Phytoremediation of Contaminated Water by Cadmium (Cd) Using Two Cyanobacteria Species (*Anabaena Variabilis* and *Nostoc Muscorum*)

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Research

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

Posted Date: August 24th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-817938/v1>

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Abstract

Background: Water pollution with heavy metals is a severe dilemma that worries the whole world related to its risk to nature ecosystem and human being health. The main objective is to evaluate the removal efficiency of Cd with various concentrations from contaminated aqueous solution by two Cyanobacteria species (*Nostoc muscorum* and *Anabaena variabilis*). For this purpose, a designed laboratory pilot scale was applied using two cyanobacteria species (*Nostoc muscorum* and *Anabaena variabilis*), four different initial concentrations (0, 0.5, 1.0 and 2.0 mg L⁻¹) for 21 days.

Results: *N. muscorum* was more efficient than *A. variabilis* for removing Cd (II), because the optimum value of residual Cd achieved by *N. muscorum* after 21 days at (0.5 mg L⁻¹) was (0.033 mg L⁻¹), where the removal efficiency was 93.4%, while the residual Cd (II) by *A. variabilis* under the same conditions was (0.054 mg L⁻¹), and the achieved removal efficiency was 89.13%. Algal growth parameters and Photosynthetic pigments were estimated for both Cyanobacteria species through 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.

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 (Gautam et al., 2014). 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 (Halttunen, 2008).

Cadmium (Cd) is one of the most noxious heavy metals that could reach the food chain through absorption by plants from the soil (Pourret and Hursthouse, 2019; Penido et al., 2019). Human activities (e.g., mining, electronics and metallurgical industries) are sources of Cd that causes contamination of water and soil (Lam et al., 2019; Karunanayake et al., 2018; Guo et al., 2019). Nordberg et al. (2018) 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 (Khan et al., 2020). Cadmium causes bone disease, kidney damage and cancer. It is reported that high levels of Cd exposure causes osteoporosis, renal dysfunction and liver damage (Ghoneim et al., 2014).

Alkaline batteries manufacturing consumes about three-quarters of Cd production. The remaining one quarter of Cd production is used by processes including coating materials and as a plastic stabilizer (Jaishankar et al., 2014). Cadmium is extremely poisonous, causes plant nutrient deficiency and oxidative stress, and also impacts on the enzymatic systems of cells (Jaishankar et al., 2014; Irfan et al., 2013). Therefore, WHO (2008) recommends that the concentration of Cd in potable water should be limited to 3 µg L⁻¹ (Zinicovscaia, 2016). For the short- and long-term irrigation water use, the preferred threshold of Cd concentration should be 0.05 and 0.01 mg L⁻¹, respectively (Fipps, 2015; Rowe and Abdel-Magid, 1995).

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 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 (Tchounwou et al., 2012; Dadar et al., 2016; Avigliano et al., 2015). Many different methods of wastewater treatment (physical, chemical treatment, biological and phytoremediation) have been applied to reduce cadmium concentration in water to the recommended international standards (Chen et al., 2020). Some of the wastewater treatments applied include physicochemical processes (ion exchange and chemical precipitation), electrochemical treatments (electrocoagulation, electrodeposition and elector-floatation), adsorption (carbon nanotubes, activated carbon and wood sawdust adsorbents), and the current methods are photocatalysis, membrane filtration, and nanotechnology (Azimi et al., 2017).

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 (Lamet et al., 2019; Khan et al., 2020). Moreover, most of the conventional techniques do not provide 100% heavy metal removal with restrictions on pH variation (Ahluwalia and Goyal, 2007; Kumar et al., 2015), are efficient at only smaller concentrations (Kumar et al., 2015; Antunes et al., 2003), produce toxic sludge and waste products, and need high exploitation and recycling costs (Obboh et al., 2009; Kumar et al., 2015). 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 (Fulekar, 2010; Singh et al., 2014). 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 (Vieira, 2000; Kumar and Pavithra, 2018).

Phytoremediation is any process that use algae to bioremediate contaminated water and wastewater (Phang et al., 2015). 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 (Kumar and Gunasundari, 2018). 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 (Garnham et al., 1992; Prakash and Awasthi, 2013). Cyanobacteria are exemplary biosorbents and are commonly found in ecosystems of water and soil (Chojnacka and Noworyta, 2004; Cepoi et al., 2016).

There were three objectives of this study; i) evaluation of the removal efficiency of Cd in aqueous solution by two cyanobacteria species (*Nostoc muscorum* and *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 world health organization (WHO) standard for reuse for agrarian activities.

Materials And Methods

2.1. Algae and culturing conditions

Two cyanobacteria species (*Nostoc muscorum* and *Anabaena 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. (1979). Erlenmeyer flasks were used with daily alternation of an average of 8 hours

of darkness and 16 hours of light. The temperature was controlled during the experiment at $27 \pm 2^\circ\text{C}$, while the cool white light intensity ranged from 3000–3500 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 minutes

2.2. 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 stirred 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.

2.3. 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 (OD_{678}). The samples were taken from plastic containers at a rate of 5 ml every four days to measure the optical density (OD), while samples were taken in volumes of 50 ml every 7 days (D_0 , D_7 , D_{14} , D_{21}) for the determination of the photosynthetic pigments, and a sample of 50 ml was taken at end of the incubation period of 21 days to measure biomass content.

2.4. 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 atomic absorption spectrometer (Perkin Elmer Analyst 400). The removal efficiency (RE) was calculated as:

$$\text{Heavy metal removal efficiency} = \left(\frac{C_0 - C_1}{C_0} \right) \times 100 \quad (1)$$

In Eq. (1) C_1 and C_0 are, respectively, the residual and initial concentrations of Cd in mg L^{-1} .

2.5. Photosynthetic pigments analysis

The samples were subjected to 10 minutes 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 one minute to reach homogeneity, after which it was heated for 10 minutes 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 $\mu\text{g ml}^{-1}$ according to the Ritchie (2008) and Davies (1976) methods, respectively.

2.6. 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 (Snedecor and Cochran, 1976). MSTAT software (Mstat-c., 1989) was for the statistical analysis.

Results And Discussions

3.1. 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 ppm for the initial concentration of 0.5, 1 and 2 ppm, respectively, translating to heavy metal removal efficiency of 93.4, 82.5 and 74.5%, respectively. Terminal residual Cd values achieved by *A. variabilis* were slightly higher at 0.054, 0.26 and 0.632 ppm, for the initial concentration of 0.5, 1 and 2 ppm, 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 ppm, respectively.

Our results of heavy metal removal were in agreement with Khan et al. (2017) who showed that the removal efficiency of Cd by 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. Ahad et al. (2017) 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. (2015) reported Cd removal efficiency of 82% after 30 h using 5 ppm initial concentration by *N. muscorum*. Dixit and Singh (2013) achieved a ceiling sorption of Cd of 85.2% at $60 \mu\text{g ml}^{-1}$ concentration within 30 min.

Anabaena sphaerica yielded removal efficiency of Cd between between 29 and 85% (Heimann and Cirés, 2015). Abdel-Aty et al. (2013) 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. (2015) 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 ppm. Siva et al. (2012) 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 mins, then reached equilibrium within 1 day with 93.9% of metal ions adsorbed by *Spirulina (Arthrospira) indica*. Inthorn et al. (2002) presented the following removal efficiencies of Cd by the different species as; T5 (94%), *Chlorococcum* sp. (94%), *Scenedesmus acutus* (88%) *Fischerella* sp. (91%) *Chlorella vulgaris* var. *vulgaris* (89%), *Nostoc* sp. (94%), *Lyngbya heironymusii* (97%), *Oscillatoria jasorvensis* (94%), *Gloeocapsa* sp. (96%), and *Phormidium molle* (95%).

Additionally, Inthorn et al. (1996) 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. (2003) reported Cd removal efficiency of 84% and 92%, respectively, in non-treated and NaOH-treated cells of *Nostoc paludosum*, and in *Phormidium angustissimum* as 86% and 94%, respectively. The results confirmed that 30 min contact time for significant Cd removal and to reach the equilibrium state. Further experiments indicated 10 mins is enough for significant removal capacity and to attain the equilibrium state.

3.2. Alga Growth parameters

3.2.1. Alga biomass

The highest biomass value for *N. muscorum* was 533.3 mg L^{-1} at the end of the 21 days of 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 approximately reached under Cd concentrations of 1 and 2 ppm after 16 days of incubation period. A biomass of 300 mg L^{-1} was recorded for *A. variabilis* in the control treatment while the lowest biomass was 50 mg L^{-1} for the initial 2 ppm Cd concentration at the end of the experiment.

The highest initial concentration of Cd reduced growth of biomass and led to cyanobacteria death (Hazarika et al., 2015). 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 (Kupper et al. 1996, 1998). Siva et al. (2012) 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 (2009) 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 (*Anabeana flos-aquae*) needed just 0.15 mg L^{-1} concentration of Cd to inhibit its growth by 50% (Heng et al., 2004). Meanwhile, Abdul Rahman and Shakoori (2004) observed *Chlorella* in the control experiment grew gradually but, when treated with a culture of $8 \text{ } \mu\text{g ml}^{-1}$ of Cd, the growth of cells decreased.

3.2.2. 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 at the middle of the experiment for both cyanobacteria species (Fig. 3). Rangsayatorn et al. (2002) 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.

3.2.3. 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 ppm in the cases of the two cyanobacteria species. 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 pigments were in agreement with Atri and Rai (2003) who stated that higher dosages of Cd reduced the Chlorophyll a and carotenoids of *Anabaena*, *Microcystis*, and *Nostoc*. Similarly, Goswami et al. (2015) showed that higher concentrations of cadmium decreased the pigments of *Anabaena doliolum*. Mota et al. (2015) reported a decline in chlorophyll a content for the cultures treated with Cd for *Cyanothece* species CCY 0110 at 24 h exposure, and the decline gradually continued thereafter.

Lamaia et al. (2005) observed that high concentrations of Cd could eliminate chloroplasts in *Cladophora fracta* (Lamaia et al., 2005). In a similar study, Arunakumara and Zhang (2009) showed that pigments content (Chlorophyll a and carotenoids) decreased with increasing Cd concentration, and the damage to the photosynthetic pigments is related to Cd toxicity (Leborans and Novillo, 1996).

3.3. Statistical Analysis

The results of *A. variabilis* and *N. muscorum* were significantly different at the 0.05 level 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 thus preferred. Nevertheless *A. variabilis* showed superior quality in OD values.

Table 1. ANOVA table of the mean square values.

Source of variation	D.F.	Mean Square			Source of variation	D.F.	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 ($P \leq 0.05$).

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 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).

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 indicates no significant differences at the 5% significance level ($P \leq 0.05$).

It turned out that *A. 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 (R^2) between residual Cd and biomass for *N. muscorum* and *A. variabilis* were 37.02% and 10.88%, respectively. Meanwhile, R^2 values between residual Cd and OD₆₇₈ were 17.54% and 6.66%, respectively, for *N. muscorum* and *A. variabilis*, the corresponding values for biomass and OD₆₇₈ were 78.58% and 69.5%, respectively.

Conclusion

The study has presented the removal efficiency of Cd by two cyanobacteria species (*Anabaena variabilis* and *Nostoc muscorum*) from contaminated water. At the end of the 21 days study period, *N. muscorum* achieved a

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

Declarations

Ethics approval and consent to participate: All authors are approval in participation in this research.

Consent for publication: All authors are consent of publication of the research paper.

Availability of data and materials: The researchers provided the experiment requirements of raw materials, algae, and system design through their own financial resources, while the data for previous studies and research was done through the Cairo University platform, which provides research on a regular basis.

Conflicts of interest: The authors declare no conflicts of interest.

Funding: No funding source.

Author contributions: Mona Abd El-Hameed, Shady Abdel Mottaleb and Gomaa Bakeer made the practical experiments, Mohamed Abuarab designed the experiments layouts and supervision all work related to his seniority, Mohamed Abuarab and Ali Mokhtar collected and analyzed the research data and writing original draft preparation, Nadhir Al-Ansari and Yeboah Gyasi-Agyei read and edited on the final manuscript.

Acknowledgement: 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.

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Figures

Figure 1

Residual concentration of Cd by *A. variabilis* (a) and *N. muscorum* (b) for three different initial concentrations.

Figure 2

Effect of Cd concentrations on Biomass.

Figure 3

Effect of Cd concentration on optical density.

Figure 4

Effect of Cd concentrations on Chlorophyll a.

Figure 5

Effect of Cd concentrations on Carotenoids.