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Effect of Blue-Green Algae Levels on the Bioremediation of Heavy Metals in Anbar City, Iraq

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ABSTRACT

This study investigated the capacity of two blue-green algae species, *Oscillatoria amoena* and *Nostoc linckia*, to accumulate heavy metals. Cultures were exposed to lead (Pb) and copper (Cu) at concentrations of 0.25, 0.50, 0.75, 1.0, and 2.0mg/ L for 14 days. The results showed that *O. amoena* exhibited a higher accumulation capacity for lead, reaching 1.501µg/ g dry weight at the highest treatment level (2.0mg/ L), compared with *N. linckia*, which accumulated 0.665µg/ g dry weight under the same conditions. In contrast, *N. linckia* demonstrated a greater ability to accumulate copper, reaching 1.08µg/ g dry weight at 2.0mg/ L, while *O. amoena* accumulated only 0.59µg/ g dry weight. A clear positive correlation was observed between heavy metal concentration in the medium and the amount accumulated by the algae. Statistical analysis confirmed significant differences in the accumulation of both Pb and Cu across treatments.

INTRODUCTION

Water pollution is one of the most critical environmental challenges, taking multiple forms and causing serious ecological and economic consequences. It can be defined as any alteration that degrades water quality, disrupts the balance of aquatic ecosystems, and reduces their ability to perform natural functions. Polluted water becomes harmful for use and loses much of its economic value, particularly regarding fisheries and other aquatic resources. Water pollution is often described as the contamination of rivers, lakes, oceans, groundwater, and rainwater, rendering them unsafe for humans, animals, plants, and other organisms **(Pandit & Kumar, 2019)**.

Among the various types of pollutants, heavy metals are considered the most hazardous because of their persistence, non-biodegradable nature, and ability to form stable complexes with organic and inorganic compounds in living organisms. This facilitates their accumulation within ecosystems **(Singh, 2008)**. Lead (Pb) is one of the most frequently encountered heavy metals in urban and agricultural environments. It is released in large amounts through fertilizers, detergents, paints, coatings, plastics, and

emissions from car service stations. In treated irrigation water, Pb concentrations range from 5mg/ L (long-term use) to 10mg/ L (short-term use), while plant tissue concentrations vary between 3 and 20mg/ kg **(Adriano, 1986; Kalra, 1998)**. Copper (Cu), another heavy metal, is essential in trace amounts but becomes toxic at higher concentrations. It is primarily concentrated in muscles, liver, and brain tissues, and excess levels are normally excreted through urine and feces **(Keller & Eickhoff, 1955)**.

Heavy metals exert a direct impact on algae, and their concentrations in aquatic systems depend both on environmental conditions and the biological characteristics of the organisms present. Due to increasing industrial, agricultural, and domestic discharges into aquatic environments, traditional chemical detection methods such as spectrophotometry have become insufficient. Consequently, researchers have turned to biological monitoring, using organisms such as algae as bioindicators of pollution **(Shaqweer & Abbas, 2005; Sithik *et al*., 2009)**.

Blue-green algae (Cyanobacteria) are a diverse group of photoautotrophic prokaryotes capable of oxygenic photosynthesis. They often occur in colonies of varying forms, with filamentous species dominating microbial mats in extreme environments such as hot springs, hypersaline lakes, deserts, and polar regions, while also thriving in more typical ecosystems **(Dodds *et al*., 1995; Weiss, 2006)**. Cyanobacteria are among the oldest organisms on Earth, with fossil records dating back 3.5 billion years, and play key roles in global biogeochemical cycles. They form the base of aquatic food webs and contribute significantly to global carbon and nitrogen cycling. However, some cyanobacteria produce harmful blooms that release cyanotoxins such as microcystin, saxitoxin, and cylindrospermopsin, posing threats to ecosystems, wildlife, and human health. The frequency and intensity of such blooms have been increasing worldwide **(Field *et al*., 1998; Scanlan *et al*., 2009)**.

In marine environments, cyanobacteria contribute nearly half of Earth's primary production. Certain lineages, such as *Crocosphaera* and related nitrogen-fixing species, dominate the open ocean and play critical roles in regulating productivity and carbon export by converting atmospheric nitrogen into ammonium, which is then assimilated into amino acids and proteins **(Flombaum *et al*., 2013)**.

Given their ecological importance, algae have attracted attention as agents of bioremediation. They are capable of treating wastewater by assimilating nutrients such as carbon and nitrogen derived from industrial, agricultural, and domestic waste. This makes them effective candidates for recycling pollutants and restoring ecological balance **(Sivakumar *et al*., 2010)**.

Accordingly, the present study aimed to evaluate the bioremediation potential of two species of blue-green algae, *Oscillatoria amoena* and *Nostoc linckia*, for their efficiency in removing heavy metals from aquatic environments.

MATERIALS AND METHODS

Study locations

Three study sites were chosen on Lake Habbaniyah in the city of Ramadi to complete the current study:

• **Site 1: Al-Dhuban Canal:** This canal serves as the outlet for Lake Habbaniyah, returning stored water to the Euphrates River in the summer. Its length reaches approximately 9.3 kilometers. This site was selected to represent an area with lower, more stable levels of contamination.

• **Site 2: Galaxy Channel:** This site, with its muddy banks and growth of some aquatic plants and algae, was chosen to represent a moderately contaminated area.

Site 3: Al-Warar Canal: This canal is the main inlet from the Euphrates River into Lake Habbaniyah. It is about 8.5 kilometers long and has a muddy bank with aquatic plants such as reeds. This location was specifically chosen to represent a highly contaminated area due to the significant inflow of untreated household and animal wastes.

Algae sample collection

Algae samples were collected on a monthly basis and for a full year, from January to December 2024. Water samples were collected using clean plastic bottles with a capacity of 500 cm^3 and transported to the laboratory. One part of each sample was fixed with 4% formalin for later microscopic examination, while the other part was left unfixed for cultivation.

The agricultural medium

The cultivation medium 10 (Chu) - referenced by **Al-Aaragy (1996)** was used to grow the algae *Oscillatoria amoena* and *Nostoc linckia*, which was prepared in the form of stock solutions. The medium was then sterilized using an Autoclave type Hirayama produced by Hirayama Company at a temperature of 121°C and a pressure of 1.5 psi for 20 minutes.

Algae isolation and identification

After collecting the algae samples, they were concentrated using a centrifuge at a speed of 3000 rpm. The filtrate was discarded, and the sediment was examined using an optical microscope. To obtain unialgal cultures, both the streaking method (using a sterile loop) and the dilution method was used for liquid media were used, as explained by **Stein (1973)**. The resulting isolates were then transferred to a cultivation room. Optimal growth conditions were maintained with a light intensity of 130-150 μmol m^{-2} s^{-1}, a 16:8 hour light/dark cycle, and a temperature of 25°C. The algae were subsequently identified based on the source **(Prescott, 1975)**.

Algae purification

Single algal isolates were purified from bacterial and fungal contaminants following the method of **Wedeman *et al*. (1984)** to obtain axenic cultures. The pure

isolates were maintained on Chu-10 culture medium. Transfers were made aseptically: from liquid medium using sterile pipettes and from solid medium using sterile plates. Each isolate was inoculated into sterile glass beakers, whose mouths were sealed with sterile cotton, and incubated in the algal cultivation room under controlled conditions.

Proliferation of algal isolates

To obtain sufficient algal biomass, sterile glass beakers (1000 cm^3) were filled with 700 cm^3 of sterile Chu-10 culture medium. Each beaker was inoculated with 70 cm^3 of the stored algal culture according to the method of **Stein (1973)**. Cultures were maintained under the same environmental conditions described above until adequate algal biomass was obtained for the subsequent experiments.

Preparation of heavy metal solutions

Stock solutions of lead (Pb) and copper (Cu) were prepared at 1000mg/ L by dissolving lead acetate and copper nitrate, respectively, in deionized distilled water. From these stock solutions, test concentrations of 0.25, 0.50, 0.75, 1.0, and 2.0mg/ L were prepared for each metal.

Experimental setup

Sterile 250 cm^3 glass flasks were prepared, each containing 150cm^3 of sterile Chu-10 medium. Each flask was inoculated with 15cm^3 of pure algal culture and was allowed to adapt for two days. After adaptation, the appropriate concentrations of Pb and Cu were added. Cultures were incubated for 14 days in a shaking incubator at 20 ± 2 °C with a 16:8 h light/dark photoperiod.

Determination of heavy metal concentrations

At the end of the incubation period, algal biomass was separated from the culture medium using pre-weighed Millipore filter paper (0.45µm). The filtrate was collected in clean plastic bottles for subsequent analysis of residual Pb and Cu concentrations, which were measured using a Phoenix 986 flame atomic absorption spectrophotometer with element-specific cathode lamps.

To determine metal accumulation in algal biomass, the filter paper containing algae was dried in a freeze dryer, re-weighed to obtain algal dry weight, and analyzed for Pb and Cu following the methods described by **APHA (2021)**.

Statistical analysis

Data were analyzed using a factorial design with two factors and three replicates. The first factor was algal species (*Oscillatoria amoena* and *Nostoc linckia*), and the second was heavy metal concentration at five levels (0.25, 0.50, 0.75, 1.0, and 2.0mg/ L). Treatments were randomly assigned. Statistical analyses were performed using SPSS software, and mean comparisons were conducted using the Least Significant Difference (LSD) test at a significance level of $P \leq 0.05$ **(Al-Rawi & Abdul Aziz, 1980)**.

RESULTS

• Measuring biochemical levels in the two study groups:

Table 1. Concentrations of lead (Pb) accumulated in the algae *Oscillatoria amoena* and *Nostoc linckia* and their culture medium

[Pb]	Mean ± SD				*P*-Value
	Oscillatoria amoena Dry weight mcg/g	*Oscillatoria amoena* In Agricultural medium mg/L	*Nostoc linckia* Dry weight mcg/g	*Nostoc linckia* In Agricultural medium mg/L	
0.25 (mg/L)	0.060	0.018	0.081	0.019	$P \leq 0.05$
.050 (mg/L)	0.107	0.044	0.169	0.032	$P \leq 0.05$
.075 (mg/L)	0.370	0.058	0.421	0.042	$P \leq 0.05$
1.0 (mg/L)	0. 808	0.133	0.867	0.018	$P \leq 0.05$
.20(mg/L)	1.501	0.068	0.665	0.064	$P \leq 0.05$

Table (1) presents the concentrations of lead (Pb) accumulated in *Oscillatoria amoena* and *Nostoc linckia*, as well as their corresponding culture media, across treatments of 0.25, 0.50, 0.75, 1.0, and 2.0mg/ L after 14 days of exposure. The results demonstrated that *O. amoena* accumulated the highest amount of Pb at the 2.0mg/ L treatment, reaching 1.501µg/ g dry weight, whereas *N. linckia* accumulated only 0.665µg/ g dry weight under the same conditions.

For copper (Cu), the highest accumulation was recorded in *N. linckia* at the 1.0mg/ L treatment, with a concentration of 0.867µg/ g dry weight. Under the same treatment, *O. amoena* accumulated slightly less Cu, at 0.808µg/ g dry weight.

Table 2. Concentrations of copper (µg/g dry weight) accumulated in the algae *Oscillatoria amoena* and *Nostoc linckia* and its culture medium

[Cu]	Mean ± SD				*P*-Value
	Oscillatoria amoena Dry weight mcg/g	*Oscillatoria amoena* in Agricultural medium mg/L	*Nostoc linckia* Dry weight mcg/g	*Nostoc linckia* in Agricultural medium mg/L	
0.25 (mg/L)	0.06	0.04	0.04	0.07	$P \leq 0.05$
.050 (mg/L)	0.09	0.11	0.06	0.17	$P \leq 0.05$
.075 (mg/L)	0.27	0.19	0.18	0.22	$P \leq 0.05$
1.0 (mg/L)	0.53	0.47	0.33	0.051	$P \leq 0.05$
.20(mg/L)	0.59	0.78	1.08	0.91	$P \leq 0.05$

Table (2) presents the concentrations of copper (Cu) accumulated in *Oscillatoria amoena* and *Nostoc linckia*, as well as in their culture media, at exposure levels of 0.25, 0.50, 0.75, 1.0, and 2.0mg/ L after 14 days. The results indicated that at the 2.0mg/ L treatment, *O. amoena* accumulated 0.78mg/ L of Cu in the culture medium, whereas *N. linckia* accumulated a slightly higher concentration of 0.91mg/ L in the medium.

Regarding Cu accumulation in algal biomass, *N. linckia* demonstrated the highest capacity, reaching 1.08µg/ g dry weight at the 2.0mg/ L treatment, compared with only 0.59µg/ g dry weight in *O. amoena* under the same conditions.

DISCUSSION

The results of the present study revealed clear differences between the two algal species in their ability to concentrate lead during the incubation period. Such variation in the amounts of accumulated heavy metals can be attributed to both the type and concentration of the element and the biological characteristics of the algal species involved **(Torres *et al*., 1998)**. Previous studies have shown that certain algae are capable of concentrating large quantities of heavy metals, even when these elements are present in relatively low concentrations in their environment **(Whitton *et al*., 1985)**.

The findings indicated that *Oscillatoria amoena* possessed a higher capacity for lead and copper accumulation compared with *Nostoc linckia*. This enhanced ability may be due to the larger adsorption surface area created by its filamentous structure and numerous branches **(Murugesan *et al*., 2008)**. Additionally, differences in cell membrane composition and absorption mechanisms may contribute to this variation, as *N. linckia* is comparatively smaller in size and may offer fewer adsorption sites **(Brown & Depledge, 1998)**. These observations are consistent with **Al-Shahari and Kamel (2013)**, who reported that both *O. amoena* and *N. linckia* can accumulate metals at higher concentrations within their tissues than those present in the surrounding culture media.

The study also demonstrated that increasing concentrations of lead and copper in the culture medium significantly influenced the accumulation ability of both algal species. Metal uptake generally increased proportionally with concentration, likely due to the greater availability of free ions for adsorption and intracellular binding. However, at the highest concentrations tested, a plateau or slight decline in accumulation was sometimes observed, possibly reflecting saturation of binding sites or the onset of metal toxicity, which may impair metabolic activity.

Analysis of the factorial design further revealed a significant interaction between algal species and metal concentration. This interaction showed that the response to increasing concentration was not uniform across species. *O. amoena* displayed a sharper increase in accumulation efficiency with rising metal levels, reflecting greater tolerance and adsorption capacity under heavy metal stress. In contrast, *N. linckia* exhibited a more moderate accumulation response, which may be explained by its smaller surface area and limited binding capacity.

Blue-green algae are frequently exposed to heavy metal pollution as a result of industrial, domestic, and agricultural discharges into waterways. Their persistence in contaminated environments demonstrates their ability to tolerate and adapt to high concentrations of toxic elements **(Maclean *et al*., 1972)**. Phytoplankton, and particularly cyanobacteria, are known to accumulate heavy metals efficiently. Among algal groups, cyanobacteria are generally the most sensitive to heavy metals, followed by green algae and then diatoms **(Al-Baydani, 2009; Tafej, 2014)**.

The differences observed between lead and copper accumulation in the two algae species may be due to species-specific physiological responses to heavy metals. These include variations in ion uptake across cell membranes, which regulate the entry of cations into the cell **(Sauvant *et al*., 1999)**. Moreover, selective absorption mechanisms, together with the distinct physical and chemical properties of each metal, may explain the differential accumulation patterns observed between *O. amoena* and *N. linckia* **(Wilk *et al*., 2006)**.

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