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Removal efficiency of lead, chromium and nickel by the cyanobacterium species: Synechoccocus elongates

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Abstract

This study investigates the impact of varying concentrations of Pb, Ni, and Cr heavy metal ions on the growth of *Synechococcus elongatus* (*S. elongatus*) and evaluates the effectiveness of *S. elongatus* in removing these heavy metal ions through biosorption, with a focus on identifying the residual ions that are not removed by this process. This study was conducted using five bioreactors filled with BG11 growth medium, each containing a different concentration of each heavy metal ion. The removal efficiency and the remaining ions of these heavy metals were determined by using an atomic absorption spectrophotometer by measuring the concentration of heavy metal ions in the initial solution and then every two days up to the end of the experiment. Maximum removal was achieved fourteen days of incubation with exposure to heavy metals at the concentrations of 0.5 ppm and 1.0 ppm (99.93% and 99.69% for Pb, 99.68% and 99.49% for Ni and 94.35% and 92.85% for Cr). At the concentration of 2.0 ppm, 70% absorption for Pb and Ni, 54.82% absorption for Cr occurred after fourteen days of incubation. At the concentrations 5.0 ppm and 10.0 ppm, microalgal growth troubled; this means that the medium was toxic.

Keywords: *Synechoccocus elongates*, Cyanobacterium, Biosorbent, Bioindicator, Wastewater

كفاءة إزالة الرصاص والكروم والنيكل بواسطة البكتيريا الزرقاء: *Synechoccocus elongates*

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الخلاصة

تبحث هذه الدراسة تاثير تراكيز مختلفة من أيونات المعادن الثقيلة: الرصاص والنيكل والكروم على نمو *S. elongatus* (*Synechococcus elongatus*) وتقييم فعالية *S. elongatus* في إزالة أيونات المعادن الثقيلة هذه من خلال الامتصاص الحيوي، مع التركيز على تحديد الأيونات المتبقية التي لم تتم إزالتها خلال هذه العملية. أجريت هذه الدراسة باستخدام خمسة مفاعلات حيوية

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تحتوي على وسط نمو 11BG، يحتوي كل منها على تركيز مختلف لكل أيون من ايونات المعادن الثقيلة. تم تحديد كفاءة الإزالة والأيونات المتبقية لهذه المعادن الثقيلة باستخدام مقياس الامتصاص الذري من خلال قياس تركيز أيونات المعادن الثقيلة في المحلول الأولي ثم كل يومين حتى نهاية التجربة. حدثت أقصى إزالة بعد أربعة عشر يومًا من الحضانة مع التعرض للمعادن الثقيلة بتركيزات 0.5 جزء في المليون و1.0 جزء في المليون (99.93% و99.69% للرصاص، 99.68% و99.49% للنيكل و94.35% و92.85% للكروم). عند تركيز 2.0 جزء في المليون، حدث امتصاص 70% للرصاص والنيكل، و54.82% امتصاص للكروم بعد أربعة عشر يومًا من الحضانة. عند التركيزات 5.0 جزء في المليون و10.0 جزء في المليون، كان نمو الطحالب الدقيقة مضطربًا، وهذا يعني أن الوسط كان سامًا.

1. Introduction:

In recent years, environmental pollution has emerged as a major concern globally, posing significant threats to human health, ecosystems, and ecological balance, while also impacting the availability of natural resources [1]. Human activity is continually contributing to increasing concentrations of different pollutants in the environment [2]. Heavy metals are commonly found in all ecosystems within organic and inorganic pollutants. All metals become hazardous at high concentrations [3]. Their buildup in the environment, their amplification through the food chain, and the risk (carcinogenic or mutagenic) they affect humans due to their non-biodegradable nature [4, 5].

Heavy metals are regarded as the most widespread class of pollutants and one of the main pollutants of freshwater due to their toxicity, persistent, unmanageable, and non-biodegradable nature [6]. Alarmingly, approximately eighty percent of wastewater that has not been treated ends up in water basins including freshwater bodies. This is contributing to global water stress which is expected to affect 60% of the world population by the year 2025 [7]. The presence of heavy metals in wastewater results from both man-made activities such as the discharge of numerous industrial metal-based manufacturing solid and liquid waste such as electroplating activity, textiles, dyes, mining, batteries, autos, and many more. Additionally, natural occurrences like volcanic eruptions or other events release Heavy metals into the environment and generate effluents with high concentrations of heavy metal [8, 9, 10]. These toxic substances can also enter the food chain and accumulate inside the human body, which hurt human health [11-13] and have negative effects on wildlife, flora, and human availability of water in both rural and urban locations [14].

Cyanobacteria, a diverse group of photoautotrophic bacteria (commonly known as blue green algae), are a diverse group of photoautotrophic bacteria that are found worldwide. They offer a wealth of biotechnological tools for sustainable development due to their metabolic variety [15]. This ability can focus on the cellular mechanisms involved and may even lead to their use as a clean, green technology for the degradation or detoxification of contaminants [16]. Numerous studies have been accomplished on the use of cyanobacteria in soil and water bioremediation [15, 17, 18, 19] this process is also known as cyanoremediation. The large-scale of cyanobacteria cultivation in ponds and their ability to fix atmospheric nitrogen and carbon dioxide (for certain genera) allow them to be self-sufficient in terms of growth, adaptation, and maintenance in contaminated or controlled environments [20]. Additionally, their high photosynthetic efficiency, large surface area, simple structure, good adsorption ability, high uptake capacity, low cost, and environmental friendliness which is further improved by their ability to withstand environmental fluctuations [20]. Furthermore, cyanobacterial biomass produced during this process can be used as a feedstock for producing a variety of bio based products with a several applications [21]. However, there remains a lack of

research focused on the value-adding of cyanobacterial biomass that results from heavy metal bioremediation [21-23].

Cyanobacteria employ various strategies, including biosorption, bioaccumulation, and biotransformation, to absorb heavy metals from contaminated environments and reduce their impact. A crucial aspect of cyanobacteria's defense against heavy metals is played by metal-binding metallothionein (MT) proteins and photoheating (PCs), enzymatic and non-enzymatic antioxidants, and enzymes that convert heavy metals into lower toxic forms [24] and Extracellular polymeric substances (EPS) which play a significant role in heavy metal absorption [25-28].

Cyanobacteria can effectively remove heavy metals from the environment by both bioaccumulation which is an active process that requires living cells, and biosorption which is a passive process that may be completed by either living or dead cell [29, 30]. Bioaccumulation is considered to be the vital mechanism for eliminating heavy metals from wastewater. A few of the processes involved in this process are ion exchange, adsorption, surface complexation, precipitation, and chelation [28]. However, to optimize adsorption capacity, several factors that are known to impact the biosorption process such as pH, temperature, the quantity of biosorbent utilized and pretreatment must be observed [31]. The ability to use the cells for several desorption/adsorption cycles, extending their shelf life and, consequently, their economic worth, is another benefit of biosorption [32, 33, 34].

Synechoccocus elongatus is a cyanobacterium species that performs as a biosorbent and can also serve as a bioindicator for the contamination of water with heavy metal ions. This study aims to examine the potential of *S. elongatus*; as a biosorbent for the removal of different concentrations of Pb, Ni and Cr heavy metals, was chosen based on research performed on sewage water of four hospitals in Baghdad, and use this method as an environmental bioindicator to analyze wastewater prior to releasing it into the environment or water bodies.

2. Material and methods

2.1 Examinations of the real sewage

Sewage samples were collected once in September 2022 from four hospitals in Baghdad: Baghdad General Hospital, Specialized General Hospital, Digestive Hospital, and Specialized Burns Hospital. These samples were taken and analyzed using an atomic Absorption spectrometer (model 7000 SHEMADZU AA) in the research center and food pollution/environment and water research at the Ministry of Science and Technology. The analyses revealed the presence of five types of heavy metals with high rates: arsenic, nickel, chromium, lead and cadmium. Nickel, chromium and lead were the highest percentage, therefore selected to verify its removal.

2.2 Preparation of Stock Solutions

Based on the initial findings from the sewage examination, stock solutions of heavy metals were prepared by using their pure salts ($Pb(NO_3)_2$, $NiCl_2$ $5H_2O$ and $K_2Cr_2O_7$) by dissolving the necessary amount of each salt in distilled water to get final concentration of 100 ppm and kept in dark until needed. Then five concentrations (0.5, 1, 2, 5 and 10) ppm of each heavy metal were prepared from stock solution. Both NaOH (0.1 N) and HCl (0.1 N) solutions were made to achieve the selected pH range for the heavy metal solution.

2.3 Isolation of Cyanobacterium species

A strain of cyanobacterium, *Synechococcus elongatus*, was utilized in this experiment. This strain of microalgae was isolated from the Iraqi environment (Tigris River), and

purified by using the serial dilution method by taking 1 mL of specimen inoculated into 9 mL of nutrient solution, and the procedure repeated many times with microscopic examination till unialgal species was obtained [35, 36].

2.4 Preparation and Sterilization of culture Media

The culture media used in this study was BG-11 which was prepared according to the Guidelines and recommendations given by (BioReady TM media, China) by dissolving 1.627 grams of BG-11 in 1 Liter of distilled water. The pH value was adjusted by using (0.1 N) NaOH or HCl to achieve the required value of pH (7). Media sterilization is an essential step in this process. This step focuses on eliminating any undesirable microorganisms to reduce contamination [37]. This was accomplished through autoclave sterilization; the autoclave took 15 minutes to reach its maximum temperature of 121 ℃ and pressure of approximately 1bar about ~ 15 pound/inch2.

2.5 Cultivation of microalgal species

Microalgal strains were grown in 500 mL sterilized BG-11 medium, incubated in 500 mL conical flask, in controlled-environment illuminated incubator with cool white fluorescent lights, with light intensity 168 μE m^{-2} s^{-1} and constant temperature (26± 2 °C), (12 h light/12 h dark) till experimental setup [38, 39].

2.6 Experimental Setup and Measurements

Laboratory experiments included preparing 500 mL volume of: control (without any change), 0.5 ppm concentration of each heavy metal, 1 ppm concentration of each heavy metal, 2 ppm concentration of each heavy metal, 5 ppm concentration for each heavy metal, and 10 ppm concentration for each heavy metal. Each conical flask contains 5 mL of algal culture media, specific volume of nutrition media (BG-11) which was prepared and sterilized to achieve a final volume of 500 mL for each heavy metal concentration (0.5, 1, 2, 5 and 10 ppm) with two duplicate to obtain more accurate results, and a control flask which contain a final volume of 500 mL without adding heavy metal stock solution. The flasks were manually shaken during growth period to prevent clumping of microalgae cells and to maintain cellular propagation [40]. All the flasks exposed to the same experimental conditions: light intensity 168 μE m^{-2} s^{-1} and constant temperature (26± 2 °C), (12 h light/12 h dark). Samples were taken at different periods throughout the period of the experiment (0, 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 6^{th}, 8^{th}, 10^{th}, 12^{th} and 14^{th} day) for the quantification of algal growth in different concentrations of heavy metals as well as for the analysis of heavy metals removal and physicochemical parameters.

2.7 Measurement of microalgal growth:

Samples of microalgal culture from each concentration were measured by using a UV spectrophotometer (APEL - JAPAN PD-303) at wavelength 730 nm during the experimental period to observe the growth kinetics from the lag phase (zero time) until the stationary phase at which the experiment was stopped.

2.8 Measurement of heavy metal concentration:

Heavy metal concentration was measured throughout the experimental period by using an atomic absorption spectrophotometer (Model 7000 SHEMADSU AA).

2.9 Measurement of removal percentage of heavy metal ions:

The removal percentage of heavy metals in the culture medium was determined by measuring the concentration of heavy metals in the initial solution and then every two days until the end of the experiment [41]. The removal percentage of heavy metals was calculated according to the following equation [42]:

$$\% \text{ Removal of heavy metal} = (C_i - C_f)/C_i \times 100\%, \quad (1)$$

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

3. Results and Discussion

Table 1 shows a decrease in the concentration of heavy metal ions as the contact time with the biosorbent increased. Throughout the prolonged exposure time, more active groups were able to bind heavy metal ions and increase the amount of metal ions absorbed, increasing the potential for interactions between the heavy metal ions and the biosorbent material. As the contact period increased, the biosorption continued until the equilibrium point was attained. The biosorbent surface's ability to bind metal ions before it reached saturation was influenced by the duration of the contact period. Due to the saturation of the cell wall surface, the biosorbent will not bind any heavy metals after it has reached the equilibrium point.

Based on Table 1 and the formula of removal percentage, Table 2 displays the computation results for the removal percentage of Pb, Cr and Ni heavy metal ions.

Table 1: Absorption of Pb, Cr and Ni heavy metal ions by *S. elongatus* with alteration in concentration through time

HM	concentrations					
Day	**0.0 ppm**	**0.5 ppm**	**1.0 ppm**	**2.0 ppm**	**5.0 ppm**	**10.0 ppm**
Pb						
0	0.00	0.48 ± 0.02	0.89 ± 0.07	1.96 ± 0.04	4.98 ± 0.02	9.89 ± 0.06
2	0.00	0.39 ± 0.01	0.78 ± 0.03	1.85 ± 0.02	4.80 ± 0.01	9.72 ± 0.02
4	0.00	0.21 ± 0.02	0.51 ± 0.02	1.43 ± 0.03	4.48 ±0.01	9.58 ± 0.01
6	0.00	0.12 ± 0.01	0.23 ± 0.01	1.18 ± 0.02	4.26 ± 0.03	9.28 ± 0.00
8	0.00	0.03 ± 0.00	0.19 ± 0.02	1.05 ± 0.01	3.82 ± 0.02	9.11 ± 0.01
10	0.00	0.00 ± 0.00	0.19 ± 0.02	1.01 ± 0.01	3.48 ± 0.00	8.92 ± 0.02
12	0.00	0.00 ± 0.00	0.00 ± 0.00	0.93 ± 0.01	3.19 ± 0.02	8.65 ± 0.01
14	0.00	0.00 ± 0.00	0.00 ± 0.00	0.58 ± 0.02	2.89 ± 0.02	8.23 ± 0.01
Cr						
0	0.00	0.49 ± 0.01	0.98 ± 0.02	1.97 ± 0.03	4.98 ± 0.02	9.97 ± 0.03
2	0.00	0.40 ± 0.02	0.85 ± 0.01	1.89 ± 0.01	4.83 ± 0.02	9.88 ± 0.03
4	0.00	0.28 ± 0.01	0.61 ± 0.02	1.65 ± 0.02	4.49 ± 0.01	9.61 ± 0.02
6	0.00	0.17 ± 0.02	0.58 ± 0.01	1.35 ± 0.01	4.40 ± 0.02	9.49 ± 0.02
8	0.00	0.09 ± 0.01	0.33 ± 0.02	1.18 ± 0.01	4.27 ± 0.03	9.18 ± 0.01
10	0.00	0.06 ± 0.00	0.19 ± 0.00	1.10 ± 0.01	4.09 ± 0.02	9.10 ± 0.01
12	0.00	0.03 ± 0.01	0.10 ± 0.01	1.06 ± 0.02	3.82 ± 0.01	8.81± 0.01
14	0.00	0.00 ± 0.01	0.07 ± 0.00	0.89 ± 0.01	3.51 ± 0.02	8.59± 0.01
Ni						
0	0.00	0.49 ± 0.01	0.99 ± 0.01	1.98 ± 0.02	4.99 ± 0.01	9.97± 0.03
2	0.00	0.39 ± 0.01	0.90 ± 0.02	1.87 ± 0.01	4.85 ± 0.02	9.85± 0.01
4	0.00	0.20 ± 0.02	0.71 ± 0.01	1.64 ± 0.02	4.52 ± 0.01	9.48± 0.02
6	0.00	0.15 ± 0.00	0.53 ± 0.02	1.40 ± 0.01	4.30 ± 0.01	9.31± 0.01
8	0.00	0.08 ± 0.00	0.37 ± 0.01	1.19 ± 0.02	4.05 ± 0.01	9.21± 0.01
10	0.00	0.00 ± 0.00	0.18 ± 0.02	1.05 ± 0.01	3.81 ± 0.02	9.04± 0.01
12	0.00	0.00 ± 0.00	0.03 ± 0.00	0.81 ± 0.02	3.66 ± 0.01	8.78± 0.01
14	0.00	0.00 ± 0.00	0.00 ± 0.00	0.36 ± 0.01	3.18 ± 0.02	8.49± 0.01

Table 2: Percentage of Pb, Cr and Ni heavy metal ions removal by S. elongatus with alteration in concentration through time.

Heavy metal	Concentration (ppm)					
Day	Removal (%)					
	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
2	00.00	18.75	12.35	05.61	03.06	00.70
4	00.00	56.25	42.69	27.04	10.04	03.13
6	00.00	75.00	74.15	39.79	14.45	06.16
8	00.00	93.75	78.65	46.43	23.29	07.88
10	00.00	98.75	96.62	48.46	30.12	09.71
12	00.00	100.00	99.32	52.55	35.94	12.53
14	00.00	100.00	100.00	70.40	41.96	16.78
Cr						
0	00.00	00.00	00.00	00.00	00.00	00.00
2	00.00	18.36	13.26	04.06	02.61	01.20
4	00.00	42.85	37.75	16.24	09.23	03.61
6	00.00	65.30	40.81	31.47	11.64	04.81
8	00.00	81.63	66.32	40.10	14.25	07.92
10	00.00	87.75	80.61	40.16	17.87	08.72
12	00.00	93.87	89.79	46.19	23.29	11.63
14	00.00	94.35	92.85	54.82	29.51	13.84
Ni						
0	00.00	00.00	00.00	00.00	00.00	00.00
2	00.00	20.40	09.09	05.55	03.20	00.90
4	00.00	57.14	28.28	17.17	10.02	03.61
6	00.00	69.38	46.46	28.78	13.82	05.80
8	00.00	83.67	62.62	39.89	18.83	07.62
10	00.00	98.77	81.81	46.96	23.64	09.32
12	00.00	99.18	96.96	59.09	26.65	11.65
14	00.00	100.00	100.00	71.71	36.27	14.84

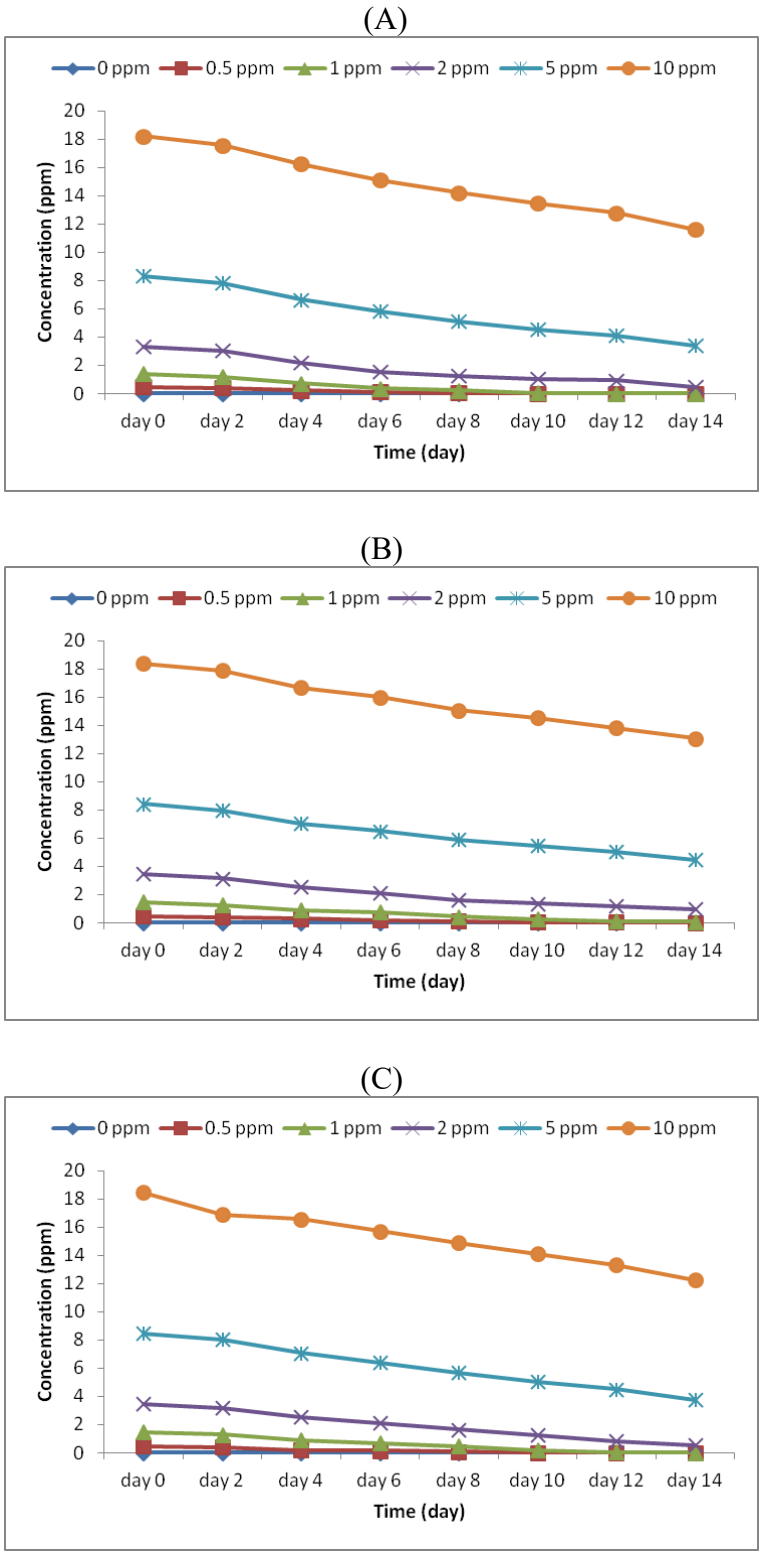


Figure 1: Pb, Cr and Ni heavy metal concentrations with respect to time: (A) Pb heavy metal, (B) Cr heavy metal and (C) Ni heavy metal.

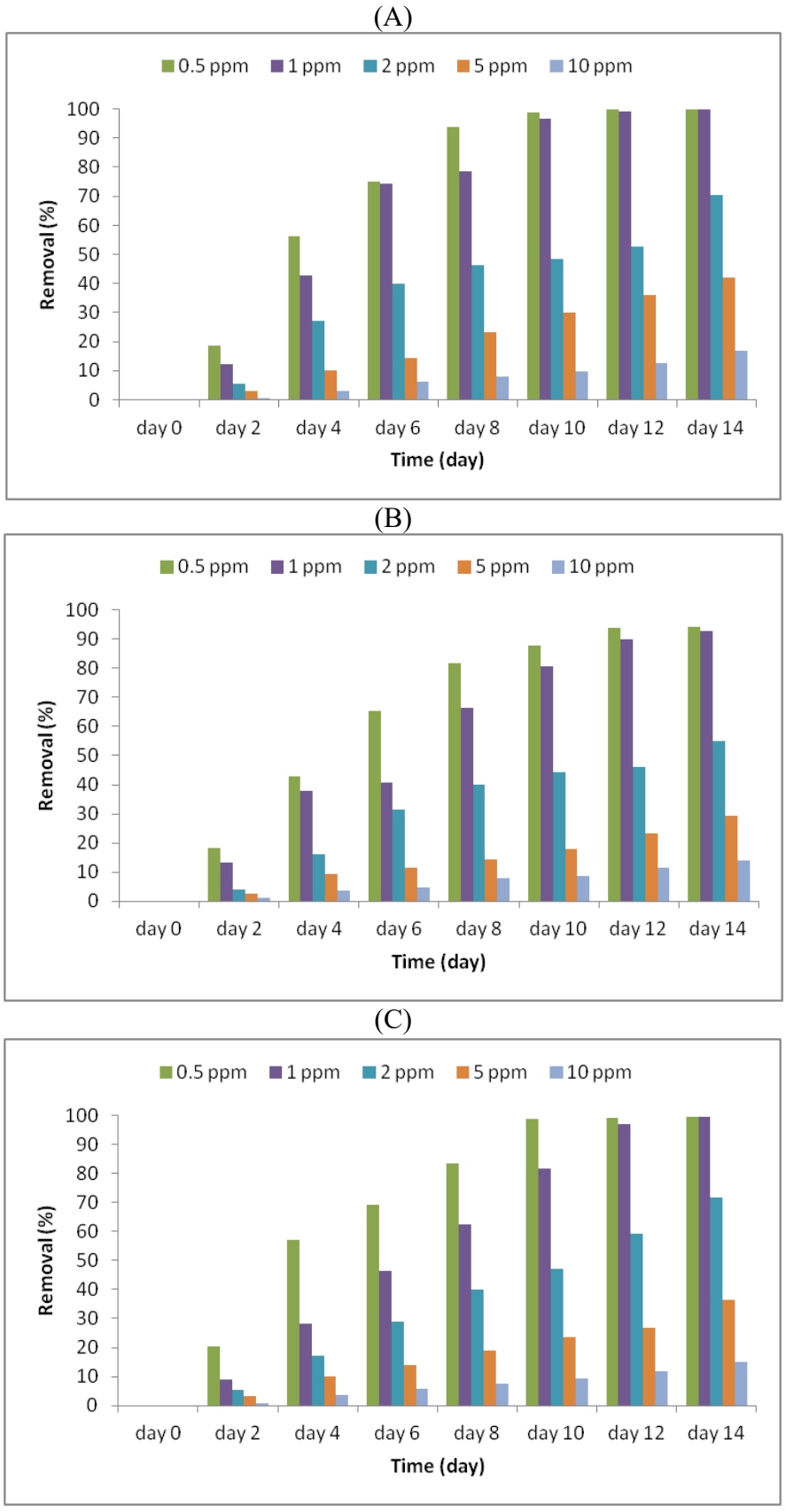


Figure 2: Pb, Cr and Ni heavy metal removal percentage with respect to time (a) Pb heavy metal, (b) Cr heavy metal, (c) Ni heavy metal.

Figures 1 and 2 demonstrate that the growth medium exposed to 0.5 ppm and 1.0 ppm of Pb, 0.5 ppm and 1.0 ppm of Ni could absorb nearly completely or 99.93%, 99.69%, 99.68% and 99.49% respectively, while the medium exposed to 0.5 ppm and 1.0 ppm of Cr absorb almost 94.35% and 92.85% respectively. These results suggesting that the microalgae could grow rapidly and proliferate in water with these heavy metal concentrations, as the growing media is not detrimental to the growth of microalgae *S. elongatus*.

At concentration of 2.0 ppm of Pb, Ni and Cr, the similar tendency to increase heavy metal ions absorption through the period of the experiment. However, the quantity of heavy metal ions absorbed was lower than the concentration of 0.5 and 1.0 ppm exposure, which meant that heavy metal ions were still absorbed but were toxic. Due to non-competitive inhibition of the metal ion cofactor required by its enzymes and an excess tolerance limit for the complex reagents that exchange metal ions from the enzyme, S. elongatus's growth was impaired when exposed to these levels of heavy metal ions [43, 44].

At concentration of 5.0 and 10.0 ppm, the absorption of Pb, Ni, and Cr significantly decreased, showing that no microbial development was taking place and the fluid was already extremely poisonous to the microalgae. The causes of the decrease in ion concentration in the growth media due to the following: first, the biosorption with bonds between metallothionein thiol groups, namely polypeptides containing approximately 30% of the amino acid cysteine [45], and secondly the non-competitive inhibitory effect of heavy metal ions to form mercaptide salts with sulfhydryl groups of enzyme proteins:

$$M + R - SH \rightleftarrows MSR + H^{+}, \quad (2)$$

where M = Metal; R = microalgal Protein radicals and SH = Sulfhydryl.

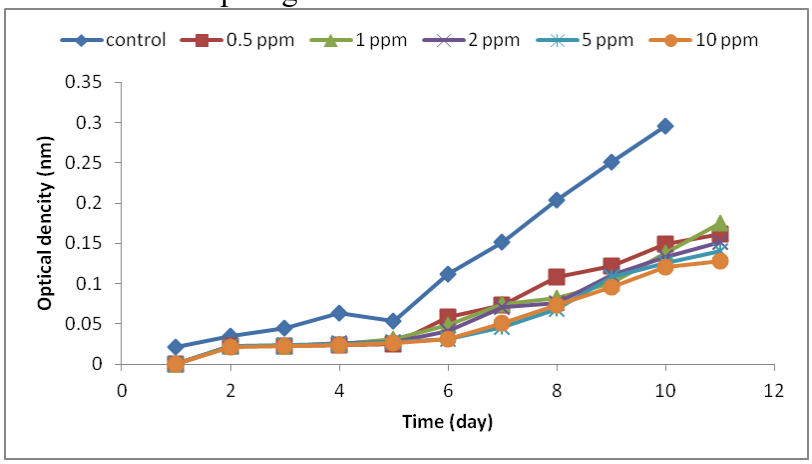
This situation prevents the enzyme from acting because it differs from the cofactor in its ability as an enzyme activator [45].

Extracellular polymers of cyanobacteria played a key role in the heavy metals biosorption, by which furthermore function as a barrier of defense from dangerous external factors. Algae's cyanobacterial extracellular polymer components include a wide range of biopolymers, contain one or two uronic acids, ester-linked acetyl groups, sulfate-containing sugars, and six or more different types of monosaccharides, they are highly anionic and differentiate them from other bacteria. The main driving force participates to EPS's position in metal sequestration is the presence of negatively charged groups that can bind to and chelate positively charged heavy metals, which include sulfate, phosphate, amide, carboxyl, hydroxyl, and carbonyl groups [25, 26, 27, 28].

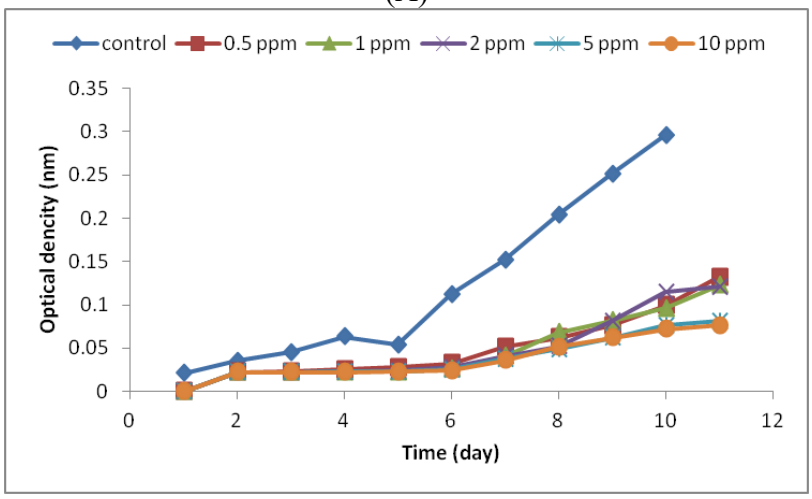
Figure 3 illustrates the vigorous growth of microalgae in the growth medium containing 0.0 ppm of heavy metal ions (control), which did not exhibit any heavy metal ion presence, and the media displayed a deep green color. During this period, the absorption process took place in the growth media contaminated with 0.5 and 1.0 ppm of Pb, Ni, and Cr from day two today fourteen. By day fourteen, the concentration of remaining ions in the growth medium was nearly reduced to 0 ppm (99.93% and 99.69% for Pb, 99.68% and 99.49% for Ni, and 94.35% and 92.85% for Cr, respectively absorbed). The cyanobacteria exhibited effective absorption at these concentrations; however, the only significant disruption observed was in their growth.

The medium exposed to 2.0 ppm of Ni and Pb, had its heavy metal concentration reduced by 70% and the medium exposed to 2.0 ppm of Cr, had its Cr concentration reduced by only about 54.82% in the growth medium after fourteen days of incubation. The growth medium exposed to Pb, Ni and Cr at 5.0 ppm experienced reductions in heavy metal concentration of approximately 41.69%, 36.27% and 29.51% respectively in growth medium after fourteen days, while the growth medium exposed to Pb, Ni and Cr at 10.0

ppm had its heavy metal concentration reduced by about 16.78%, 14.84% and 13.84% respectively which indicated poor growth of microalgae, and both concentrations (5.0 and 10.0 ppm) of these heavy metal ions have the potential to be toxic to microalgal growth and the medium show pale green color .



(A)



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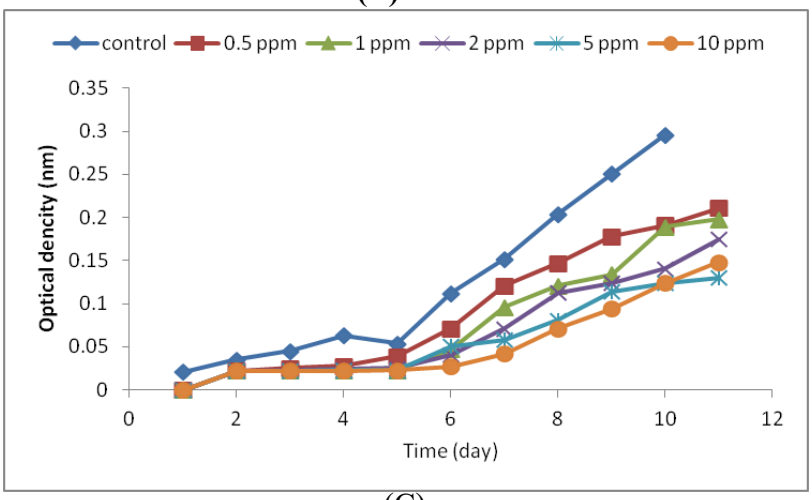

(C)

Figure 3: Growth of *Synechococcus elongatus* exposed to: (A) Pb heavy metal, (B) Cr heavy metal, (C) Ni heavy metal.

The findings of this stud could facilitate the development of a novel bioindicator instrument by organizations that generate waste streams containing heavy metal ions. The growth color of the microalgae serves as an indicator of high heavy metal waste, which displays the palest color (slowest growth). The wastewater treatment system that would enhance the stream carrying heavy metals generated by industrial activity is developed by a pond covered with *S. elongatus* microalgae. When *S. elongatus* microalgae develop rapidly and turn the water into a rich green color, the waste is ready to be released. If *S. elongatus* microalgae development is hindered and the water appears pale green or colorless, more treatment is required before it may be released.

The findings of this study may open the door for the adoption of a novel bioindicator device by facilities that generate a system for waste treatment with streams that include heavy metal ions. High level heavy metal waste was detected by the growth color of the microalgae, which exhibits the slowest growth and a lighter green color. An artificial pond populated with *S. elongatus* microalgae can enhance the wastewater treatment system that handles streams contaminated with heavy metals from industrial activities. The waste quality is appropriate for release if *S. elongatus* microalgae are growing rapidly and turning the growth color to deep green. Conversely, the water requires additional treatment before being released if the growth of *S. elongatus* microalgae is disturbed and shows a pale green color or no color at all.

Conclusions

S. elongatus microalgae show good absorption for Pb, Ni and Cr heavy metal ions with 0.5 and 1.0 ppm concentrations (99.93% and 99.69% for Pb, 99.68% and 99.49% for Ni and 94.35% and 92.85% for Cr) after fourteen days of incubation. Incubating for fourteen days in a medium with 2.0 ppm heavy metal ions resulted in absorption rates of only 70% absorption for Pb and Ni, 54.82% absorption for Cr. The mediums with 5.0 ppm and 10.0 ppm heavy metal ions were toxic to the microalgae, with very little absorption of heavy metals. Industries that produce heavy metal ion waste in their operations may use this method as an environmental bioindicator to analyze their wastewater prior to releasing it into the environment or water bodies.

5. Acknowledgment

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6. Conflict of Interest

The authors declare that they have no conflicts of interest**.**

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