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Hexavalent chromium [Cr(VI)] tolerance and reduction activity of *Synechococcus* sp. and *Synechocystis* sp. isolated in West and South Bay of Laguna de Bay, Philippines

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Abstract. *Pujalte ECO, Posadas KDB, Morales TC, Chang ACG. 2024. Hexavalent chromium [Cr(VI)] tolerance and reduction activity of* Synechococcus *sp. and* Synechocystis *sp. isolated in West and South Bay of Laguna de Bay, Philippines. Asian J Trop Biotechnol 21: 1-9.* Cyanobacteria are prevalent in terrestrial and aquatic ecosystems which can tolerate stress caused by heavy metals. In the Philippines, various anthropogenic activities have contributed to the heavy metal contamination in water systems. Laguna de Bay is the largest inland body of water in the Philippines that functions as a multipurpose lake; however, heavy metal contamination such as hexavalent chromium [Cr(VI)] has progressed through the years due to various anthropogenic activities. This study evaluated the capability of cyanobacterial strains isolated from Laguna de Bay to tolerate and reduce varying concentrations of Cr(VI) using different parameters. Cyanobacterial isolates from Tadlac and Jamboree Lake were subjected to tolerance assay in varying Cr(VI) concentrations, followed by the reduction assay utilizing 1,5-Diphenylcarbazide (1,5-DPCZ) at OD_{540}. Through morphological characterization, two genera were identified: *Synechococcus* sp. from West Bay and *Synechocystis* sp. from South Bay. This study revealed that both isolates could tolerate and reduce high Cr(VI) levels within optimum pH of 7 and 8, respectively. The data acquired from the tolerance assay showed that a Cr(VI) concentration of 1000 mg/L still permitted the growth of the two cyanobacteria genera. Percentage reduction of the isolates at their respective optimal pH showed variation wherein *Synechococcus* sp. at pH 7 exhibited a 58% Cr(VI) average reduction compared to *Synechocystis* sp. at pH 8, which then exhibited a 66% Cr(VI) average reduction. The present study's findings indicate the potential of the two indigenous cyanobacteria in the bioremediation of Cr(VI) in Laguna de Bay.

Keywords: Bioreduction, biosorption, Cr(VI), cyanobacteria, tolerance

INTRODUCTION

Microorganisms such as cyanobacteria have long been acknowledged for their ability to remove heavy metals in terrestrial and aquatic environments. These microorganisms undergo oxygenic photosynthesis, allowing them to amalgamate and produce algal blooms in extreme environmental conditions (Huertas et al. 2014). Cyanobacteria are cost-effective, eco-friendly, low maintenance, and fast-growing; thus, they are excellent agents in bioremediation (Kulal et al. 2020). Aside from being highly diverse organisms, cyanobacteria are also known to inhabit various aquatic systems, such as those with heavy pollution, owing to their simple growth requirements and ability to acclimate to changing environmental factors (Abed et al. 2009).

Cyanobacteria, under the presence of heavy metals in a water source, can experience an exerted impact on their physiological processes; however, these species can adopt strategies at the cellular and molecular level to be able to combat the stress caused by heavy metal ions (Al-Amin et al. 2021). In addition, the ability to excrete heavy metal ligands, allow cyanobacteria to adapt to high metal concentrations (Huertas et al. 2014).

Heavy metals are a group of metals and metalloids characterized by their relatively high atomic number and atomic density, such as Mn, Pb, As, Cr, and Cu (Raychaudhuri et al. 2021). Even in low concentrations, most heavy metals are classified as toxic, carcinogenic, and naturally mutagenic. During prolonged exposure, humans and animals can acquire heavy metal poisoning from contaminated sources via dermal contact, inhalation, and consumption of contaminated food (Kumar and Bharadvaja 2020). Due to their toxicity, heavy metals are known environmental pollutants, and contamination can be transmitted to humans by consuming contaminated organisms such as Nile tilapia (*Oreochromis niloticus*) (Alam et al. 2019). Heavy metal pollution from anthropogenic activities such as agriculture and urban runoff increases public health risks due to their persistence in aquatic systems and bioaccumulation in aquatic flora and fauna (Ahmad et al. 2020).

Moreover, with the increase of pollutants in both aquatic and terrestrial ecosystems, biotechnologists and researchers innovated the use of bioremediation–a process in which chemical reactions facilitated by microorganisms and/or biological organisms reduce or transform contaminants into less toxic forms; this process can either be performed in situ or in vivo (Fennell et al. 2011).

Cyanobacteria have a high binding affinity to metal ions due to the presence of negatively charged groups which act as metal-binding sites. Hence, these microorganisms are expected to be effective bioremediation agents against heavy metals as they can detoxify wastewater through phytoremediation (Al-Amin et al. 2021).

In the Philippines, Laguna de Bay is Southeast Asia's largest freshwater lake and the third largest freshwater lake (LLDA 2016). The lake offers importance for its unique ecosystem and high economic importance; however, its water quality has continuously declined due to waste contaminants such as heavy metals brought by anthropogenic activities along its tributary rivers (Sacdal et al. 2022). Heavy metal runoff has been one of the main pollutants of surface and groundwater in areas with high urbanization and industrialization, especially in the western and southern parts of the lake (Vardhan et al. 2019). The presence of metal ion pollutants enabled several species of microalgae to develop resistance against metal ions found in Laguna de Bay (Rai et al. 1981; Nacorda et al. 2007).

Therefore, with the potentiality of cyanobacteria isolated from the West and South Bays of Laguna de Bay as a phytoremediation agent, this present study intends to detect and further investigate its capability to thrive in harsh environments, particularly with Cr(VI) exposure. Lastly, this study aims to evaluate the biosorption activity of the harvested cyanobacterial cultures in varying Cr(VI) concentrations and pH levels. These objectives prompt the need to conduct the study and further evaluate the nature of cyanobacterial strains as phytoremediation agents.

MATERIALS AND METHODS

Sampling site

The sampling site was in Laguna de Bay (14.3935° N, 121.1939° E), Philippines, particularly in the West Bay (Jamboree Lake; 14°23′8″N 121°2′8″E) and South Bay (Tadlac Lake; 14°10′57″N 121°12′23″E) (Figure 2) which are active sites for industrial, household, and agricultural pollutants. Laguna de Bay is known to be the largest inland body of water in the Philippines, having a total surface area of 900 km^2 with a highest elevation point of 12.50 meters and lowest elevation point of 10.50 meters (LLDA 2016).

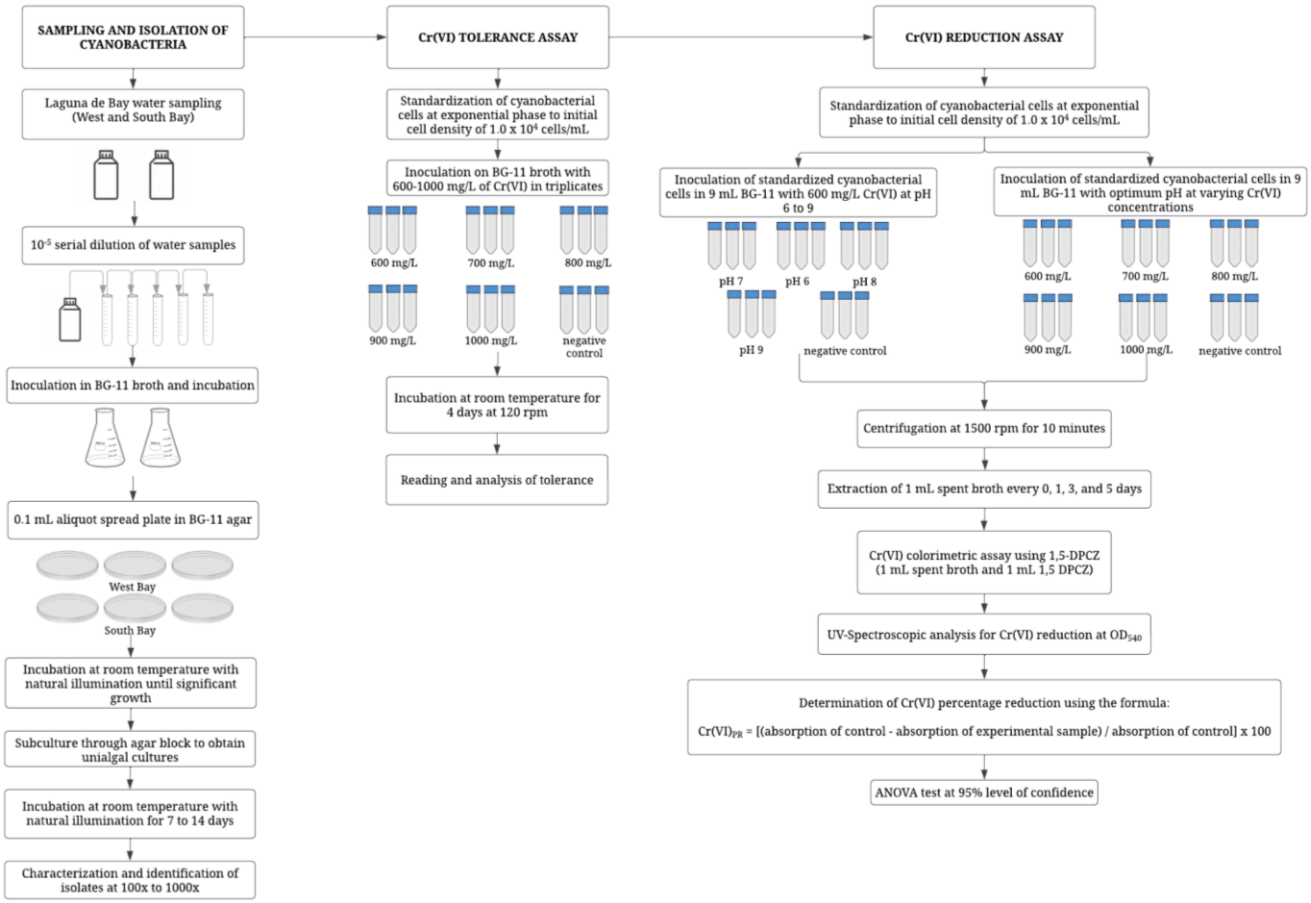


Figure 1. An experimental framework for the Cr(VI) tolerance and reduction activity of *Synechococcus* sp. and *Synechocystis* sp. isolated in the West and South Bay of Laguna de Bay, Philippines

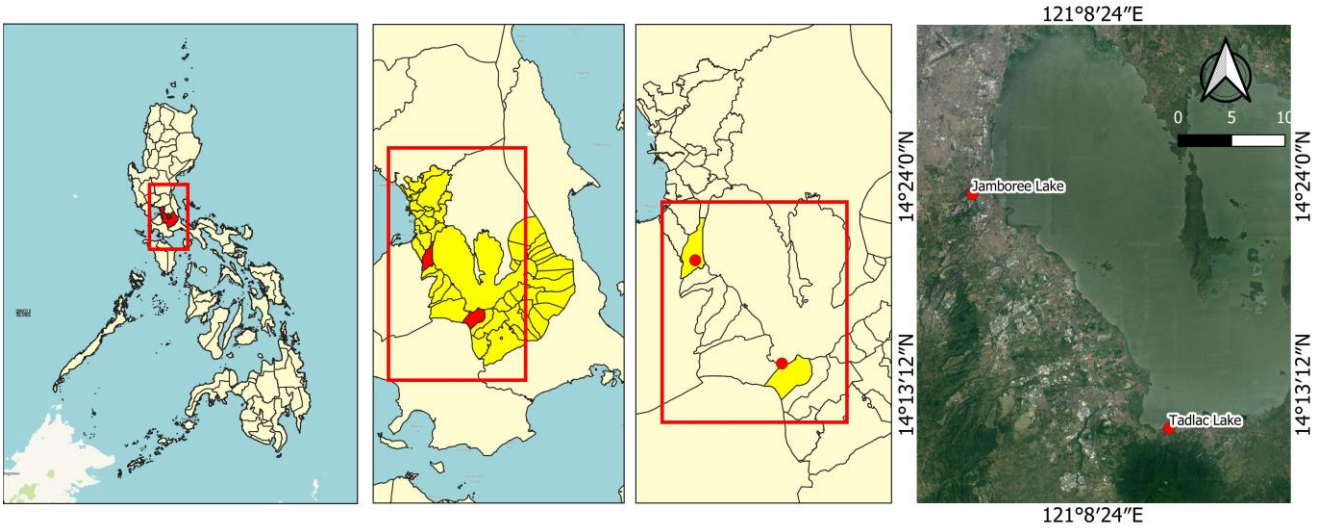


Figure 2. Map of Laguna de Bay, Philippines, and the sampling sites of the cyanobacterial strains: Jamboree Lake, Muntinlupa, and Tadlac Lake, Los Baños, Philippines

Standard sampling protocols

The standard procedure for sampling cyanobacteria by the Center for Freshwater Biology (2010) was followed throughout the collection. Water sampling was conducted in October, mid-day between 10 AM and 3 PM. It was collected in Jamboree Lake (West Bay) and Tadlac Lake (South Bay) at a 5-20 cm depth using a plastic bucket, which was then transferred into four 500 mL sterile HDPE bottles with proper labeling. The presence of visual surface "blooms" of cyanobacteria served as an indication of cyanobacterial accumulation in the location. Handling of water samples was conducted aseptically. Lakewater pH from both sampling sites was determined in duplicates using pH test strips.

Isolation and characterization of cyanobacteria species

The isolation method was derived with modification from De Sotto et al. (2015) study. One milliliter (1 mL) of the collected water sample was serially diluted to 10^{-5} (Figure 1). The standard formula for BG-11 was used for broth and plate media preparation that contained: 1.5 g/L $NaNO_3$, 0.04 g/L K_2HPO_4, 0.075 g/L $MgSO_4•7H_2O$, 0.036 g/L $CaCl_2•2H_2O$, 0.006 g/L citric acid, 0.006 g/L ferric ammonium citrate, 0.001 g/L $Na_2•EDTA•2H_2O$, 0.02 g/L Na_2CO_3, 2.86 g/L H_3BO_3, 1.81 g/L $MnCl_2•4H_2O$, 0.222 g/L $ZnSO_4•7H_2O$, 0.39 g/L $NaMoO_4•2H_2O$, 0.079 g/L $CuSO_4•5H_2O$, 0.0494 $Co(NO_3)_2•6H_2O$ (Kirrolia et al. 2012). Adjustment of pH for broth and plates via the addition of sterilized 0.1 M NaOH and/or 0.1 M HCL was performed to maintain a pH value of 7-8. Next, 1000 µL serially diluted water samples were extracted from the BG-11 broth and plate cultures for incubation and characterization. Prepared samples were done in triplicates and incubated at room temperature for 7-14 days.

A natural illumination cycle was followed during the cultivation. After successful cultivation, cyanobacterial colonies were purified via spread plate inoculation and agar block method. Inoculated cyanobacterial isolates were then viewed through a compound light microscope at 100x to 1000x magnification and identified through the use of dichotomous keys from Nienaber and Steinitz-Kannan (2018) and Casamatta and Hašler (2016). After identification, one cyanobacteria genera from West Bay and South Bay were selected to evaluate Cr(VI) tolerance and reduction.

Growth studies and measurements

The cultivated cyanobacterial strains were cultured and maintained in 300 mL Erlenmeyer flasks using BG-11 broth medium in pH 7-8 and were agitated at 100-120 rpm using an orbital shaker at room temperature under natural illumination. Growth of the samples was measured using a UV-visible spectrophotometer (at 650 nm every 5 days) and terminated for microscopic examination of the cells and cell quantification using a hemocytometer and a compound light microscope.

Cr(VI) tolerance

Nine (9) mL of BG-11 medium and 1 mL of sample from each bay at the exponential growth phase were subjected to serial dilution of $1x10^4$ cells/mL for standardization through hemocytometer cell counting. The standardized cells were then subjected to varying concentrations (600, 700, 800, 900, and 1000 mg/L) of Cr(VI) in triplicates. The prepared mixtures were placed in an orbital shaker at 100-120 rpm at room temperature for 4 days. Unexposed cyanobacterial isolates inoculated in BG-11 broth were used as a negative control. Tubes were observed for tolerance through turbidity and quantified by UV-visible spectroscopy analysis and viable cell count.

Cr(VI) reduction

One (1) mL of cyanobacterial culture from each bay was vortexed and extracted into a falcon tube containing 9 mL BG-11 medium to perform the standardization of cyanobacterial cells to a serial dilution value of $1x10^4$

cells/mL through hemocytometer cell counting. Optimization of pH for Cr(VI) reduction was done by drawing out 1 mL of the standardized cyanobacterial cells into falcon tubes containing 600 mg/L Cr(VI) with varying pH (6, 7, 8, and 9). All prepared samples were done in triplicates. The second part of the reduction assay was performed by inoculating 1 mL of standardized cyanobacterial cells into 9 mL BG-11 medium under optimum pH at varying Cr(VI) concentrations (600, 700, 800, 900, and 1,000 mg/L). All prepared samples were also done in triplicates. The prepared samples from both reduction assay parts were centrifuged at 1,500 rpm for 10 minutes. Cr(VI) colorimetric assay was performed by extracting 1 mL of spent broth on days 0, 1, 3, and 5, followed by the addition of 1 mL of 1,5-Diphenylcarbazide (0.5 g 1,5-DPCZ in 100 mL absolute ethanol and 400 mL 3.6 N H_2SO_4) to achieve a ratio of 1:1 in each solution. Those solutions were mixed by pipetting up and down before transferring to a cuvette for UV-visible spectroscopy analysis of Cr(VI) reduction at OD_{540}. One (1) mL of standardized cyanobacterial cells was inoculated into 9 mL of BG-11 medium without Cr(VI), which served as the negative control. The percentage reduction of Cr(VI) was determined using the formula (Bennett et al. 2013):

$$Cr(VI)_{PR} = \frac{(absorbance\ of\ control - absorbance\ of\ experimental\ sample)}{absorbance\ of\ control}\ x\ 100$$

The obtained percentage reduction of Cr(VI) was further evaluated using the ANOVA test at a 95% confidence level and the Tukey Honest Significant Difference (HSD) and post-hoc test.

RESULTS AND DISCUSSION

pH lake water sampling

The lake water pH from the sampling sites was measured twice, wherein the water in Tadlac Lake, Los Baños (South Bay), had a pH of ~8 during both trials, while the pH of the water from Jamboree Lake, Muntinlupa (West Bay) had a pH of ~7 on both trials (Table 1).

Species description and morphology

The cyanobacterial isolates from West and South bays, Laguna de Bay, were observed on a compound light microscope at 100x to 1000x magnification, as shown in Figure 3. Identification through morphology was done using dichotomous keys from Casamatta and Hašler (2016) and Nienaber and Steinitz-Kannan (2018).

***Synechococcus* sp.** This species was isolated from West Bay, Laguna de Bay. The cells are unicellular, rod-shaped, light-green to blue-green, and without a mucilaginous sheath.

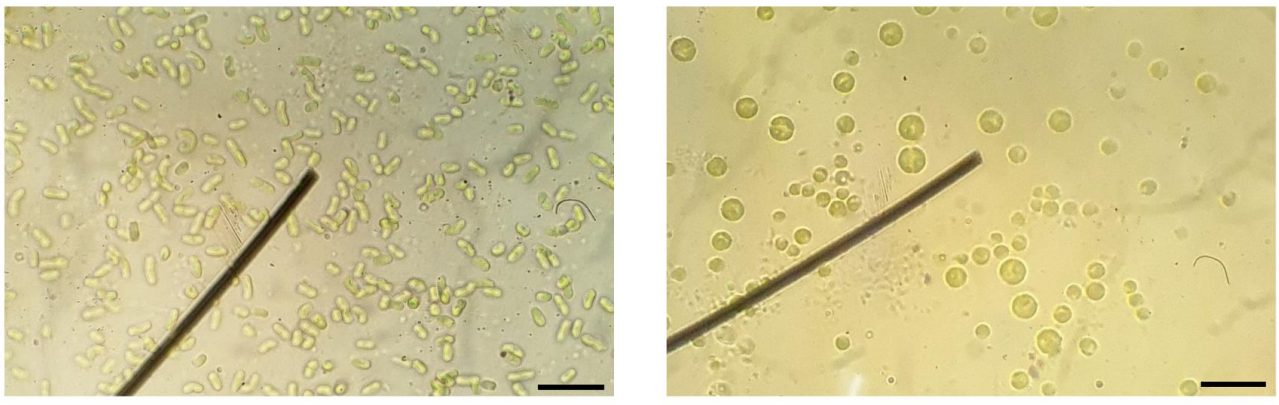
***Synechocystis* sp.** This species was isolated from South Bay, Laguna de Bay. The cells are unicellular, spherical, bright green in color, and have a colorless mucilaginous sheath.

Growth studies and measurements

Cyanobacterial cells at their exponential phase were utilized in this study. *Synechococcus* sp. and *Synechocystis* sp. were subjected to a UV-Visible spectrophotometer at 650 nm to verify their growth phase. Both isolates were found to be at their exponential phase at day 35 (Figure 4), which makes the cells viable for experimentation.

Table 1. Physico-chemical parameters in the sampling sites in Laguna de Bay, Philippines

Parameters	West Bay	South Bay
Exact location	14°23'8" N 121°2'8" E	14°10'57" N 121°12'23" E
pH	6.5 - 7.0	7.5 - 8.0



A. *Synechococcus* sp. 1000 X

B. *Synechocystis* sp. 1000 X

Figure 3. Microscopic observation of cyanobacterial isolates from A. West and B. South Bay, Laguna de Bay, Philippines (each scale bar = 10 µM; magnification = 1000x)

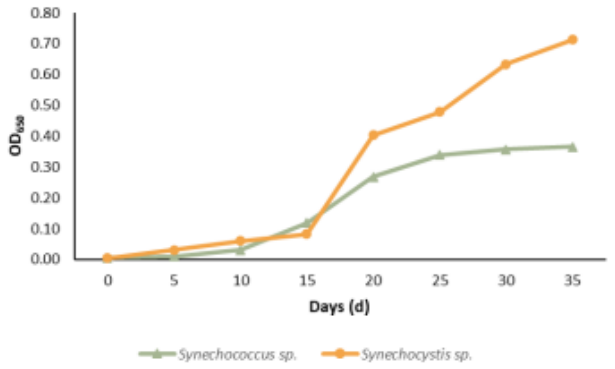


Figure 4. Growth curve of *Synechococcus* sp. and *Synechocystis* sp. isolated from West and South Bay, Laguna de Bay, Philippines

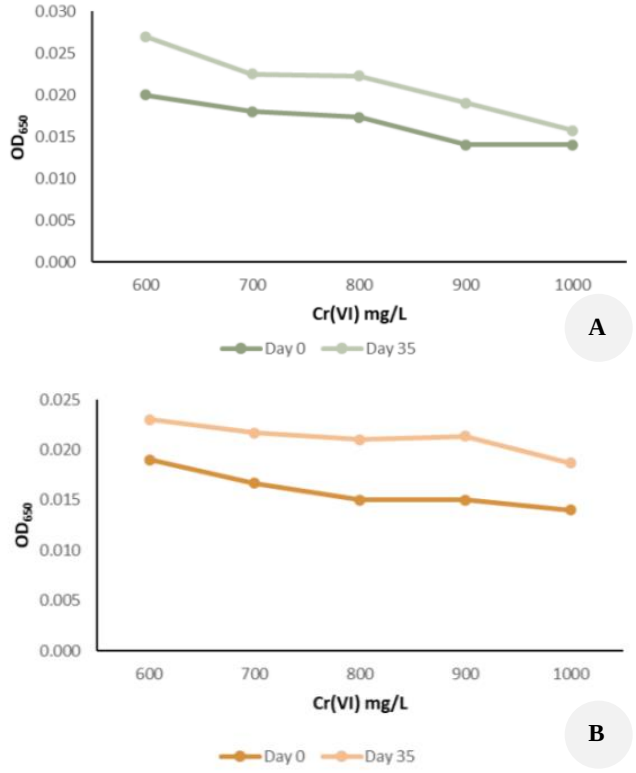


Figure 5. Comparison of Cr(VI) tolerance of A. *Synechococcus* sp. and B. *Synechocystis* sp. in varying Cr(VI) concentrations at Day 0 and Day 35

Tolerance of cyanobacterial isolates against Cr(VI)

After 35 days, the cyanobacterial isolates that were amended with varying Cr(VI) concentrations were observed wherein visible cyanobacterial growth was observed in all tubes amended with varying Cr(VI) concentrations viz. 600, 700, 800, 900, and 1000 mg/L for *Synechococcus* sp. and *Synechocystis* sp. After this, tubes were observed for turbidity, and 1 mL aliquots of each tube were quantified through absorbances at OD_{650} to indicate cyanobacterial cell growth (Figure 5.A and 5.B). From the acquired data, it can be inferred that a Cr(VI) concentration of 1000 mg/L still permitted the growth of the cyanobacterial cultures isolated from the West and South Bays of Laguna de Bay. This verifies the isolated cyanobacterial cells' tolerance capacity against heavy metals such as Cr(VI).

Optimization of pH for Cr(VI) reduction

Moreover, to assess the optimum pH for Cr(VI) reduction, the cyanobacterial isolates were exposed to varying pH levels of 6, 7, 8, and 9 using the lowest experimental Cr(VI) concentration at 600 mg/L. As shown in Figure 6, pH 7 showed the highest Cr(VI) reduction for *Synechococcus* sp. (West Bay), while pH 8 had the highest Cr(VI) reduction for *Synechocystis* sp. (South Bay). The results illustrate similarity with the pH values obtained during water collection in both bays (West Bay: pH 7; South Bay: pH 8). This may indicate that the Cr(VI) reduction of cyanobacteria may be ideal at the pH of the natural environment where the species thrived. Statistically, both isolates showed no significant difference ($p > 0.05$) in varying pH levels at 600 mg/L Cr(VI).

Cyanobacterial Cr(VI) reduction

The results showed the Cr(VI) percentage reduction of the isolates in varying heavy metal concentrations at optimal pH 7 for *Synechococcus* sp. and pH 8 for *Synechocystis* sp. isolates. There was an 8% higher Cr(VI) removal for *Synechococcus* sp. (58% Cr(VI) average reduction) over *Synechocystis* sp. (66% Cr(VI) average reduction). This can be correlated to the significant differences in Cr(VI) reduction for both isolates ($p < 0.05$); post-hoc analysis was performed for verification wherein percentage reduction between 600 mg/L and 1000 mg/L, along with 800 mg/L and 1000 mg/L of Cr(VI) concentrations showed significant differences. Therefore, it can be elucidated that Cr(VI) reduction in *Synechococcus* sp. (42-65% Cr(VI) reduction) and *Synechocystis* sp. (45-78% Cr(VI) reduction) is constant at 600-900 mg/L and starts to decrease at 1000 mg/L significantly. Additionally, Figures 8.A and 8.B show the Cr(VI) percentage reduction of both isolates from days 0 to 5, which generally shows a trend of decreasing heavy metal reduction across all Cr(VI) concentrations (600-1000 mg/L) as contact time increases (Figures 7.A and 7.B).

Discussion

Heavy metals in Laguna de Bay are caused by various anthropogenic sources such as urban and industrial activities, which are common most especially on the western side of the lake. The southern bay, specifically Tadlac Lake, is a volcanic crater lake that is now used for recreational purposes (Sacdal et al. 2022). Hexavalent chromium [Cr(VI)] is amongst these toxic heavy metals found in the lake and is considered carcinogenic. Moreover, an association between Cr(VI) exposure and lung cancer was found through inhalation (World Health Organization 2017). The study of Sacdal et al. (2022) recorded the presence of other heavy metals present in Laguna de Bay such as Ni, Co, Cu, Mn, and As. Elevated concentration of these heavy metals can resulted in

deterioration of cell morphology and also competition for essential nutrients and binding sites for enzymes and transporters being threatened, eventually causing lysis and cell death (Tottey et al. 2012; Tiwari et al. 2019; Kalita and Baruah 2023). Among the mentioned heavy metals, Cr was found to be the most distributed heavy metal in Laguna de Bay, hence the researchers opted for this to be used in the study. One of the toxic characteristics of Cr(VI) is its ability to bind extracellularly to various functional groups found in the microbial cell wall, which can damage microbial enzymes. Consequently, microbes, including cyanobacterial communities, have developed defensive systems to cope with heavy metal stress (Sharma et al. 2022). Cyanobacterial communities in mining sites in the Philippines were detected via isolation-dependent methods, which heavily suggests the potential of cyanobacteria as a phytoremediation agent due to their tolerance in extreme environments contaminated with heavy metals (Damatac II and Cao 2022).

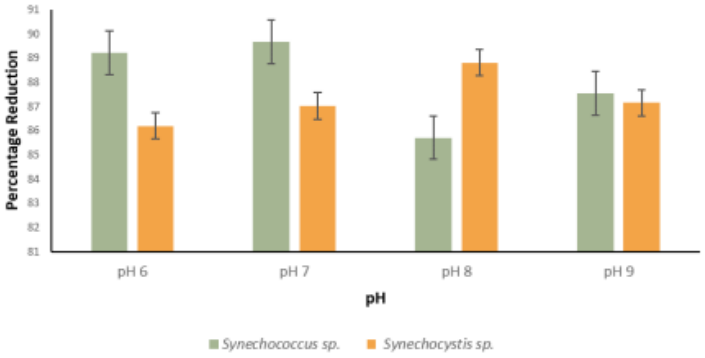


Figure 6. Cr(VI) percentage reduction of *Synechococcus* sp. and *Synechocystis* sp. in varying pH levels at 600 mg/L Cr(VI)

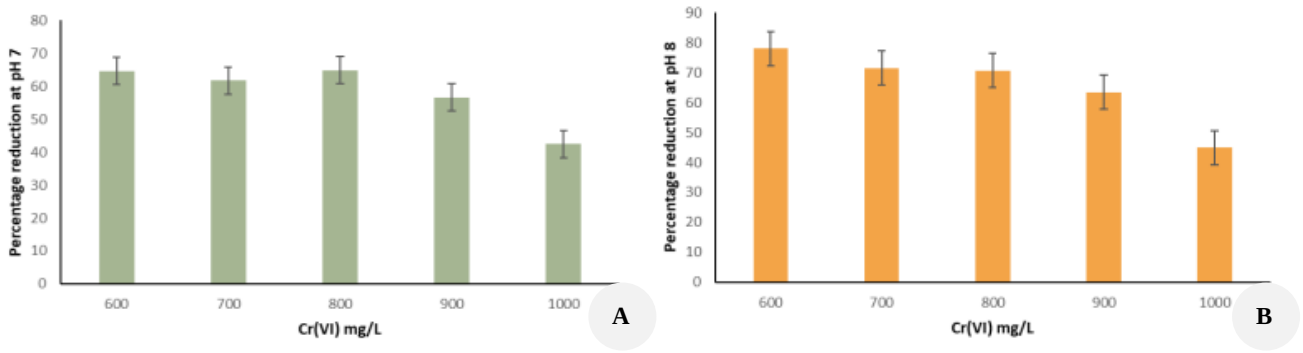


Figure 7. A. Cr(VI) percentage reduction of *Synechococcus* sp. in varying Cr(VI) concentrations at pH 7 and B. Cr(VI) percentage reduction of *Synechocystis* sp. in varying Cr(VI) concentrations at pH 8

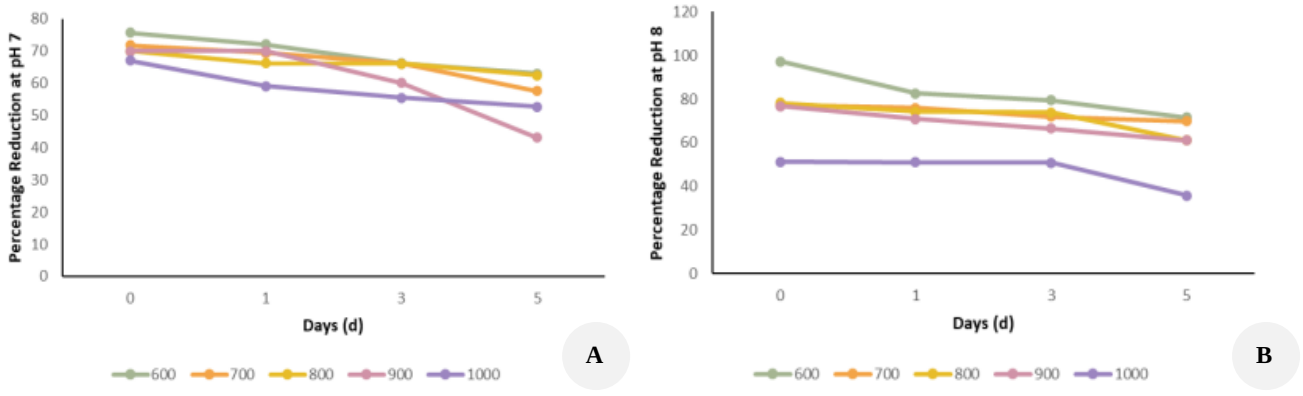


Figure 8. A. Cr(VI) percentage reduction of *Synechococcus* sp. at pH 7 from Day 0 to 5 and B. Cr(VI) percentage reduction of *Synechocystis* sp. at pH 8 from Day 0 to 5

Cr(VI) is known to cause oxidative stress; hence, it is essential to understand cyanobacterial cells' ability to tolerate Cr(VI) levels. The characteristics of cyanobacteria, such as their ubiquity, rapid growth rate, simple growth conditions, and heavy metal tolerance and removal, contribute to these microorganisms' potential as bioremediation agents. Tolerance to heavy metals such as Cr(VI) can be influenced by the type of cyanobacterial species used. Additionally, *Synechococcus* sp. was found to be the most sensitive to Cr(VI); however, sensitivity gradually decreased as the cyanobacterial strains acquired tolerance to heavy metal ions due to prolonged exposure (Munagamage et al. 2016). This further implicates the existence of specificity regarding the sensitivity of cyanobacteria to different types of heavy metal ions present in the environment.

Laguna de Bay is one of the most important freshwater lakes in the country due to it being a multipurpose lake, however, the presence of heavy metal contaminations such as Cr(VI) can potentially threaten its function. In this study, the different cyanobacterial strains, *Synechococcus* sp., and *Synechocystis* sp., were amended with varying Cr(VI) concentrations to determine their tolerance against the heavy metal. The test result showed that at 1,000 mg/L of Cr(VI) concentration, cyanobacterial isolates from both bays could still grow under such harsh conditions (Figures 5.A and 5.B). Along with this, previous studies suggested that *Synechococcoccus* and *Synechocystis* cultures can tolerate high levels of Cr(VI) concentrations, wherein *Synechococcus* showed twice the number of cells compared to *Synechocystis* while both were found to be photosynthetic-dependent. Additionally, *Synechococcus* had higher photosynthetic activity and stronger oxidative stress tolerance mechanisms (Gupta et al. 2013; Khattar et al. 2014; Gupta and Ballal 2015; Gupta et al. 2021).

It is also important to note that since the cultures were isolated from polluted environments, the possibility that the organisms have adapted to heavy metal toxicity and their continuous exposure to heavy metals, such as chromium, may have led them to tolerate high levels of concentrations (Khattar et al. 2014). This further verifies the alarming chromium contamination in the surface water of Laguna de Bay, particularly in West Bay and South Bay, along with the potentiality of cyanobacteria to pave the way for in-situ bioremediation of heavy metals in the environment (Al-Homaidan et al. 2015; Kwak et al. 2015; Cui et al. 2020).

Among the mechanisms that enable these species to thrive under harmful conditions and maintain cellular homeostasis is their ability to perform biosorption. In recent studies, ion exchange has become an emerging factor concerning the factors that influence the biosorption process of these microorganisms. During the process of ion exchange in cyanobacteria, proton and metal ions compete with each other for the binding site, which is governed by the endogenous pH of the cell (Schiewer and Volesky 2000; Tiwari et al. 2019).

Chromium is known to have a pH-dependent equilibrium in aqueous solutions (Mendam et al. 2022). The pH facilitates the metal binding in aqueous mediums (Dixit and Singh 2014). This study investigated the effects of varying pH levels 6, 7, 8, and 9 regarding the Cr(VI) reduction efficiency of *Synechococcus* sp. and *Synechocystis* sp. at 600 mg/L (Figure 6). It was found that the highest Cr(VI) reduction was at pH 7 for *Synechococcus* sp. (90%) and at pH 8 for *Synechocystis* sp. (88.8%); this may be attributed to the favorable growth conditions of the cyanobacterial biomass in the respective pH levels. *Synechocystis* sp. possesses heavy sheaths known to be chelating agents for the biosorption of positively charged heavy metal ions in aqueous solutions (De Philippis et al. 2011). On the other hand, *Synechococcus* sp., though lacking the presence of a heavy sheath, has also been demonstrated to have biosorption abilities towards heavy metal ions such as lead and uranium (Acharya et al. 2008; Anburaj et al. 2020). At a higher pH, there are fewer concentrations of H^+ ions compared to low pH, resulting in the reduced competition of H^+ ions and Cr(VI) ions existing in the solution that would bind in the cellular structure of the cyanobacteria (Sen et al. 2018). In addition, with increased pH, adsorbing sites may undergo deprotonation due to low concentration of H^+ ions that causes cation exchange of H^+ with Cr(VI) ions, allowing better metal removal capability (Miranda et al. 2012; Sen et al. 2018). This may explain why the present study's optimum Cr(VI) reduction for obtained cyanobacterial isolates was at pH 7 and 8. A trend was observed in several studies wherein pH beyond the optimum results in a decrease in metal adsorption due to the formation of hydroxylated complexes of the metal that would compete with the active adsorbing sites of cells (Volesky and Holan 1995; Pardo et al. 2003; Gabr et al. 2008).

In this study, it was also found that at optimum pH (7 and 8), there was efficient Cr(VI) reduction from *Synechococcus* sp. (42-65%) and *Synechocystis* sp. (45-78%) at Cr(VI) concentrations of 600-1,000 mg/L (Figures 7.A-B). At the highest concentration of 1000 mg/L, there was a significant decrease in Cr(VI) reduction. It can be inferred that cyanobacterial isolates can effectively reduce Cr(VI) at their respective optimum pH; however, there is a specific maximum value of initial Cr(VI) wherein isolates can effectively bind Cr(VI) ions existing in the solution, beyond this threshold value can have detrimental effects to the organism leading to reduced adsorption (Sen et al. 2018). An increasing number of studies have been conducted on the subject of transformation of the water-soluble and eminently toxic chromium (VI) to a less toxic form and insoluble chromium (III) (Shukla et al. 2012). Furthermore, as shown in Figures 8.A and 8.B, the percentage reduction of varying Cr(VI) concentrations generally decreased from days 0 to 5 for both isolates. Similar trends were observed from Gupta and Rastogi (2008) and Sen et al. (2018), wherein there was a gradual decrease in Cr(VI) biosorption as contact time increased. This may be due to the uptake of Cr(VI) on the adsorption sites of the cell surface; as metal ions progressively cover more sites, the adsorption rate becomes slower until equilibrium uptake is achieved (Miranda et al. 2012). The results of the present study show that *Synechococcus* sp. and *Synechocystis* sp. isolated from Laguna de Bay possess

a high reduction capability for Cr(VI) at optimum pH 7 and 8 and may be efficiently used as biosorbents for the removal of heavy metal from aquatic systems. Various studies from other countries presented the tolerance and removal capacity of *Synechocystis* sp. wherein Ozturk et al. (2009) concluded that the cyanobacterial exopolysaccharide from cyanobacteria isolated in Mogan Lake and Bafa Lake, Turkey, including its monomer components, contributes to its tolerance and reduction capacity. Additionally, *Synechocystis* strains isolated from Lahore, Pakistan, showed chromium resistance, reduction, and the production of non-protein thiols when exposed to chromium stress (Hameed and Hasnain 2012). On the other hand, the Cr(VI) tolerance and bioreduction capacity of *Synechococcus* sp. has not been widely studied.

This study revealed *Synechococcus* sp. and *Synechocystis* sp. isolated from the West and South Bays of Laguna de Bay, Philippines, to tolerate and efficiently reduce Cr(VI) under unfavorable conditions. As such, the study provided further evidence regarding the potential of cyanobacteria as a phytoremediation agent, particularly against heavy metal pollution such as Cr(VI). Furthermore, the isolates can be used in biotechnological applications to reduce heavy metal pollutants in contaminated aquatic environments. Cyanobacterial species can be genetically modified to increase and enhance growth, photosynthetic efficiency, and tolerance against environmental factors (Priyanka et al. 2020). Furthermore, the photoautotrophic capacity of cyanobacteria makes them advantageous as a long-term bioremediation agent due to the absence of secondary pollution during the reutilization cycle of biomass (Mulbry et al. 2008; Brar et al. 2017). However, the study by Al-Amin et al. (2021), highlighted that the use of cyanobacteria as bioremediation agents can jeopardize indigenous aquatic systems if toxic cyanobacterial species are used.

However, this study is only limited to the isolated cyanobacterial strains viz. *Synechococcus* sp. and *Synechocystis* sp. were isolated in the West and South Bays of Laguna de Bay, Philippines. Hence, using other cyanobacterial strains from other parts of the lake could further help assess the potentiality of cyanobacteria in tolerating and reducing Cr(VI). Other heavy metals could also be assessed to verify further their capability in reducing heavy metal pollutants. In addition, the measurement of Cr(VI) uptake and the effect of the inoculum size are also important parameters to be considered for heavy metal reduction which could be done in future studies.

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