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Wastewater Treatment by the Cyanobacterium Species: *Synechococcus elongatus* as Biosorption Material for Pb, Cr and Ni Heavy Metals

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Abstract: This research studies the effect of carbon dioxide on microalgae during the biological treatment of wastewater. *Synechococcus elongatus* was cultivated to eliminate heavy metal pollutants (lead, chromium and nickel) at different concentrations (0.5, 1.0, 2.0, 5.0 and 10.0ppm) under rich-CO_2 environment. 50 ml of microalgae cultural medium were bubbled with 2 liters/ minute of CO_2 gas, three times a week for two weeks. The results demonstrate that the effectiveness of microalgae growth and pollutant removal is directly impacted by CO_2 gas dosages, as quickly and efficiently cell adaption in comparison to control groups. The highest pollutant removal was 100% (during day 10 of cultivation) with the presence of CO_2 and 99.93% (during day 14 of cultivation) without the presence of CO_2 for Pb at 0.5 ppm.

Key words: Bioremedation, carbon dioxide, wastewater, heavy metal, pollutants, removal efficiency.

Introduction

Two significant elements that make wastewater management challenging throughout the purification process are biological oxygen demand and chemical oxygen demand. The pre-treatment process poses a risk to the environment because of the high concentration of suspended particles and sludge (Sekar et al., 2021). Furthermore, because heavy metals are present, using wastewater for value-added purposes like agriculture is not feasible. Therefore, eliminating harmful heavy metals is crucial to reusing wastewater. Using microalgae is among the finest and most cost-effective methods for removing metals from wastewater. Microalgae is a significant source for the production of biomass (Ganesan et al., 2020) also regarded to be a superior option because of its remarkable productivity and its favourable environmental characteristics (Anderson et al., 2021; Kim et al., 2021). Compared to terrestrial plants, the photosynthesis rate is approximately fifty times greater.

Microalgae typically develop easily in a wide variety of wastewaters without any difficulties. However, the higher concentration of hazardous heavy metals influenced growth (Zhu et al., 2018). Because dangerous contaminants are consumed in significant quantities, the microalgae growth rate is impeded (Spain et al., 2021). When discharged improperly, heavy metals can accumulate in living things and pose a threat to public health. The most prevalent HMs are lead, nickel,

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arsenic, cadmium, mercury, chromium, iron, zinc and copper (Mitra et al., 2022).

Human activities like mining, smelting, manufacturing, use of home metal, and farming utilization emit these heavy metals into the environment (Fu and Wang, 2011; Flouty and Estephane, 2012). Through home sewage, nonpoint runoff, industrial discharges, and precipitation in the atmosphere, these heavy metals enter water bodies (Kim et al., 2021). When large amounts prevent water from self-purifying, heavy metal pollution in water bodies arises. Many environmental laws and guidelines implemented to protect ground and surface water from heavy metals (Yan et al., 2022).

Due to microalgae's ability to treat wastewater in a single motion using numerous techniques to maintain the nitrogen, carbon, and phosphorus ratios, treatment using microalgae appears to be more effective than traditional wastewater treatment. Additionally, it can also be a green option because it can convert CO_2 into chemical elements and fuel without contaminating the environment, it can reduce the emission of greenhouse gases, making it an environmentally favorable option (Danouche et al., 2021). A very effective alternative method for reducing CO_2 emissions could be the fixation of CO_2 by different species of microalgae in combination with biofuel generation and wastewater treatment (Abed et al., 2018).

This study aims to examine the ability to use *Synechococcus elongatus*, isolated and purified from the Iraqi environment as biosorbent material for different concentrations of heavy metals chosen according to research on sewage water of four hospitals in Baghdad and study the effect of using CO_2 as a carbon source in the growth and removal efficiency for these pollutants during the cultivation period.

Material and Methods

Examination of Wastewater

Wastewater samples were collected from four hospitals in Baghdad: Baghdad Teaching Hospital, Ghazi Al-Hariri Hospital for Surgical Specialties, Digestive Hospital, and Specialized Burns Hospital, and examined at the Scientific Research Authority/Environment and Water Centre, using an Atomic Absorption Spectrophotometer (model 7000 SHEMADZU AA). The assessment showed High rates of arsenic, nickel, chromium, lead, and cadmium in wastewater; the percentages of nickel, chromium, and lead were the greatest, therefore we chose them to confirm the microalgae's removal capability.

Preparation of Stock Solutions

Heavy metals stock solutions were prepared from their pure salts ($Pb(NO_3)_2$, $NiCl_2$ $5H_2O$ and $K_2Cr_2O_7$) by dissolving each salt in distilled water to get a final concentration of 100 ppm, then storing in the dark until needed. Five concentrations (0.5, 1.0, 2.0, 5.0, and 10.0 ppm) of each heavy metal were prepared. 0.1 N of NaOH and HCl was used to adjust the solution's pH.

Isolation of Cyanobacterium Species

Synechoccous elongatus is a cyanobacterium species, isolated from the Tigris River in Baghdad-Iraq and purified using serial dilution technique (Abed et al., 2014; Kothari et al., 2011).

Preparation and Sterilisation of Culture Media

The culture medium used was BG-11, prepared by using 1 L of distilled water for dissolving 1.627 grams of BG-11. 0.1 N of NaOH or HCl was used to get the pH value 7. Sterilising the media is a crucial step for getting rid of any unwanted microorganisms (Abed et al., 2019) by using an autoclave at 121°C/2 bar of pressure for 15 minutes.

Cultivation of Cyanobacterium Species

Microalgal strains were cultivated in sterilised BG-11, incubated in a 500 ml conical flask, in a standard-environment illuminated incubator with cool white fluorescent lights, with light intensity 168 $\mu Em^{-2}s^{-1}$ and constant temperature (26± 2 °C) (AL-Mashhadani et al., 2015; Hassan et al., 2021).

Bubbling System of CO_2

CO_2 was used as a carbon source for microalgae cultures by using a bubbling system which consisted of a gas source connected to 100 ml sterilized containers, and the gas was pumped at a rate of 2L/min, for 5 minutes, three times a week, for two weeks to 50 ml of microalgae culture media.

Measurements and Experimental Configuration

Experiments include a dual-component setup, both parts kept in an incubator that has lighting and a temperature appropriate to microalgae growth. The first part is divided into two groups each group includes preparing 500 ml volume flasks, containing algal culture, BG11 growth media and five concentrations (0.5, 1.0, 2.0, 5.0 and 10.0 ppm) for each heavy metal (Pb, Cr and Ni) with two duplicates for each concentration, and control (without adding heavy metal). The first group with CO_2 and the second group without CO_2. The second group

contains a gas source bubbling system connected to 100 ml sterilised containers, to equip pure CO_2 gas with a 2 L/min flow rate, for 5 minutes, three times a week, for two weeks.

2 L/ min of CO_2 gas was pumped into the 100 ml containers (containing 50 ml of microalgal medium with heavy metals) for 5 minutes, three times a week, in order to prevent a pH decrease. All the flasks were exposed to light intensity (168 $\mu Em^{-2}s^{-1)}$ and constant temperature (26± 2 °C), and manually stirred to keep the microalgae cells from clumping and to sustain cellular proliferation (Chiu et al., 2009). Samples were taken at various times during the experiment (0, first, second, third, fourth, sixth, eighth, tenth, twelfth and fourteenth day) for quantifying algal growth, metal removal and physicochemical parameters in different heavy metal concentrations.

Measurement of Microalgae Growth

Using a UV spectrophotometer (APEL - JAPAN PD-303) at a wavelength of 680 nm, samples of microalgae cultures from each concentration were measured to monitor growth kinetics from zero time (lag phase) to stationary phase, where the experiment was terminated.

Specific Growth Rate

The specific growth rate (Sg) in the exponential development phase was computed using (Rai and Gupta, 2017):

$$S_g = \ln (X_t/X_0)/(t\text{-}t_0)$$

S_g: specific growth rate (day^{-1}), X_t : cell density at time t (cel ml^{-1}) and X_0: cell density at time t_0 (cel ml^{-1}).

The term "doubling time" (t_d) refers to the amount of time needed to double the mass of the cell when the cell is set to be doubled ($xn = 2xn0$) and (t_0=0) (Makki et al., 2023), and can be calculated as follows:

$$t_d = ln2/Sg$$

Measurement of Heavy Metal Concentration

Using an atomic absorption spectrophotometer (PARKIN ELMER – 1100B), the concentration of heavy metals was determined during the period of the experiment.

Measurement the Removal Percentage of Heavy Metal Ions

The removal percentage was performed by measuring the heavy metal concentration in the initial solution and then every two days till the experiment ended. The removal percentage was calculated using the following equation (Mojiri et al., 2021):

$$\% \text{ Removal of heavy metal} = (C_i - C_f)/Ci \times 100\% \quad (2)$$

where C_i and C_f are the initial and final concentrations of heavy metals (ppm) respectively.

Statistical Analysis

All measurements made throughout the experiments were done in triplicate, and the means of the three replicates are used to illustrate the results, The least significant difference was used to significantly compare between means (ANOVA/Two way) in this study.

Results and Discussion

Synechococcus elongatus exposed to Pb, Cr, and Ni heavy metal pollutants did not exhibit any appreciable harm. Microalgae growth rate was unaffected by the contaminants.

This research studies the impact of Pb, Cr and Ni heavy metals on micro algae growth with the presence and absence of CO_2, and examines how CO_2 affects the development of microalgae and how well they remove heavy metals from the environment. All the cultures' steady growth provided evidence that microalgae cultures might handle this kind of contamination in the future.

The outcomes of the experiment show that *Synechococcus elongatus* after 2 weeks recorded the highest optical density (0.468, 0.457 and 0.409) for the concentrations (0.5, 1.0, and 2.0 ppm, respectively) of Pb with $CO_2$when compared to control group which recorded optical density (0.390) in the presence of CO_2, while it recorded (0.149, 0.138 and 0.133 respectively) without CO_2 for the same concentrations when compared to control group which recorded (0.251) in the absence of CO_2, indicating better growth and pollution consumption when the CO_2 is present. The lowest optical density (0.197, 0.196 and 0.175) was recorded for the concentration (2.0, 5.0 and 10.0 ppm, respectively) of Cr with CO_2, while it recorded (0.115, 0.076 and 0.072, respectively) without CO_2 for the same concentrations (Figure 1).

The studies (Mahdi et al., 2021; Makki et al., 2023) also indicate the multiplication of algal cultures and their adaptation to the environment equipped with CO_2 gas, by providing all the ideal environmental conditions of temperature, intensity of illumination, pH values and nutrients and the culture media equipped with CO_2 is important in terms of rapid growth while

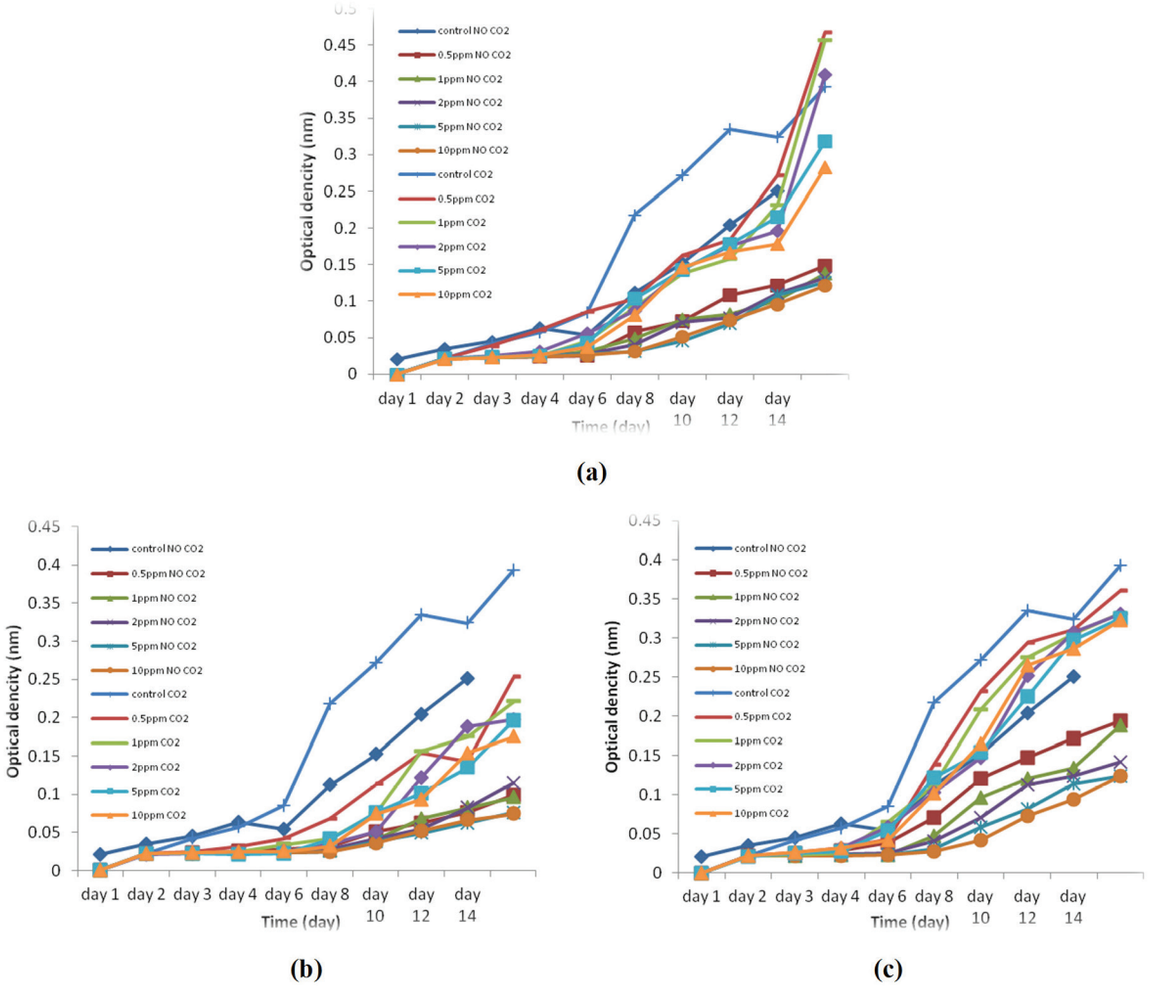

Figure 1: Growth of *Synechococcus elongatus* (without and with CO_2) (a) with Pb (b) with Cr (c) with Ni.

providing the rest of the nutrients. Besides, the selection of microalgae species is important because it directly influences photosynthetic efficiency and thus carbon-fixing performance and biomass production (Biesta et al., 2010).

Table 1 show the specific growth rate and generation time for *Synechococcus elongates* at different concentrations of Pb, Cr and Ni heavy metal (0.5, 1.0, 2.0, 5.0 and 10.0 ppm). The results show that different concentrations of heavy metal pollutants did not affect the generation time and specific growth rate response. Also, there is a significant enhancement in *Synechococcus elongatus* doubling time and specific growth rate with the presence of CO_2. The control group recorded a specific growth rate (0.2289 hr^{-1}) with CO_2, and (0.1920 hr^{-1}) without CO_2, and doubling time (3.031 and 3.609 hr) with and without CO_2 respectively which come quite close to what was obtained in algal culture media. The kinetics of growth rate is affected, according to these results, when these heavy metal pollutants are present with algal culture media and the type of microalgae cultivated may also have an impact on this effect. The growth of microalgae biomass was shown to be limited by the availability of carbon and resulted in a multiplication of growth rate, which agrees with a previous study (Cheng et al., 2017; Khudhair, 2017a; Mahdi et al., 2021; Makki et al., 2023) which focussed on carbon source as limiting factor for microalgae growth and leads to enhance the growth rate by many times by employment of different microalgae cultures.

The majority of microorganisms, including microalgae, experience unfavourable environmental circumstances when pure CO_2 is utilised in cultivation systems because of the quick pH drop. In order to prevent meeting, the carbon requirements of microalgae,

Table 1: Specific growth rate and generation time of *Synechococcus elongatus* in 14 days

Heavy metal	*Specific growth rate (day^{-1})*		*LSD value*	*Doubling time (hr^{-1})*		*LSD value*
Concentration	*CO_2*	*Without CO_2*		*CO_2*	*Without CO_2*	
			Pb			
0.5 ppm	0.2070	0.1785	0.057 NS	3.34	3.89	0.578 NS
1.0 ppm	0.2318	0.1526	0.068*	2.98	4.54	0.705*
2.0 ppm	0.1988	0.1558	0.055 NS	3.48	6.18	0.884*
5.0 ppm	0.2001	0.1504	0.052 NS	3.46	4.60	0.802*
10.0 ppm	0.2024	0.1538	0.057 NS	3.42	4.50	0.819*
			Cr			
0.5 ppm	0.2302	0.1263	0.071*	3.01	5.48	0.894*
1.0 ppm	0.1872	0.1429	0.055 NS	3.70	4.84	0.711*
2.0 ppm	0.2064	0.1567	0.054 NS	3.35	4.42	0.703*
5.0 ppm	0.2187	0.1195	0.076*	3.16	5.79	1.02*
10.0 ppm	0.1946	0.1141	0.064*	3.56	6.07	1.19*
			Ni			
0.5 ppm	0.2192	0.1690	0.059 NS	3.16	4.10	0.766*
1.0 ppm	0.2225	0.1755	0.057 NS	3.11	3.94	0.728*
2.0 ppm	0.2152	0.1435	0.067*	3.22	4.82	0.902*
5.0 ppm	0.2140	0.1404	0.063*	3.23	4.93	1.15*
10.0 ppm	0.2099	0.1404	0.061*	3.30	4.93	1.085*
Control	0.2286	0.1920	0.048 NS	3.03	3.60	0.602 NS

* ($P \leq 0.05$), NS: Non-Significant

the inflow CO_2 concentration must be less than the critical threshold as well. Nonetheless, it should never surpass the upper limit to avoid significant CO_2 loss, which results in needless environmental contamination since the microalgae culture releases CO_2 into the environment that the microalgae species are unable to use. To avoid significant loss of CO_2 that is released by the microalgae culture to the environment and cannot be used by the microalgae species, excessive CO_2 release should never exceed the maximum limit. Moreover, through intracellular carbon anhydrase, high concentrations suppressed the photosynthetic activity of microalgae, lowering the amount of CO_2 residues in flue gases (Mohsenpour and Willoughby, 2016). The pH of the microalgae growing medium in the photobioreactor completely correlates with the availability and solubility of CO_2 (Xiong et al., 2019).

Synechococcus elongates attained the maximum effectiveness (100%) in removing heavy metal pollutants during the fourteen days with the presence of CO_2 as shown in Figure 2, which indicates that CO_2 gas speeds up the process of removing these types of contaminants. When CO_2 gas is present, the removal capacity increases. The maximum removal efficiency was 100% at 0.5 ppm (on day 10 of cultivation for Pb and Cr) and 1.0 ppm (on day 12 of cultivation for Pb, Cr and Ni) in the presence of CO_2, and 99% at 0.5 ppm (in day 12 of cultivation for Pb and Ni) and 1.0 ppm (in day 14 of cultivation for Pb and Ni) without the presence of CO_2, suggesting a faster rate of consumption at lower doses compared to higher values. The lowest removal percentage for *Synechococcus elongatus* is 14.04% with the presence of CO_2 and 13.84% without the presence of CO_2 for Cr at 10.0 ppm on day 14 of cultivation (Table 2). The rise in biological deterioration may suggest that microalgae hydrolysing enzymes is a practical technique. Heavy metals are biodegraded by a number of enzymatic routes, including hydroxylation, methylation, nitrosation, and deamination (Cheng et al., 2019). Both the extracellular polymeric substances in particles

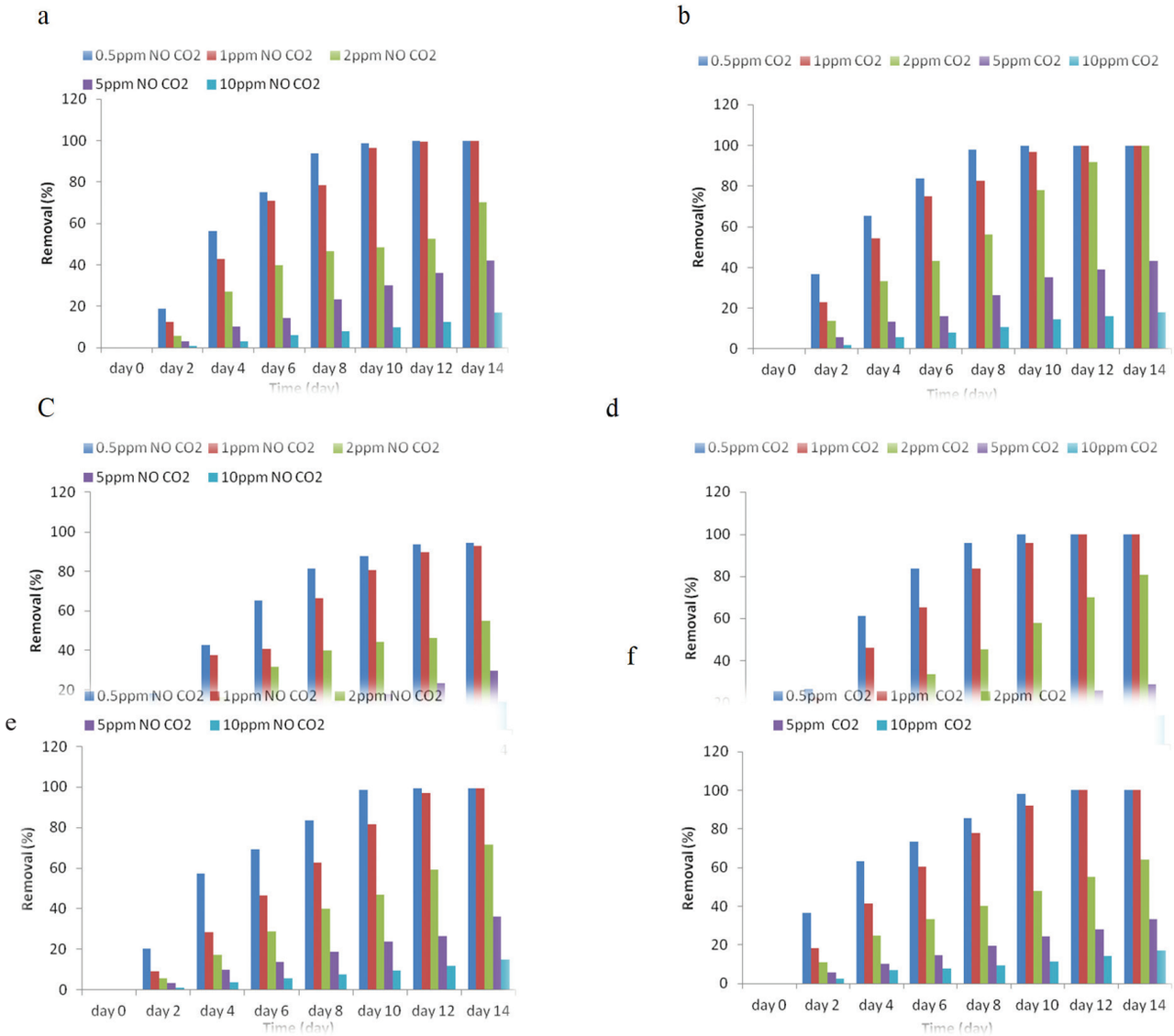

Figure 2: Heavy metal removal by *Synechococcus elongatus* without and with CO_2 (a, b) Pb, (c, d) Cr, (e,f) Ni.

and the pH of culture media have an impact on the pollutant absorption by microalgae (Anushree et al., 2017).

Conclusion

This study revealed that it was possible to successfully cultivate *Synechococcus elongatus* despite the presence of Pb, Cr, and Ni heavy metals at various concentrations (0.5, 1.0, 2.0, 5.0, and 10.0 ppm), and under a rich_CO_2 environment (three times a week, gas was bubbled at a rate of 2 L/min for 5 minutes into 50 ml volume of microalgae cultures, for 2 weeks). The experiment shows direct CO_2 influence on the development and removal effectiveness of microalgae species, compared to control groups which showed slower growth and less removal efficiency. All the concentrations of heavy metals showed an impact on algal-specific growth rate and doubling time, and the carbon-rich environment affected the growth process and removal efficiency. The highest removal percentage was 100% (on day 10 of cultivation) with the presence of CO_2 and 99.93% (on day 14 of cultivation) without the presence of CO_2 for Pb at 0.5 ppm concentration. Therefore, processes that produce heavy metal ion waste in their operations can use microalgae as an effective method to analyze their wastewater prior to releasing it into the environment or water bodies.

Table 2: Heavy metal removal by *Synechococcus elongatus* after 14 days of cultivation with the presence of CO_2

Heavy metal	*Concentration (ppm)*						
Day	*Removal (%)*						*LSD value*
	0.0 ppm	*0.5 ppm*	*1.0 ppm*	*2.0 pm*	*5.0 ppm*	*10.0 ppm*	
				Pb			
0	00.00	00.00	00.00	00.00	00.00	00.00	0.00 NS
2	00.00	36.73	22.82	13.77	05.62	01.90	6.95 *
4	00.00	65.30	54.34	33.16	13.25	05.71	8.41 *
6	00.00	83.67	75.00	43.36	16.06	08.01	8.96 *
8	00.00	97.95	82.60	56.12	26.30	10.72	8.65 *
10	00.00	100.00	96.73	78.06	35.34	14.62	9.02 *
12	00.00	100.00	100.00	91.83	39.15	16.03	8.62 *
14	00.00	100.00	100.00	100.00	43.17	18.03	8.57 *
LSD value	00.00 NS	11.26 *	10.63 *	10.05 *	8.75 *	6.38 *	---
				Ni			
0	00.00	00.00	00.00	00.00	00.00	00.00	0.00 NS
2	00.00	36.73	18.18	11.11	06.01	02.70	6.41 *
4	00.00	63.26	41.41	24.74	10.22	06.92	8.56 *
6	00.00	73.46	60.60	33.33	14.62	08.02	8.91 *
8	00.00	85.71	77.77	40.40	19.43	09.42	9.22 *
10	00.00	97.95	91.91	47.97	24.64	11.63	9.85 *
12	00.00	100.00	100.00	55.05	28.25	14.44	10.04 *
14	00.00	100.00	100.00	64.14	33.46	16.95	8.93 *
LSD value	00.00 NS	10.54 *	10.07 *	8.66 *	7.23 *	5.96 *	---

* ($P \leq 0.05$), NS: Non-Significant.

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