the cells, D. salina removes chromium ions, while N. salinicola employs both intracellular and extracellular mechanisms to remove lead and chromium (Elleuch, et al., 2021; Li etal, 2023).

Bioremediation occurs in two stages: the first is rapid passive adsorption, which takes about 30 minutes and is attributed to the surface adsorption of the cell wall components, followed by the slower active absorption process that can take more than a month due to the transfer of metal ions through the membrane into the cytoplasm (Nowicka, 2022; Shenghrg et al, 2024). These absorbing biological activities may result from the contents of the microalgae, such as fluorocoloids, citrates, phosphorus, and nitrogen, in the dried algal cell. (Giuagliano et al. 2024) demonstrated the active and dried Chlamydomonas ability to adsorb both mono- and multivalent ionic solutions in wastewater.

The transfer of heavy metals within the vacuoles or cytoplasm is a mechanism that contributes to heavy metal tolerance by minimizing cytoplasmic metal concentrations as much as possible. This is achieved by binding or forming metal ions into sulfide, iron, or phosphate complexes in the cytosol and transporting them into the vacuoles. The acidic pH helps displace the metal, allowing the complex to return to the cytosol, while the metal is trapped in the vacuole by organic acids, which are typically present in high concentrations within the vacuoles. Microalgae carry out this role as a cell protection from heavy metals (Shen et al., 2024).

3.3 the binding spots between the heavy metals and the functional groups

Environmental heavy metal contamination poses a great threat to terrestrial and aquatic ecosystems. Microalgae have been identified as an effective bioremediation process where heavy metals are adsorbed through the process of biosorption (Yadav, et al., 2019).

The chemical functional group binding sites on the microalgae cell walls, i.e., amine, hydroxyl, carboxyl, and sulfate groups, are accountable for the removal of heavy metals (Abdel Fattah, et al., 2023). The presence of these functional groups carrying a negative charge on the outer layer of the membrane facilitates this. (Singh, et al., 2021).

Table (4) illustrates the functional groups in the Dictyosphaerium sp. cell walls, which include amine, alkane, carboxyl, carbonyl amide, sulfate, alcohol, and phenol groups. Slight changes appeared in these functional groups after treatment with heavy metals. The frequency of 1329 cm^{-1} vanished for the amine group during acid hydrolysis before and after treatment of the algae using copper and chromium. Nonetheless, a new amine band was seen at a frequency of 1066 cm^{-1} after chromium treatment but not in the control sample or samples treated with nickel and copper. The alkene group at 2927 cm^{-1} disappeared in all samples treated with nickel, copper, and chromium. The carbonyl group at 1651 cm^{-1} in the control sample was not detected in any of the treatments.

The alkene group at 2927 cm^{-1} disappeared in all samples treated with nickel, copper, and chromium. The carbonyl group at 1651 cm^{-1} in the control sample was not detected in any of the treatments. The alkene CH_3 group at 1416 cm^{-1} appeared in the control sample and the nickel treatment, while it appeared at 1832 cm^{-1} in the chromium treatment. Additionally, the sulfate group at 1329 cm^{-1} disappeared when treated with copper and chromium. No changes were observed in the alcohol and phenol groups at frequencies of 1087 cm^{-1} and 1045 cm^{-1} in either the control sample or the treated samples with nickel, copper, and chromium.

Table 2. Functional groups detected by IR technique in the cell walls algae of Dictyosphaerium sp.

Sample after treatment Cr	Sample after treatment Cu	Sample after treatment Ni	Sample before treatment	Functional groups
3353	3322	3322	3336	Amines
-----	-----	1329	1329	3400-3300
1274	1274	1274	1274	1350-1000
1066	-----	-----	-----	
1044	1045	1045	1045	
2973	2973	2973	2973	Alkanes
-----	------	------	2927	3000-2850
2973	2973	2973	2973	Carboxylic Acid
2927	2927	2927	2927	3300-2500
2866	2866	2866	2866	1320-1210
1274	1274	1274	1274	
				Aldehydes Ketones
				1740-1720
-----	------	-------	1651	Carboneal
				Amid
				1680-1630
1382	------	1416	1417	Alkancs
				CH3(bend)
				1375-1450
-----	------	1329	1329	Sulfones
1087	1087	1087	1087	Sulfates
				1350-1140
1045	1045	1045	1045	Alcohols&
1087	1087	1087	1087	Phenols
				970-1250

The second algae under study, Chlorosarcinosis eremi, showed that the functional groups present in its cell wall played a significant role in the algae's ability to adsorb heavy metals. The key functional groups observed were amine groups, alkene groups, carboxyl acid, aldehyde, carbonyl amide, sulfate, alcohol, and phenol (Table 6). Clear differences were noted in the amine group at a frequency of 3331 cm^{-1}, which was observed in both the control group and the sample treated with nickel. This group appeared at a frequency of 3352 cm^{-1} in the copper-treated sample. For the chromium-treated sample, the amine group appeared at a frequency of 3344 cm^{-1}. The alkene group appeared at 2973 cm^{-1} in all samples, while in the control and nickel-treated samples, it appeared at 2831 cm^{-1}, and in the copper-treated sample, it appeared at 2869 cm^{-1}. A distinct peak at 2894 cm^{-1} was observed in the chromium-treated sample. The carboxyl group showed similar frequency patterns to the alkene group. It was noted that the carbonyl amide group disappeared from both the control sample and the nickel-treated sample, while it was present at a frequency of 1467 cm^{-1} in the copper and chromium-treated samples.

The sulfate group was present in all samples with a frequency of 1274 cm-1. The alcohol and phenol groups were detected at two frequencies: 1087 cm-1 and 1045 cm-1 in all samples, except for the sample treated with nickel, where only the first frequency was observed.

Table 3. Functional groups identified by IR spectroscopy in the cell walls of the alga Chlorosarcinosis eremi

Sample after treatment Cr	Sample after treatment Cu	Sample after treatment Ni	Sample before treatment	Functional groups
------	-----	3329	3331	Amines
-----	3352	------	~~~~	3400-3300
3344	~~~~~	------	------	1350-1000
2973	2973	2973	2973	Alkanes
~~	~~~~	2881	2881	3000-2850
2894	------	-------	~~~~~	
~~	2869	~~~~~	~~~~~	
2973	2973	2973	2973	Carboxylic Acid
~~	~~~~	2881	2881	3300-2500
2894	~~~~~	------	-----	1320-1210
~~	2869	-------	------	
				Aldehydes
				Ketones
				1740-1720
1467	1467	-------	-------	Carboneal
				Amid
				1680-1630
				Alkancs
				CH3(bend)
				1375-1450
1274	1274	1274	1274	Sulfones
				Sulfates
				1350-1140
-------	1087	1087	1087	Alcohols&
1044	1044	1045	1045	Phenols
				970-1250

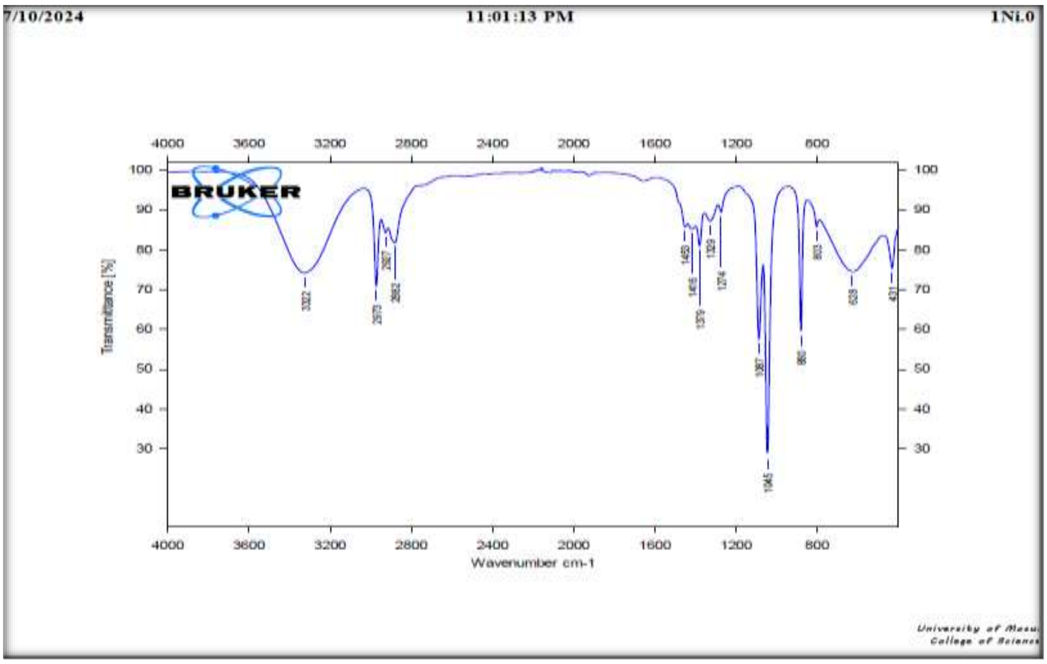


Figure 2. Binding of nickel metal to the functional groups of the wall of the alga Dictyosphaerium Sp.

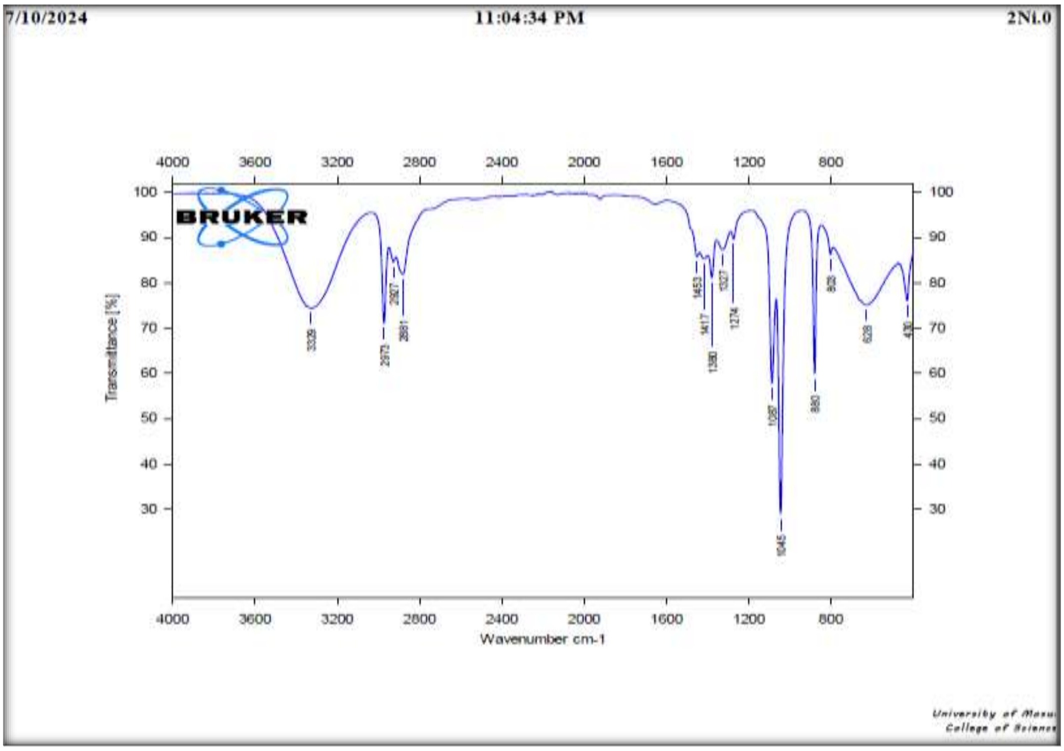


Figure 3. Binding of nickel metal to the functional groups of the wall of the alga Chlorosarcinopsis.ermi

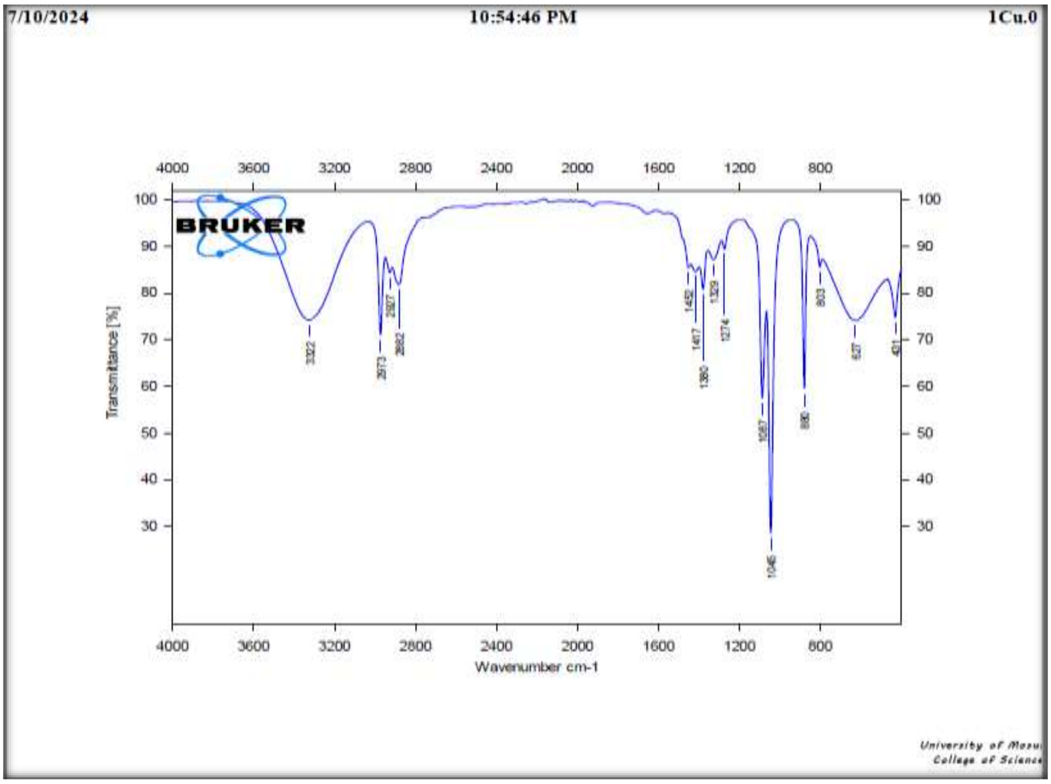


Figure 4. Copper metal binding to the functional groups of the wall of the alga Dictyosphaerium Sp.

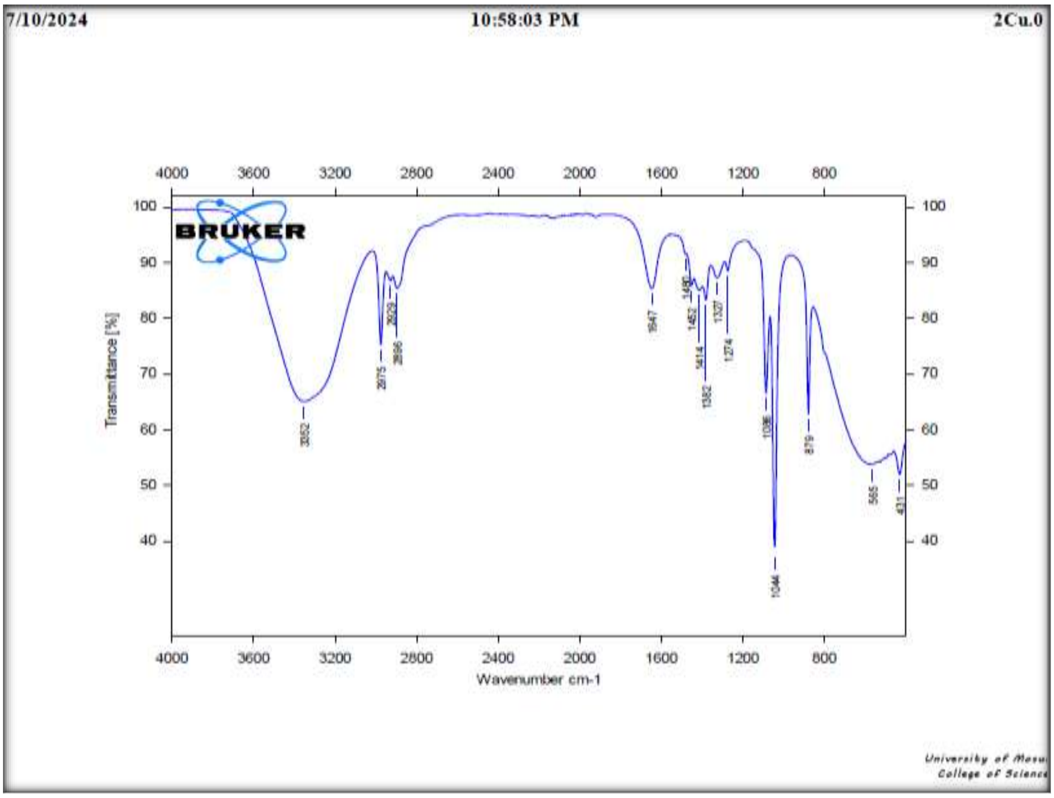


Figure 5. Copper metal binding to the functional groups of the wall of the alga Chlorosarcinopsis.ermi

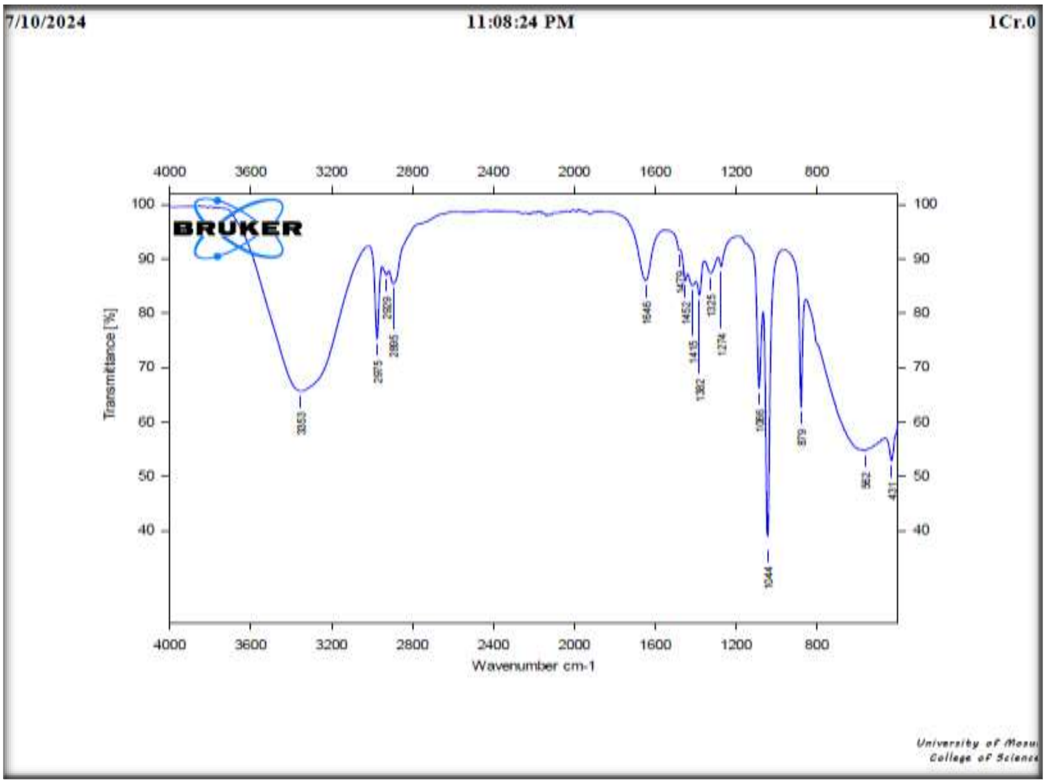


Figure 6. Binding of chromium metal to the functional groups of the wall of the alga Dictyosphaerium Sp.

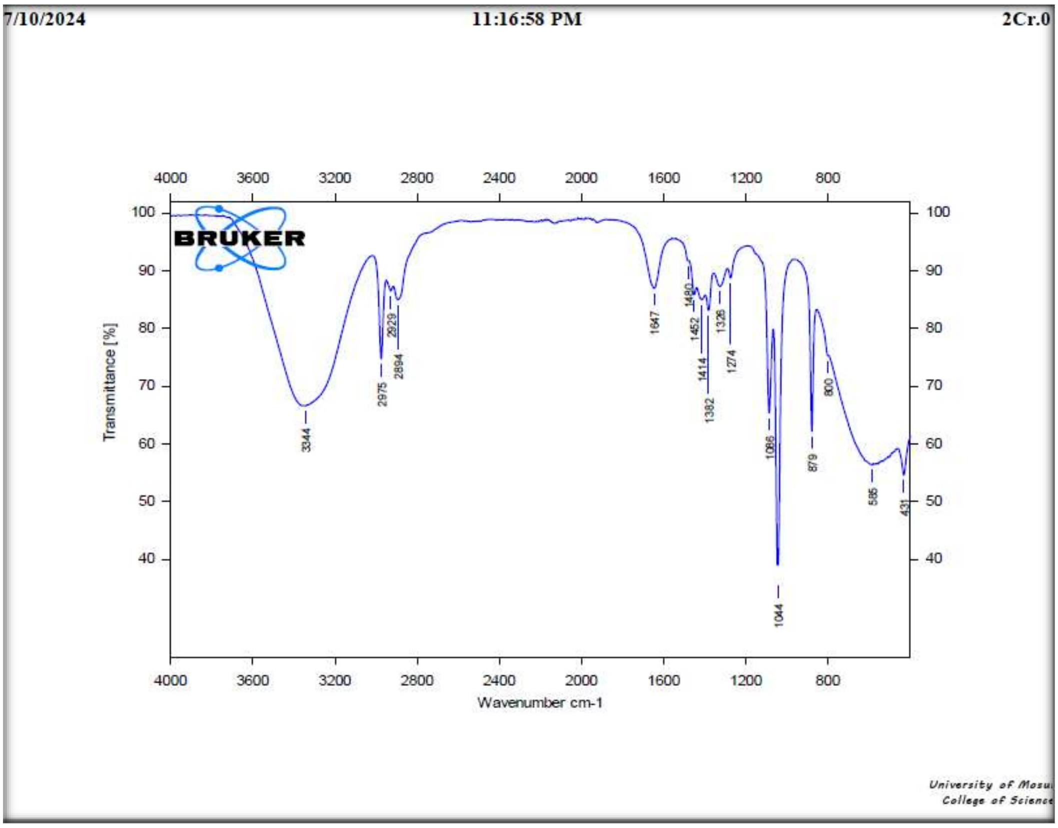


Figure 7. Binding of chromium metal to the functional groups of the wall of the alga Chlorosarcinopsis.ermi

These results in our current study are consistent with those reported by Elleuchb et al. (2024) on the biosorption capacity of *Dunaliella sp. AL1* for copper and hexavalent chromium. When the algae were exposed to near-lethal concentrations, reactive oxygen species were generated in the algal cells, and the biosorption of copper and chromium reached 95.26%, respectively. FTIR results confirmed the interaction of copper and chromium ions with N-H and C-H groups. In addition, Scenedesmus GTAflla was also determined to remove chromium with high efficiency from wastewater under non-nutrient conditions, up to 99%. (Tripathi et al., 2024).

The microalgae Sphaeroplea, Chlorella reinhardtii, Chlorella miniata, and Chlorella vulgaris were found to be effective in the removal of toxic heavy metals from wastewater (Koiterd et al., 2021). Four microalgae species were recently reported to adsorb heavy metals in recent work done by Wang et al. (2022). Microcystis aeruginosa was specifically observed to be extremely efficient in the removal of Mn and Fe from groundwater. Moreover, microalgae are resistant to heavy metal toxicity through gene expression modulation and the release of extracellular polysaccharides (Santiago et al., 2015).

Chlorella vulgaris plays a significant role in bioremediation with dead cells and its ability to decrease chromium and copper to the less toxic trivalent form by biological and non-biological means (Verma et al., 2024).

Chlamydomonas moewusii and Auxenochloris pyrenoidosa were isolated by Venkatesan et al. (2023) from wastewater and cultured in BG 11 medium. These algae demonstrated bioremediation capabilities for chromium pollution, with removal rates of 65% and 90%, respectively, for each algae species.

Nickel was removed by 100% through bioremediation using Scenedesmus obliquus and Chlorella pyrenoidosa (Ton et al., 2024). (Almomani et al. 2021) also highlighted the ability of Spirulina platensis and Chlorella vulgaris to remove nickel and copper by 63% and 87%, respectively, during acid treatment, which increased the negative electrical charge of the functional groups, enhancing the removal of heavy metals.

Dead biomass acts as an effective biosorbent for lead ions from industrial wastewater, with lead removal observed under electron microscope images (Dawood et al., 2024). Significant changes were observed in the cell wall of the algae, manifesting as extensions of the algal tips, indicating a substantial alteration on the surface of the algal biomass. These changes were associated with the formation of bonds between heavy metal ions and the carboxyl, carbonyl, and amide groups (Soco et al., 2024).

Thus, microalgae possess a range of functional groups capable of binding heavy metal ions within their biomass. The cell wall consists of lipids, polysaccharides, and proteins that provide functional binding groups for metal ions, such as amine, hydroxyl, carboxyl, sulfate, and phosphate (Fawzy et al., 2024). This was confirmed through FTIR results, which showed that the carboxyl, amide, amine, carbonyl, and alkyl groups are the main groups responsible for the biosorption process.

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