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Phycoremediation of contaminated water by cadmium (Cd) using two cyanobacterial strains (*Trichormus variabilis* and *Nostoc muscorum*)

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Abstract

Background: Water pollution with heavy metals is a severe dilemma that concerns the whole world related to its risk to natural ecosystems and human health. The main objective was to evaluate the removal efficiency of Cd of various concentrations from contaminated aqueous solution by use of two cyanobacterial strains (*Nostoc muscorum* and *Trichormus variabilis*). For this purpose, a specially designed laboratory pilot-scale experiment was conducted using these two cyanobacterial strains on four different initial concentrations of Cd (0, 0.5, 1.0 and 2.0 mg L⁻¹) for 21 days.

Results: *N. muscorum* was more efficient than *T. variabilis* for removing Cd (II), with the optimum value of residual Cd of 0.033 mg L⁻¹ achieved by *N. muscorum* after 21 days with initial concentration of 0.5 mg L⁻¹, translating to removal efficiency of 93.4%, while the residual Cd (II) achieved by *T. variabilis* under the same conditions was 0.054 mg L⁻¹ (89.13% removal efficiency). Algal growth parameters and photosynthetic pigments were estimated for both cyanobacterial strains throughout the incubation period.

Conclusions: High Cd concentration had a more toxic impact on algal growth. The outcomes of this study will help to produce treated water that could be reused in agrarian activities.

Keywords: Phycoremediation, Cyanobacteria, Cd removal, Contaminated water, Wastewater

Introduction

One of the global challenges is the pollution of water bodies by heavy metals. Any metal and metalloid element having density within the range of 3.5 to 7 g cm⁻³ is considered poisonous even if present in low concentrations [20]. An optimum low level of concentration of heavy metals such as iron (Fe), copper (Cu) and zinc (Zn) have biological usefulness while others, including lead (Pb)

and cadmium (Cd), are not useful biologically and are toxic irrespective of the level of contamination [24].

Cadmium (Cd) is one of the most noxious heavy metals that could reach the food chain through absorption by plants from the soil [48, 50]. Human activities (e.g., mining, electronics, and metallurgical industries) are sources of Cd that causes contamination of water and soil [23, 33, 41]. Nordberg et al. [46] stated Cd absorbed from the soil by field crops, such as wheat, rice, and potatoes, has negative effects on human bones. In Japan, water and soil contaminated by Cd is the major cause of the Itai-itai disease [35]. Cadmium causes bone disease, kidney damage and cancer. It is reported

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that high levels of Cd exposure cause osteoporosis, renal dysfunction, and liver damage [21].

The manufacture of alkaline batteries consumes about three-quarters of Cd production. The remaining one-quarter is used by processes including coating materials and as a plastic stabilizer [32]. Cadmium is extremely poisonous, causes plant nutrient deficiency and oxidative stress, and also impacts on the enzymatic systems of cells [31, 32]. Therefore, World Health Organization (WHO) recommends that the concentration of Cd in potable water should be limited to $3 \mu\text{g L}^{-1}$ [65]. For short- and long-term irrigation water use, the preferred threshold of Cd concentration should be 0.05 and 0.01 mg L^{-1} , respectively [17, 56].

Human health issues related to the pollution of water bodies and soil by heavy metals resulting from pesticides, fertilizers, sewage water and industrial activities have received global attention. There is a lack of precautionary measures put in place to inspect industrial facilities that discharge contaminated wastewater into agricultural drains that supply irrigation water for production of crops in many countries, be it developing or developed. Thus, people who handle the contaminated irrigation water and the resulting agricultural products put their health at risk [8, 14, 60]. Many different methods of wastewater treatment (physical, chemical treatment, biological and phytoremediation) have been applied to reduce Cd concentration in water to the recommended international standards [11]. Some of the wastewater treatments applied include physicochemical processes (ion exchange and chemical precipitation), electrochemical treatments (electrocoagulation, electrodeposition, and electro-flotation), adsorption (carbon nanotubes, activated carbon and wood sawdust adsorbents), and the most common current methods are photocatalysis, membrane filtration, and nanotechnology [9, 12, 61, 63, 64].

However, these techniques have many disadvantages. For example, they are costly, consume high energy, have potential of secondary pollution and are only valid within a range of Cd concentration [35, 41]. Moreover, most of the conventional techniques do not provide 100% heavy metal removal with restrictions on pH variation are efficient at only smaller concentrations [5, 36], produce toxic sludge and waste products, and need high exploitation and recycling costs [39, 47]. Being environmentally friendly, biotechnology techniques have received significant attention. In addition, they provide high heavy metal removal efficiency, consume less energy, and are carried out at lower pressure and temperature [18, 57]. However, availability of selected biomass types for the biosorbent is of primary importance. Biosorbents can be nature based (e.g., bacteria, fungi and algae) or from industrial and

agricultural wastes. Several studies have used biosorbents for dye and metal treatments [38, 62].

Phytoremediation is any process that use algae to bioremediate contaminated water and wastewater [49]. The characteristics of algae involve a high ratio of surface area to volume, high heavy metals tolerance, growth possibility either autotrophically or heterotrophically, the ability for genetic manipulation, phytochelatin expression and phototaxy [37]. Biosorption using blue-green algae (cyanobacteria) is rich in vitamins and proteins. The biomass can absorb and adsorb heavy metals from aquatic solution even when the cells are dead. Unlike conventional methods, cyanobacteria processes do not produce polluting sludges, are highly effective, easy to operate and cost effective for treating large quantities of wastewater with low contaminant concentrations [19, 51]. Cyanobacteria are exemplary biosorbents and are commonly found in ecosystems of water and soil [10, 13].

There were three objectives of this study: i) evaluation of the removal efficiency of Cd from an aqueous solution by the use of two cyanobacterial strains (*Nostoc muscorum* and *Trichormus variabilis* (*Anabaena variabilis*)) in a specially designed laboratory pilot-scale experiment; ii) investigation of the influence of various concentrations of Cd on algae growth parameters, and iii) treatment of wastewater to the WHO standard for reuse for agrarian activities.

Materials and methods

Algae and culturing conditions

Two cyanobacterial strains (*Nostoc muscorum* and *Trichormus variabilis*) were cultured in a BG110 liquid medium consisting of a mixture of MgSO_4 , K_2HPO_4 , CaCl_2 , Na_2EDTA , Na_2CO_3 , citric acid, and ferric ammonium citrate as presented by Ripkka et al. [54]. Erlenmeyer flasks were used with daily alternation of an average of 8 h of darkness and 16 h of light. The temperature was controlled during the experiment at $27 \pm 2 \text{ }^\circ\text{C}$, while the cool white light intensity ranged from 3000 to 3500 lx and the pH was set to 7.2. The algae cells were harvested on the 15th day, which corresponds to the middle of the logarithmic phase, and centrifuged at 3000 rpm for 10 min.

Preparation of metal/toxin stock solutions

The cadmium-contaminated aqueous solution was prepared by adding 0.684 g of cadmium sulfate ($\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$) to 100 cm^3 of distilled water and stirring well to ensure that the cadmium sulfate was completely dissolved. The prepared solution was diluted using the medium to obtain the desired concentrations of 0, 0.5, 1 and 2 mg L^{-1} used in the experiments. Three replicates for each concentration treatment were set up.

Experimental setup

The experiments were carried out using plastic containers having dimensions of 26.9 cm in length, 18.75 cm in width and 12.5 cm in depth. Each container was filled with a mixture of 2 L of the prepared aqueous solution (BG110 medium) of different Cd concentrations and 110 ml of the algae medium (OD₆₇₈). Samples were taken from the plastic containers at a rate of 5 ml every 4 days to measure the optical density (OD), while samples were taken in volumes of 50 ml every 7 days (D₀, D₇, D₁₄, D₂₁) for the determination of the photosynthetic pigments, and a sample of 50 ml was taken at the end of the incubation period of 21 days to measure biomass content.

Heavy metal removal efficiency

Five ml samples were taken from the contaminated media every four days to estimate the concentration of residual Cd caused by algae absorption using an atomic absorption spectrometer (Perkin Elmer Analyst 400). The removal efficiency (RE) was calculated as:

$$\text{Heavy metal removal efficiency} = \left(\frac{C_o - C_1}{C_o} \right) \times 100, \tag{1}$$

where C₁ and C_o are, respectively, the residual and initial concentrations of Cd in mg L⁻¹.

Photosynthetic pigments analysis

The samples were subjected to 10-min centrifugation at 4000 rpm, after which the algae medium was carefully added before the distilled water was carefully poured with the algal cell suspension into a 4 ml DMSO solution. The mixture was stirred at 1000 rpm for 1 min to reach

homogeneity, after which it was heated for 10 min in a water bath at 65 °C. Six ml of 95% acetone concentration was added to the algal cells extracted from the DMSO solution and mixed thoroughly. The photosynthesis pigments concentrations of Chlorophyll A and carotenoids were estimated in µg ml⁻¹ according to the Ritchie [55] and Davies [15] methods, respectively.

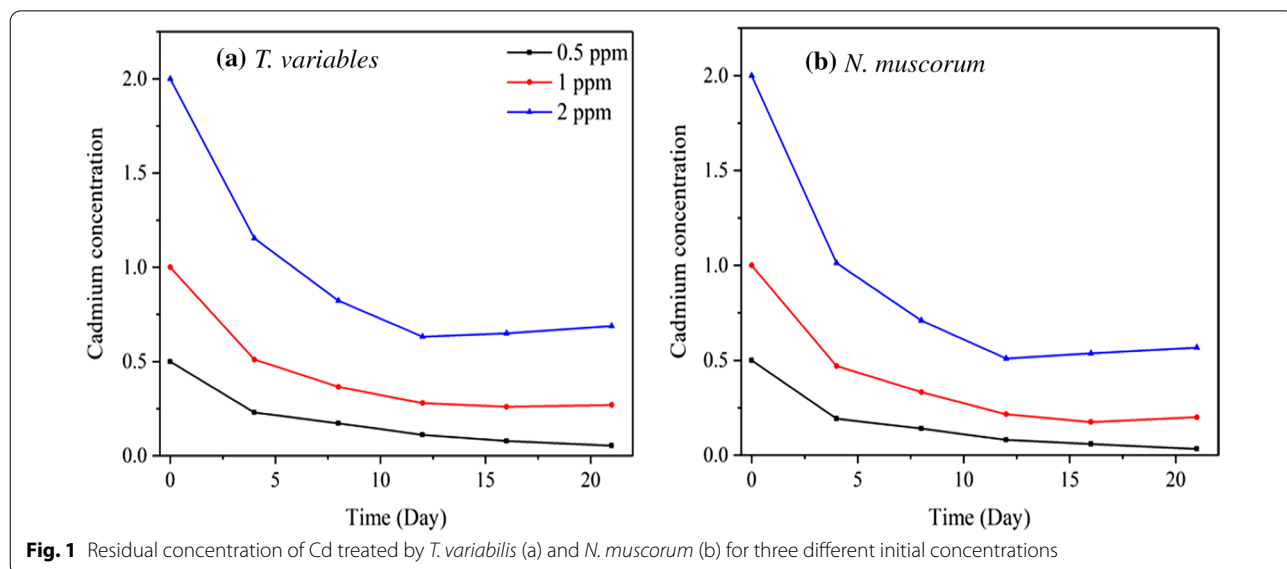
Statistical analysis

The statistical analysis involved the use of a random complete block design (RCBD) with factorial data analysis, the three factors considered being concentration (C), algae (A), and number of days (D). Three replications were implemented in order to minimize parameter errors. The least significant differences (LSD) and correlation coefficient (CC) test were applied [59]. MSTAT software [45] was used for the statistical analysis.

Results and discussion

Removal of heavy metal

There was a significant variation of residual Cd values among the different initial Cd concentrations considered. As shown in Fig. 1, the residual Cd tends to stabilize after day 12 for all initial concentrations. *N. muscorum* achieved a terminal residual Cd value of 0.033, 0.175 and 0.51 mg L⁻¹ for the initial concentration of 0.5, 1 and 2 mg L⁻¹, respectively, translating to heavy metal removal efficiency of 93.4, 82.5 and 74.5%, respectively. Terminal residual Cd values achieved by *T. variabilis* were slightly higher at 0.054, 0.26 and 0.632 mg L⁻¹ for the initial concentration of 0.5, 1 and 2 mg L⁻¹, respectively, reflecting removal efficiency values of 89.13, 74.00 and 68.38%, respectively (Fig. 1). Cadmium was released again into



the contaminated water as a result of the algae's sorption decline related to the toxic effect of Cd, and so the residual Cd marginally increased after 16 and 12 days for the initial concentrations of 1 and 2 mg L⁻¹, respectively.

Our results of heavy metal removal were in agreement with Khan et al. [34] who showed that the removal efficiency of Cd by use of four freshwater algae (*Zygnema insigne*, *Cladophora glomerata*, *Vaucheria debaryana*, and *Oedogonium westii*) increased with time elapsed and reached the highest level on the 9th day after which there was marginal change. The cyanobacterium *Nostoc* sp. JRD1 was used to remediate polluted water with different heavy metal ions from the reservoir of Hindustan Paper Corporation Limited, India, and showed a very high removal (94%) capacity for Cd(II) upon exposure to 0.5 ppm for 24 h [3].

Ahad et al. [2] achieved a removal efficiency of 92% of Cd by *N. muscorum* within 24 h from the initial concentration of 0.5 ppm. Hazarika et al. [25] reported Cd removal efficiency by *N. muscorum* of 82% after 30 h using 5 ppm initial concentration. Dixit and Singh [16] achieved a ceiling sorption of Cd of 85.2% at 60 µg ml⁻¹ concentration within 30 min.

Anabaena sphaerica yielded removal efficiencies of Cd between 29 and 85% [26]. Abdel-Aty et al. [1] indicated that the biosorption of Cd with *Anabaena sphaerica* was rapid in the first 20 min of the experiment followed by a gradual increase until attaining equilibrium at 90 min after which the biosorption was steady. Their results showed that the initial biosorption at 50 ppm of Cd was 94.3%, but decreased with increasing Cd concentration. Goswami et al. [22] reported that *Anabaena doliolum* recorded high Cd removal efficiency of 91.2% at the beginning and 68.6% at the 7th day of exposure under different concentrations ranging from 0.5 to 2.0 mg L⁻¹. Siva et al. [58] observed rapid biosorption at the beginning of the experiment with Cd concentration of 1 mg L⁻¹ achieving a removal efficiency of 74% within 6 min, then reached equilibrium within 1 day with 93.9% of metal ions adsorbed by *Spirulina (Arthrospira) indica*. Inthorn et al. [30] presented the following removal efficiencies of Cd by the use of different species, namely T5 (94%), *Chlorococcum* sp. (94%), *Scenedesmus acutus* (88%) *Fischerella* sp. (91%) *Chlorella vulgaris var. vulgaris* (89%), *Nostoc* sp. (94%), *Lyngbya hieronymusii* (97%), *Oscillatoria jatorvensis* (94%), *Gloeocapsa* sp. (96%), and *Phormidium molle* (95%).

Additionally, Inthorn et al. [29] reported that more than 90% of Cd removal efficiency was achieved within 10 min at 1 ppm initial concentration by *Tolypothrix tenuis*, after which the Cd concentration remained steady. Inthorn et al. [28] reported Cd removal efficiency of 84% and 92%, respectively, in non-treated and NaOH-treated

cells of *Nostoc paludosum*, and for similar circumstances in *Phormidium angustissimum* reported 86% and 94%, respectively. The results confirmed that 30 min contact time was required for significant Cd removal and to reach the equilibrium state. Further experiments indicated 10 min is enough for significant removal capacity and to attain the equilibrium state.

Alga growth parameters

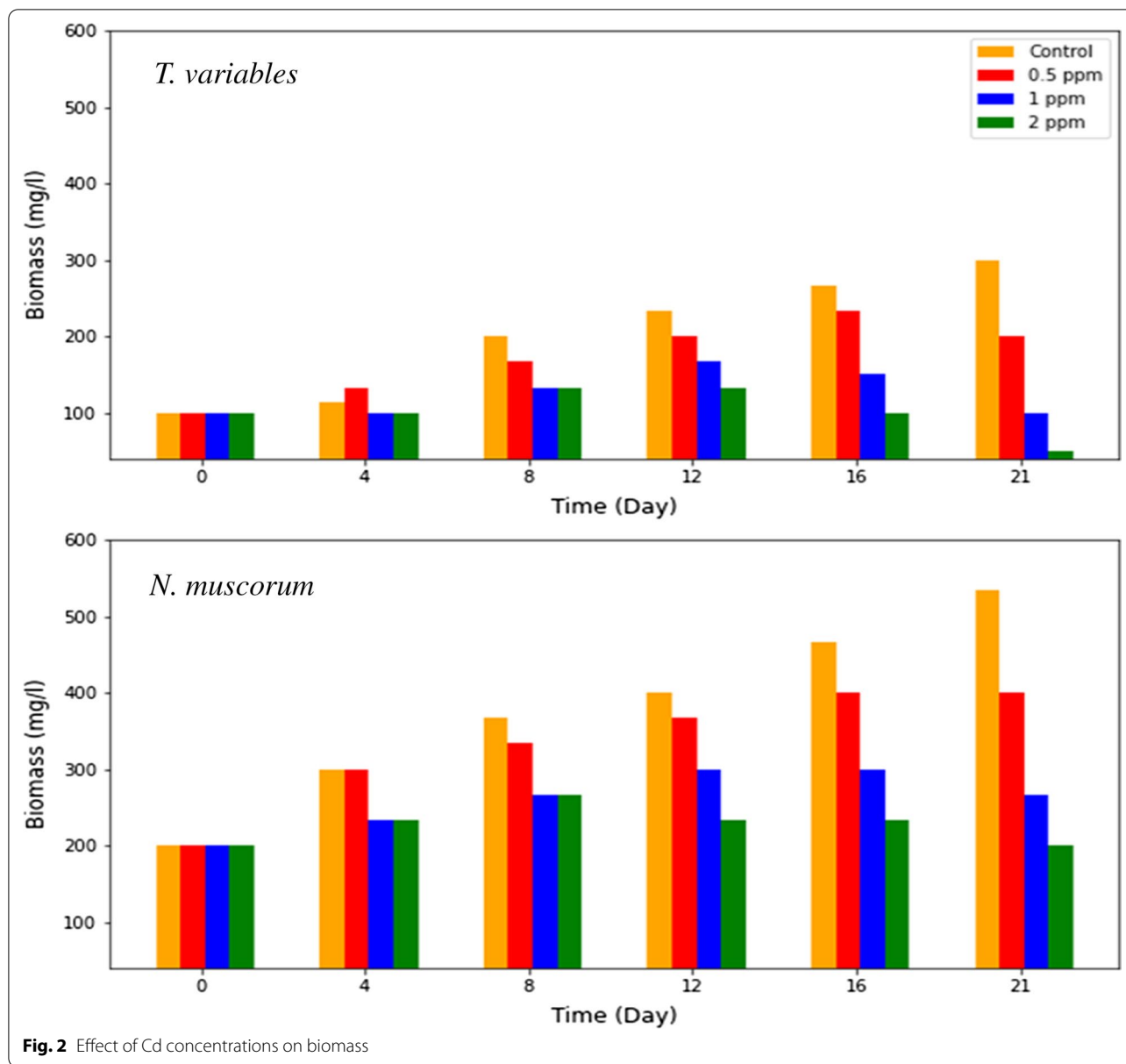
Alga biomass

The highest biomass value for *N. muscorum* was 533.3 mg L⁻¹ at the end of the 21 days of the experiment for the control treatment (0.0 concentration), while the lowest value of Cd was 200 mg L⁻¹ after 21 days for the initial concentration of 2 ppm (Fig. 2). The decline phase was approximately reached under Cd concentrations of 1 and 2 mg L⁻¹ after 16 days of incubation period. A biomass of 300 mg L⁻¹ was recorded for *T. variabilis* in the control treatment while the lowest biomass was 50 mg L⁻¹ for the initial 2 mg L⁻¹ Cd concentration at the end of the experiment.

The highest initial concentration of Cd reduced growth of biomass and led to cyanobacteria death [25]. Only 5 to 6 days of incubation period was required for cadmium to delay the algae growth. Cd replaced the Mg in the chlorophyll molecule of the algae and affected photosynthesis, leading to a reduced growth of the cells, particularly the more sensitive *N. muscorum* cells [40]. Siva et al. [58] stated that Cd concentration of 10 mg L⁻¹ is extremely poisonous and inhibited *Spirulina (Arthrospira) indica* growth, the growth inhibition increasing with the concentration of Cd in the aqueous solution. Arunakumara and Zhang [6] demonstrated that Cd concentrations of 1, 2, 4, 6 and 8 mg L⁻¹ inhibited the growth of *Synechocystis* sp. PCC 6803 at 2 days into the incubation period of 8 days. Similarly, the cyanobacterium (*Anabaena flos-aquae*) needed just 0.15 mg L⁻¹ concentration of Cd to inhibit its growth by 50% [27]. Meanwhile, Rehman and Shakoori [52] observed *Chlorella* in the control experiment grew gradually but, when treated with a culture of 8 µg ml⁻¹ of Cd, the growth of cells decreased.

Optical density (OD)

The growth rate of algae cells was affected slightly at 0.5 ppm concentration of Cd, whereas concentration levels of 1 and 2 ppm completely inhibited growth by the middle of the experiment for both cyanobacterial strains (Fig. 3). Rangsayatorn et al. [53] specified that optical density of *Spirulina platensis* was affected by higher concentrations of Cd that caused the death of cyanobacterial cells, while insignificant growth suppression was observed at lower concentrations.



Pigments

The reduction in Chlorophyll A was minimal at 0.5 ppm initial Cd concentration during the incubation period (Fig. 4), but significant reductions were detected at concentrations of 1 and 2 mg L⁻¹ in the cases of the two cyanobacterial strains. Likewise, carotenoids displayed a similar trend, even though *N. muscorum* showed nearly a constant value for the 0.5 ppm initial concentration (Fig. 5).

The results obtained for the pigments agreed with Atri and Rai [7] who stated that higher dosages of Cd reduced Chlorophyll A and carotenoids of *Anabaena*, *Microcystis*, and *Nostoc*. Similarly, Goswami et al. [22]

showed that higher concentrations of Cd decreased the pigments of *Anabaena doliolum*. Alidoust et al. [4] reported that *Nostoc entophytum* ISC32' cells exposure to 2 mg L⁻¹ Cd resulted in 65.77% reduction in Chlorophyll A.

Mota et al. [44] reported a decline in Chlorophyll A content for the cultures treated with Cd for *Cyanoshece* species CCY 0110 at 24 h exposure, and the decline gradually continued thereafter. Lamaia et al. [42] observed that high concentrations of Cd could eliminate chloroplasts in *Cladophora fracta* [42]. In a similar study, Arunakumara and Zhang [6] showed that pigments content (Chlorophyll A and carotenoids)

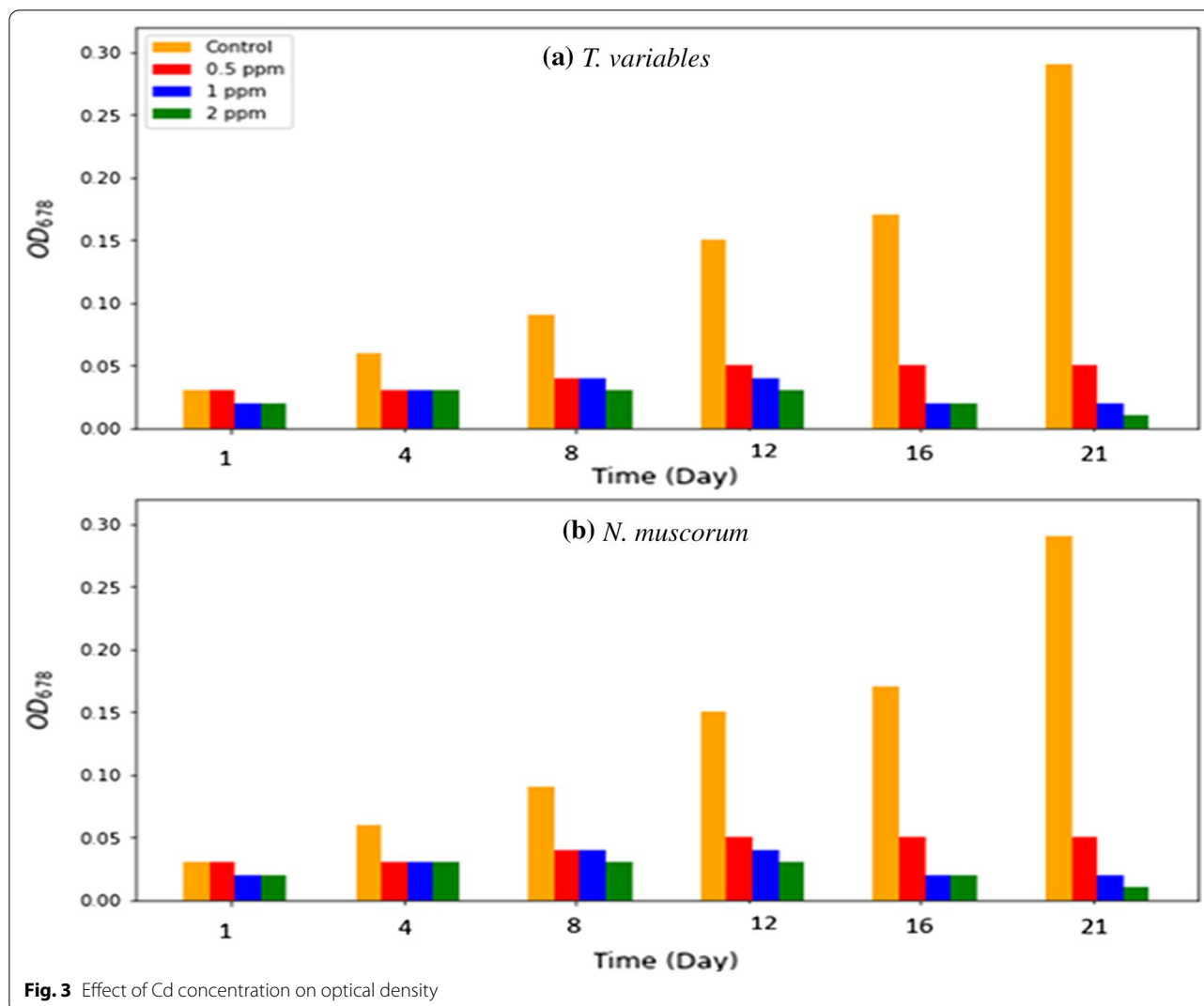


Fig. 3 Effect of Cd concentration on optical density

decreased with increasing Cd concentration, and the damage to the photosynthetic pigments is related to Cd toxicity [43].

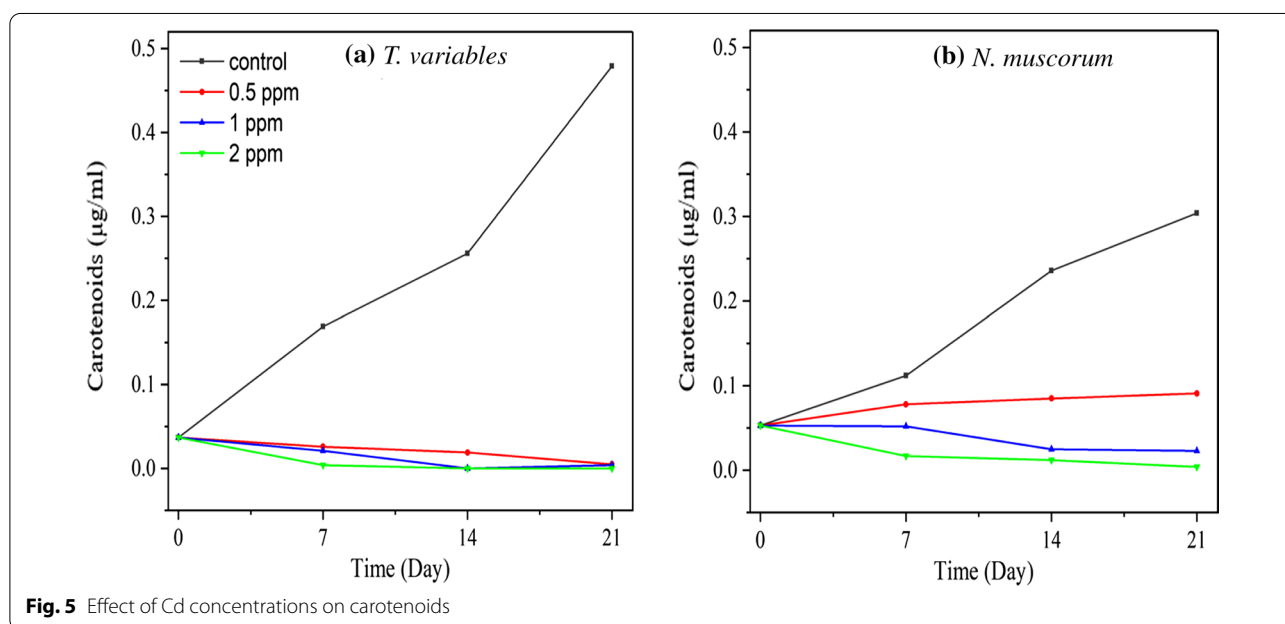
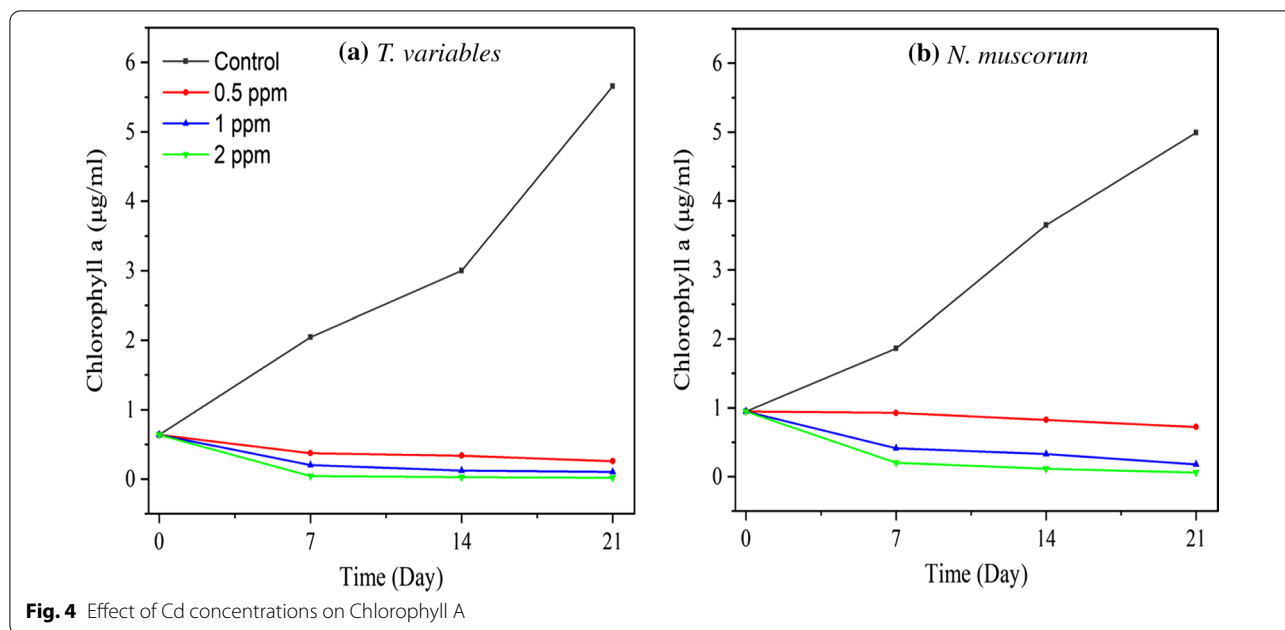
Statistical analysis

The results of *T. variabilis* and *N. muscorum* were significantly different at the 0.05 level of significance for the individual treatments (Table 1). However, the 3 factors (concentrations, days and algae) did not show any significant difference with respect to biomass at the 0.05 significance level. It is observed that *N. muscorum* has a higher removal efficiency of Cd from pigments and contaminated water and is thus preferred. Nevertheless *T. variabilis* showed superior quality in OD values.

There were significant differences between values under different initial Cd concentrations at the 0.05 significance level. It is observed that the best results were achieved in terms of residual Cd, OD, Chlorophyll A,

and carotenoids by the control treatment (C₀), followed by the 2nd concentration (C_{0.5}), while the 4th concentration (C₂) accomplished the lowest results. However, there was non-significant difference between C₁ and C₂ in relation to OD values. Days 12 (D₁₂), 16 (D₁₆) and 21 (D₂₁) did not exhibit any significant statistical differences. However, (D₂₁) achieved the best result with respect to OD. Pigments as well exhibited significant differences at the 0.05 level (Table 2).

It turned out that *T. variabilis* was the best alga in pigment towards the end of the study period with the case of no Cd treatment (A₂C₀D₂₁). The coefficient of determination (R²) values between residual Cd and biomass for *N. muscorum* and *T. variabilis* were 37.02% and 10.88%, respectively. Meanwhile, R² values between residual Cd and OD₆₇₈ were 17.54% and 6.66%, respectively, for *N. muscorum* and *T. variabilis*, while the



corresponding values for biomass and OD₆₇₈ were 78.58% and 69.5%, respectively.

Conclusion

The study has presented the removal efficiency of Cd from contaminated water by use of two cyanobacterial strains (*Trichormus variabilis* and *Nostoc muscorum*). At the end of the 21-day study period, and for the

initial metal concentration of 0.5 mg L⁻¹, *N. muscorum* achieved a maximum removal efficiency of Cd of 93.4%, whereas *T. variabilis* recorded 89.13%. It is observed that *N. muscorum* is more efficient for Cd removal compared with *T. variabilis*. Higher concentrations of Cd had a more toxic effect on the growth of algae. Our study confirms the potential of cyanobacteria for phycoremediation. The removal of Cd from the aqueous solution was attributed to biosorption of cyanobacteria.

Table 1 ANOVA table of the mean square values

Source of variation	DF	Mean square			Source of variation	DF	Mean square	
		Residual Cd	Biomass	OD			Chl. a	Carotenoids
Replications	2	0.001 ^{ns}	0.0006 ^{ns}	0.0013 ^{ns}	Replications	2	0.001 ^{ns}	0.0002 ^{ns}
Algae (A)	1	0.068 ^{**}	0.0002 ^{ns}	0.0020 ^{**}	Algae (A)	1	1.036 ^{**}	0.0020 ^{**}
Concentrations (C)	3	6.002 ^{**}	0.0061 ^{ns}	0.0720 ^{**}	Concentrations (C)	3	36.076 ^{**}	0.1910 ^{**}
AC	3	0.017 ^{**}	0.0004 ^{ns}	0.0010 ^{**}	AC	3	0.194 ^{**}	0.0130 ^{**}
Days (D)	5	1.561 ^{**}	0.0019 ^{ns}	0.0120 ^{**}	Days (D)	3	2.817 ^{**}	0.0210 ^{**}
AD	5	0.014 ^{**}	0.0015 ^{ns}	0.0002 ^{**}	AD	3	0.174 ^{**}	0.0020 ^{**}
CD	15	0.328 ^{**}	0.0003 ^{ns}	0.0110 ^{**}	CD	9	0.124 ^{**}	0.0380 ^{**}
ACD	15	0.001 ^{**}	0.0025 ^{ns}	0.0003 ^{**}	ACD	9	0.126 ^{**}	0.0030 ^{**}
Error	94	0.002	0.001	0.0002	Error	62	0.001	0.0001

(^{ns}) there was no difference between the treatments at the 5% significance level.

(^{**}) there was a high difference between the treatments at the 5% significance level ($P \leq 0.05$)

Table 2 Relative performance of the 3 factors (days, concentrations, and algae)

Treatments	Res. Cd (ppm)	OD ₆₇₈	Treatments	Chl. a (µg mL ⁻¹)	Carotenoids (µg mL ⁻¹)
Algae (A)					
A ₁	0.364b	0.048b	A ₁	1.129a	0.079a
A ₂	0.407a	0.056a	A ₂	0.921b	0.071b
F. Test	**	**	F. Test	**	**
Concentrations (C)					
C ₀	0.000d	0.118a	C ₀	2.849a	0.207a
C _{0.5}	0.179c	0.042b	C _{0.5}	0.6287b	0.049b
C ₁	0.423b	0.027c	C ₁	0.3663c	0.027c
C ₂	0.940a	0.020c	C ₂	0.2567d	0.016d
LSD	0.0222	0.00702	LSD	0.01813	0.005771
Days (D)					
D ₀	0.875a	0.023f	D ₀	0.792c	0.045d
D ₄	0.446b	0.035e	D ₇	0.758d	0.061c
D ₈	0.318c	0.047d	D ₁₄	1.051b	0.079b
D ₁₂	0.229d	0.058c	D ₂₁	1.500a	0.114a
D ₁₆	0.220d	0.066b			
D ₂₁	0.226d	0.084a			
LSD	0.02563	0.008106	LSD	0.01813	0.005771

*The same letters attached to the results indicate no significant differences at the 5% significance level ($P \leq 0.05$)

Acknowledgements

The authors would like to express their thanks to the Faculty of Agriculture, Cairo University, Agricultural Engineering Department and Plant Physiology Department for their support in this work.

Authors' contributions

MMAE-H, Shady AM and GB undertook the practical experiments. MA designed the experimental layouts and supervised all related work. MA and AM collected and analyzed the research data and wrote the original draft which was reviewed by all the authors. NA-A and YG-A edited the final manuscript. All authors read and approved the final manuscript.

Funding

No funding source.

Availability of data and materials

The researchers provided the experimental requirements of raw materials, algae, and system design through their own financial resources, while the data from previous studies and research were obtained through the Cairo University platform, which provides research on a regular basis.

Declarations

Ethics approval and consent to participate

All authors gave approval for their participation in this research.

Consent for publication

All authors consent to publication of the research paper.

Competing interests

The authors declare no conflicts of interest.

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Received: 20 August 2021 Accepted: 20 November 2021

Published online: 11 December 2021

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