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Metal tolerance of *Spirulina platensis*

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ABSTRACT

Microorganism-based bioremediation is a well-sought after method for industrial wastewater treatment and forms the primary stage. The current research suggests using *Spirulina platensis* as an organism of choice for bioremediation. This study provides an insight into the potential use of primary-treated wastewater as the growth media for *Spirulina platensis.* The tolerance of *S. platensis* was confirmed for metals such as mercury (Hg), cadmium (Cd), manganese (Mn), zinc (Zn), and copper (Cu) by using media enriched with these metals. *S. platensis* was most tolerant to Hg followed by Cd and Cu. Further, it is suggested that the biomass and bioactive compounds extracted from *S. platensis* be tested for their application in animal and aquaculture feed, supplements, and pharmaceuticals.

1. Introduction

Improper discharge of waste causes heavy metal contamination that causes detrimental and long-term effects on the ecology and human population (Wang and Chen 2009; Priyadarshini et al. 2019). Heavy metals cannot be removed entirely from the system but can be biotransformed (Juwarkar et al. 2010). Bioremediation, identified as the biological deterioration of contaminants, intensifies this process (Gouma et al. 2014).

There has been a shift in the focus to organisms capable of biosorbing toxic compounds from their environment (Dhankhar and Hooda 2011) - to overcome the drawbacks of existing technologies. Thus, bioremediation is environment-friendly and cost-effective. *Spirulina* (*Arthrospira*) *platensis* (Gomont 1892), a filamentous cyanobacterium, is recognized as a nutritious food supplement, alongside being a source for commercially valuable bioactive compounds, phycocyanin (Güroy et al. 2017; Paramanya et al. 2019). This cyanobacterium thrives in a high-salt environment, tolerant of osmotic stresses (Usharani et al. 2012).

Biosorption is a physio-chemical process involving the passive uptake and accumulation of toxicants (heavy metals) by biological materials (usually dead or inactive) from their surroundings (Yan and Viraraghavan 2003). Microorganisms have evolved many bioprocesses to exploit the chemical properties of selectively acquired metals for catalyzing reactions and maintaining protein structure (Murali and Mehar 2014).

In wastewater management, the dry biomass of *S. platensis* is used for precipitation (Fariduddin et al. 2018) and biosorption of heavy metals (Ahmad et al. 2010; Michalak et al. 2020) at low cost (Rangsayatorn et al. 2002). *Spirulina* sp. is effective in bioremediating water polluted with petroleum hydrocarbons (Ciferri 1983), pesticides (Khan et al. 2005), some estrogens (Shi et al. 2010), radioactive elements (Fukuda et al. 2014), and fluoride ions (Tabagari et al. 2019). Dead biomass is preferred for several reasons: high tolerance to toxic heavy metal ions, no necessary nutrient supply, and no limiting culture conditions (Kőnig-Péter et al. 2014). However, little is known about the use of its live biomass for biosorption.

Culturing *S. platensis* in low metal concentrations can potentially be used for tertiary treatment for metal-contaminated effluent (Murugesan et al. 2008). Commercially grown and consumed *Spirulina* supplements have traces of inorganic elements and heavy metals at concentrations that do not exceed the present regulation levels; if appropriate measures are taken, it can be considered safe food (Al-Dhabi 2013). This application has the potential to combat the issues of contaminated biomass. In addition, the efficiency of *S. platensis* in the biosorption of heavy metals makes it a potential organism for cost-effective and environment-friendly wastewater treatment.

Lu et al. (2000) suggested that mercury inhibits the quantitative photosynthetic yield of cyanobacteria. Cu and Zn directly affect photosynthetic pathways, leading to a decrease in cell growth (Lone et al. 2008). Therefore, changes and variations in biomass yield and chemical composition of *Spirulina* sp. are considered when bioactive compounds are extracted or used as dietary supplements or fertilizers.

This pilot study aims to verify the growth of *Spirulina platensis* and its tolerance to heavy metals in wastewater. Preliminary experiments were conducted to standardize and optimize the growth conditions of *S. platensis*, in turn establishing a growth curve.

2. Materials and Methods

2.1. Procurement and cultivation of Spirulina platensis PCC 7345

The strain of *S. platensis* PCC 7345 was procured from BITS Pilani, India. The pure culture was sub-cultured in the Blue-Green 11 (BG11) medium (Dineshkumar et al. 2016) and grown under photoautotrophic conditions - 28°C in constant white light (pH 7.2) - on a shaker.

2.2. Determination of Standard Growth Curve

To standardize growth, *S. platensis* was grown in triplicate in 250-mL flasks containing 200 mL BG11 culture medium (Table 1) for four weeks. 500-µL inoculum was added to the medium at an initial cell count of $2.5x10^4$ cells mL^{-1}.

Table 1. Composition of Blue-Green 11 (BG11) medium (Adapted from Moghazy 2019)

Macroelement nutrients	Concentration (g L^{-1})
$NaNO_3$	1500.00
K_2HPO_4	40.00
$MgSO_4.7H_2O$	75.00
$CaCl_2.7H_2O$	36.00
Citric Acid	6.00
Na_2CO_2	20.00
Na_2EDTA	1.00
Ferric ammonium citrate	6.00
Microelement nutrients	**Concentration (mg L^{-1})**
H_3BO_3	2.860
$MnCl_2.4H_2O$	1.810
$ZnSO_4.7H_2O$	0.222
$Na_2MoO_4.7H_2O$	0.390
$CuSO_4.5H_2O$	0.079
$Co(NO_3)_2.6H_2O$	0.0494

The Direct Cell Counting method was used to estimate the growth density of *S. platensis*. This method involves using a calibrated grid placed over the culture chamber (Neubauer hemocytometer), followed by a cell count per grid square under a microscope (Liu 2017). Samples were collected aseptically every third day from the day of inoculation and observed at 45x magnification. Counts were performed until cell density was constant. To obtain reproducible results, the seeded material had a constant dilution factor from count to count. In addition, the number of cells per millilitre was calculated (Selvakumaran and Jell 2005).

Number of viable cells mL^{-1}= The average number of viable cells per 0.1 cm^2 area × 10^4 (correction factor for the volume of shaded area) × Dilution factor

2.3. Metal Tolerance of Spirulina platensis PCC 7345

S. platensis was grown in the presence of metal ions to test its potential to tolerate metals detected in wastewater. The metal ions and their final concentrations (Table 2) in the medium were selected based on the standards for permissible heavy metal concentrations in inlet wastewater laid by the Ministry of Environment and Forests, Government of India (2010) for Common Effluent Treatment Plants (CETP) as per The Environment (Protection) Rules (1986). The inoculum was added at an initial cell count of $1x10^4$ cells mL^{-1}. The potential of *S. platensis* to grow in metal-enriched media was visually observed and later direct cell counts were done on the twenty-eighth day, from the day of inoculation.

Table 2. Final concentration of heavy metals in the medium

Sr. No.	Heavy Metal	Concentration (ppm)
1.	Cadmium (Cd)	1
2.	Cobalt (Co)	49
3.	Copper (Cu)	3
4.	Manganese (Mn)	1812
5.	Mercury (Hg)	0.01
6.	Zinc (Zn)	222

3. Results

3.1. Growth curve of Spirulina platensis PCC 7345

The Growth Curve was used to determine Exponential Growth Time. As seen in Figure 1, for the first five days there was a gradual increase in the number of *S. platensis* cells followed by a steep increase for the next three days (exponential phase). It remained nearly constant between 12-28 days (stationary phase) and decreased beyond 28 days (death phase).

3.2. Tolerance of Spirulina platensis to Metals – Qualitative

S. platensis grew in media enriched with cadmium, copper, and mercury; no observed growth in media with cobalt, manganese, and zinc (Table 3). Quantitative methods confirmed these results.

Table 3. Growth of *S. platensis* in BG11 medium with various heavy metals

Heavy Metal	Growth of *S. platensis*
Cadmium (Cd)	+
Cobalt (Co)	-
Copper (Cu)	+
Manganese (Mn)	-
Mercury (Hg)	++
Zinc (Zn)	-

+ represents growth; - represents no growth

3.3. Tolerance of Spirulina platensis to Metals – Quantitative

To quantitatively investigate *Spirulina*'s metal tolerance, the living cells cultivated in media were counted. *S. platensis* was most tolerant of Hg ($11x10^4$ cells mL^{-1}), followed by Cd and Cu, respectively (Figure 2).

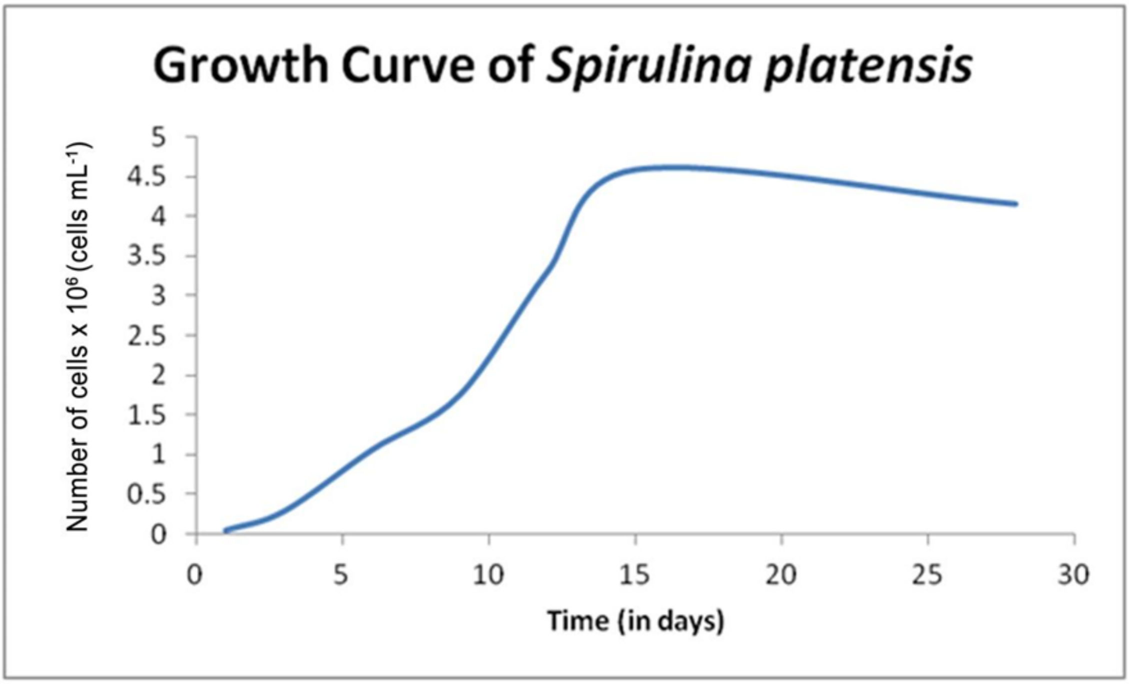

Figure 1. Growth curve of *S. platensis* in BG11 medium.

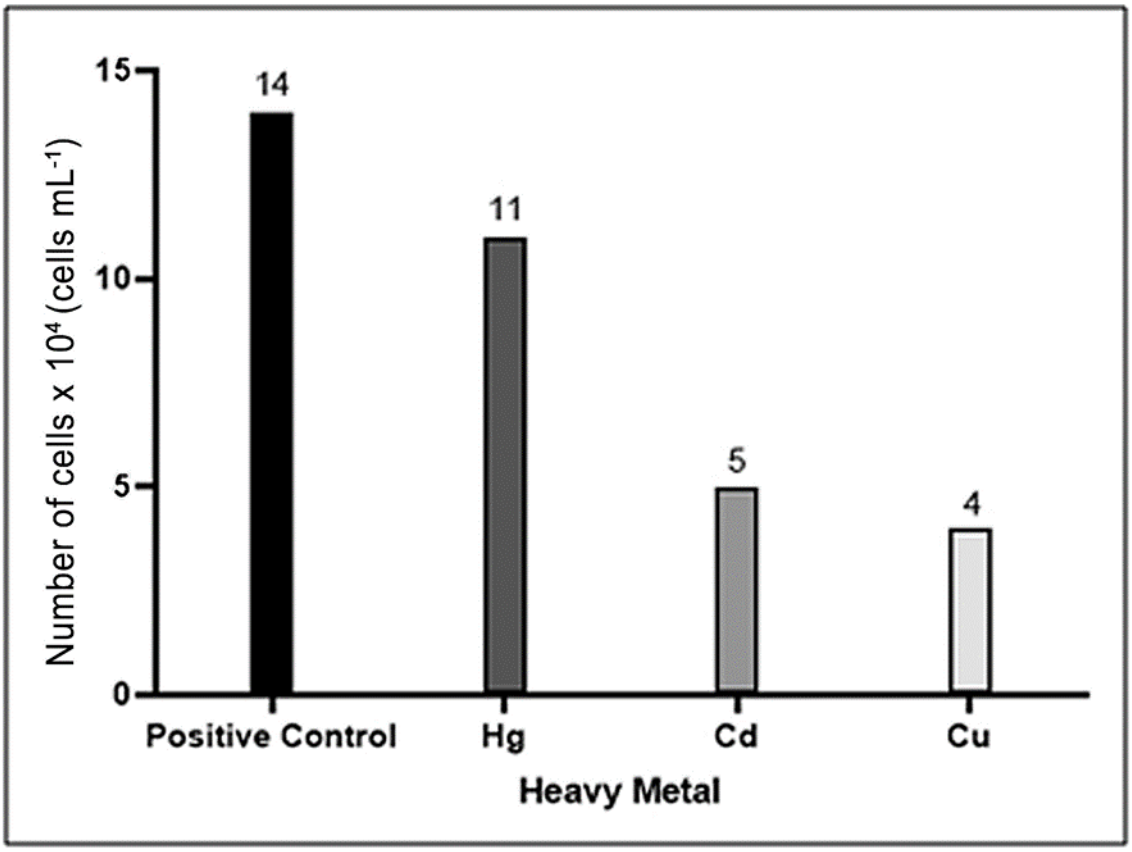

Figure 2. Number of cells counted of *S. platensis* in medium with various heavy metals.

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4. Discussion

Spirulina platensis contributes 30% of the total global algae biomass production, majorly cultivated using chemical-based culture media (Lim et al. 2021). Moreover, the excessive use of chemicals and nutrients has adverse effects on the environment. This highlights the need for an alternative culture medium such as wastewater, rich in nitrogen, phosphorus, and indigenous bacteria (Jia and Yuan 2016).

Cobalt, manganese, and zinc probably disrupted the metabolic pathways of *S. platensis*, so no growth was recorded for these media. Quantitative analysis of S. platensis tolerance to mercury was contrary to the order of toxicity proposed by Thomson and Kurup (2010); clear reasons for this were not identified. In view of these results, *S. platensis* can be used as an effective organism for the uptake of metal ions in wastewater treatment, as also suggested by Murali and Mehar (2014), Michael et al. (2019), and Lim et al. (2021).

This further paves the way for value-added products of *S. platensis,* such as food supplements, wastewater treatment (bioremediation), biofuels, animal feeds, and fertilizers (Padgaonkar et al. 2021). *S. platensis* tolerance for heavy metals could find applications in wastewater management and address the environmental issues of heavy metals in the natural ecosystem. High tolerance for mercury can imply the cyanobacteria's suitability for mercury treatment. Through further investigation, it is necessary to understand the tolerance range of *S. platensis* to Hg (II) and the subsequent effect on the organism's metabolic pathways.

The current studies support the use of sewage as the growth media (Lim et al. 2021), but studies are needed to establish proper evidence on the bioremedial potential of *S. platensis.* Since it exclusively uses light as an energy source (phototrophic organism), its use in biological reactors for bioremediation of hazardous substances is proposed as a cost-effective approach. Major cities of India generate Around 72368 million litres per day of wastewater (Central Pollution Control Board 2021), which can ensure better productivity of *S. platensis*.

5. Conclusion

The results of this study confirm the tolerance of *Spirulina platensis* to mercury (0.01 ppm), copper (3 ppm) and cadmium (1 ppm). However, the organism could not tolerate other metals such as cobalt, manganese, and zinc at the amounts found in primary wastewater. One solution to this problem could be diluting the wastewater before inoculation with the organism.

The study also shows that *Spirulina platensis* has a high tolerance to mercury (at 0.01 ppm). Its tolerance towards Cu and Cd was considerably less, but noteworthy. However, it is necessary to further determine the suitability of *S. platensis* as a bioremediator for heavy metals. The biomass can be studied for the extraction of bioactive compounds or value-added products.

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