GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(Research Article)

Phycoremediation assessment of heavy metals and nutrients from wastewater using some cyanobacteria

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GSC Biological and Pharmaceutical Sciences, 2024, 28(03), 119–132

Publication history: Received on 31 July 2024; revised on 10 September 2024; accepted on 13 September 2024

Article DOI: https://doi.org/10.30574/gscbps.2024.28.3.0327

Abstract

Cyanobacterial Phycoremediation is considered an effective agent intended for the biosorption of heavy metals (HMs) and nutrients for wastewater treatment. *Arthrospira platensis*, *Oscillatoria simplicissima,* and *Nostoc muscorum* Were investigated to remove pollutants from wastewater. Over three weeks, the cyanobacteria significantly reduced BOD, COD, and nutrients (ammonium, nitrate, total nitrogen, and total phosphorus). The effectiveness depended on the type of cyanobacteria used and the incubation time. In general, *Arthrospira platensis* is best for removing organic materials. HMs removal % varied significantly according to the incubation times and the type of heavy metal. All the tested cyanobacteria had a promising effect in removing the heavy metals under study, especially manganese, as *Nostoc Muscorum* initially removed most of the metals. In general, cyanobacteria can be a viable and environmentally friendly way to treat wastewater, and use their biomass in other fields, such as producing biofuels.

Keywords: Cyanobacterial species; Phycoremediation; Heavy metals; Wastewater; Physicochemical parameters

1. Introduction

Phycoremediation is a branch of ecological biotechnology that uses algae to treat pollutants [1], [2] as hydrocarbon waste, and other organic pollutants [3]. Algae are efficient and commercial biosorbents owing to their low nutrient requirements. [4] validated that the biosorption efficiency of algae has been reported as approximately 15.3–84.6% higher than other microbial biosorbents such as bacteria and fungi [5], [6] and [7]. [3] also confirmed that the bioremediation of different organic pollutants by microalgae and cyanobacteria is environmentally adequate green technology for the treatment of polluted water than other microorganisms and conventional methods, additionally, this contaminant promotes the growth of algal biomass and does not generate large amounts of secondary waste (sludge). Microalgae and Cyanobacteria are more efficient than plants due to their rapid development rate and low cultivation requirements [3] and are carried out in shallow contaminated areas for phycoremediation.

Phycoremediation has numerous advantages over other bioremediation processes as algal biomass can be applied in wastewater with higher metal concentration than for membrane processes [8]; no need to synthesize algal biomass; biomass can be regenerated and reused in several adsorption/desorption series; high uptake capacity and efficiency of HMs removal [9]; no sludge or toxic chemical produced; algal biomass can be applied in discontinuous and continuous regimes; by using dead biomass, no nutrient or oxygen supply needed; appropriate for anaerobic and aerobic effluent treatment units; algal biomass can be used all around year [10]; and cost-effective [11], and [12].

A variety of Cyanobacteria species have been recognized as promising candidates for HMs removal and/or detoxification, and potential low-cost alternatives to physicochemical remediation techniques [4]. The investment cost

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of biological processes is 5–20 times less than that of conventional chemical procedures. In comparison, the running cost is 3–10 times less than that of conventional procedures [13]. Furthermore, phycoremediation can be viewed as a form of permanent bioremediation, as it may result in the complete mineralization of pollutants, as well as a blue and circular economy [14] and [15].

Over five decades of research on wastewater treatment by microalgae can play a very important role in pollution bioremediation [16].HMs removal can be achieved by biosorption and bioaccumulation. The presence of heavy metal ions such as lead, copper, cadmium, zinc, and nickel as contaminants in wastewater leads to pollution of the natural environment [17]. The efficiency of HMs removal by algae is influenced by several parameters including pH, temperature, ionic strength, contact time, and presence of counter ions. Various HMs such as Mn^{2+}, Ni^{2+}, Cu^{2+}, Mo^{2+}, Fe^{2+}, and Zn^{2+} are essential to algal growth and are known as 'trace elements' or micronutrients. In contrast, other HMs, including Sn^{2+}, Au^{3+}, Cd^{2+}, Pb^{2+}, Sr^{2+}, Ti^{3+} and Hg^{2+} are toxic at high concentrations [18] and [1]. Detailed studies of the physiochemical composition of algal cells have helped in the efficacy of algae in environmental pollution control, particularly in HM removal from domestic and industrial wastewater. Some algae have shown exceptional tolerance and survival in water polluted with relatively high HM concentrations [19] and [20]. However, the efficiency of living algae cells during wastewater treatment is higher than that of dead biomass, as they can remove and retain a greater quantity of metals using both biosorption and bioaccumulation mechanisms for a longer period.

Cyanobacteria are cosmopolitan photoautotrophic bacteria that represent the largest and widest group of microorganisms. Their metabolic diversity represents a rich source of biotechnological instruments for sustainable development [21] and [22]. Their ability to survive in extreme conditions, comprising environments containing pesticides, petroleum by-products, radioactive compounds, crude oils, xenobiotics, and HMs, has drawn increasing interest from the scientific community, shedding light on the cellular mechanisms involved as well as their possible exploitation as a clean green technology for degradation or detoxification of contaminants [23]. Many studies have been carried out for soil and water bioremediation adopting cyanobacteria. Such a process is also named cyanoremediation [24] and [25]. However, the valorization of cyanobacterial biomass obtained after HMs bioremediation is still poorly investigated [26] and [27]. Our goal was to study the effectiveness of some types of cyanobacteria in eliminating or reducing the percentage of harmful heavy metals (eight heavy metals were chosen) present in wastewater and to determine the reduction rate in the plant-based treatment of wastewater to enable its safe use using selected three species of Cyanobacteria.

2. Materials and Methods

2.1. Study Area

Water samples were taken seasonally from August 2018 to August 2022 from El-Tagneed El-Gadida domestic wastewater lift station, Zagazig city branch, El Sharkia governorate. The station's coordinates are 30.34345N latitude and 31.30569E longitude.

2.2. Preparation of wastewater samples

The crude wastewater samples were collected in plastic jars (Approximately 200L) and transported immediately to the laboratory for physicochemical and heavy metals investigations. Initially, the collected samples were filtered by mesh to remove large and hard particles of raw wastewater then filtered by filter paper and stored at 4°C in the dark for further analysis.

2.3. The tested Cyanobacteria species

Cyanobacteria species were obtained from the phycology laboratory – faculty of science – Zagazig University. The tested cyanobacteria were *Arthrospira platensis, Oscillatoria simplicissima,* and *Nostoc muscorum*. These selected species were identified according to [28]; [29]; [30]; [31], and [32].

2.4. Cultivation of the tested Cyanobacteria

The selected strains of Cyanobacteria were allowed to grow into 1.5 liters of plastic bottles filled with 1 liter of crude wastewater after sterilization. Three replications of each species were set up. The algae bottles were incubated under continuous illumination with a light intensity of 3500 Lux at 30°C ± 2°C for 21 days.

2.5. Estimation of Physicochemical and nutrients parameters

Physical parameters pH, EC, TDS, BOD, and COD in wastewater samples were determined for 2 and 3 weeks of incubation as a control. The hydrogen ion concentration (pH) was measured by using a digital pH meter (CRISON pH-Meter GLP 21+) according to the method listed in [33]. Electrical Conductivity (EC) (μScm^{-1}) was evaluated by a conductivity meter (WTW cond 3110) as mentioned in [33]. Total dissolved solids (TDS) were calculated from EC readings by WTW cond 3110 [33]. The analysis of Biochemical Oxygen Demand (BOD) was performed using a VELP FOC 215 E incubator and EVO sensors [33]. Chemical Oxygen Demand (COD) is performed using the HANNA Hi 83099 COD and multiparameter bench photometer [33].

Chemical parameters were determined for 2 and 3 weeks of incubation. Ammonia (NH_3) was determined by the method of Nesslerisation according to [34] by using ST-uv-1901PC Double Beam Spectrophotometer US Lab having a range of 190 to 1100nm. Nitrite (NO_2) was determined by the Griess Reaction Method according to [33] via ST-uv-1901PC US Lab Double Beam Spectrophotometer having a range of 190 to 1100 nm. Nitrate (NO_3) was determined by the photometric method using Machery Nagel photometer PF-11. Total nitrogen (TN) was calculated as the total sum of ammonia (NH_3) + Nitrite (No_2) + Nitrate (NO_3) values. Total phosphorus (TP) was determined according to [33] using HANNA Hi 83099 COD and multiparameter bench photometer. The N/P ratio was calculated according to the data obtained from total nitrogen and total phosphorus.

2.6. Estimation of heavy metals

HMs were estimated in wastewater by using Perkinelmer Pin AAcle 900T atomic absorption device by flame technique [33]. The collected samples of wastewater were analyzed for eight elements Ag, Zn, Cd, Pb, Cu, Mn, Fe, and Ni [35].

2.7. Statistical analysis:

For data showing boxplots were carried out using PAST= Paleontological Statistics (Version 2.17). Multivariate and cluster analyses were performed to classify the three studied Cyanobacteria species, depending on all the quantitative characters obtained using PAST [36]. Data analyses of mean ± standard deviation (SD), for physicochemical parameters and N/P ratio removal were presented. Statistical analyses were performed using the SPSS 10.0 software (SPSS, Richmond, VA, USA).

3. Results

3.1. Effect of the cyanobacteria on physicochemical parameters of wastewater

The effect of the tested Cyanobacteria species on the values (**±**SD) and the reduction percentage (%) (**±**SD) of wastewater physical parameters after 2 and 3 weeks of incubation in comparison with control was listed in Table (1). The pH of the wastewater decreased after treatment with all three cyanobacteria species. The greatest reduction % in pH was observed with *Oscillatoria simplicissima* at both 2 (5.81 ± 0.94) and 3 (2.41 ±0.85) weeks. The EC of the wastewater decreased over time for all three cyanobacteria species. The maximum reduction% in EC was observed with *Arthrospira platensis* at both 2 (5.1 ± 0.06) and 3 (4.53 ± 0.006) weeks. There was a slight decrease in TDS observed between the 2 weeks and 3 weeks control period in all the tested species. The selected cyanobacteria species were effective in reducing BOD in wastewater. The 3 weeks control had a greater BOD reduction than the 2 weeks control. All three cyanobacteria species were able to reduce COD in the wastewater. The reduction was higher after 3 weeks compared to 2 weeks.

Table 1 The effect of the tested Cyanobacteria species on the values (±SD) and reduction percentage (%) (±SD) of physical parameters of wastewater after 2 and 3 weeks of incubation

Physical parameters of wastewater (±SD)		**pH**	**pH reduction %**	**E.C (μS/cm)**	**E.C reduction %**	**TDS (ppm)**	**TDS reduction %**	**BOD (ppm)**	**BOD reduction %**	**COD (ppm)**	**COD reduction %**
control / Cyanobacterial species		8.6 ± 0.02		1686 ± 1		1054 ± 1		44.8 ± 0.2		79 ± 0.2	
2 weeks	*Arthrospira platensis*	8.2 ± 0.05	4.68 ± 0.40	1600 ± 2	5.1 ± 0.06	1000 ± 2	5.12 ± 0.1	41.5 ± 0.03	7.36 ± 0.34	72 ± 0.3	8.86 ± 0.15
	Oscillatoria simplicissima	8.1 ± 0.1	5.81 ± 0.94	1650 ± 2	2.13 ± 0.06	1031 ± 1	2.18 ± 0.2	42.6 ± 0.05	4.91 ± 0.31	71 ± 0.3	4.91 ± 0.31
	Nostoc muscorum	8.4 ± 0.05	2.32 ± 0.35	1618 ± 1	4.03 ± 0.01	1011 ± 4	4.08 ± 0.3	41.7 ± 0.4	6.91 ± 0.33	65 ± 0.3	17.72 ± 0.2
control / Cyanobacterial species		8.3 ± 0.03		1500 ± 1		938 ± 2		54.6 ± 0.02		84.2 ± 0.05	
3 weeks	*Arthrospira platensis*	8.2 ± 0.04	1.2 ± 0.125	1432±1	4.53 ± 0.006	895±5	4.58 ± 0.32	20.1 ± 0.05	63.2 ± 0.08	29 ± 0.2	65.6 ± 0.22
	Oscillatoria simplicissima	8.1 ± 0.1	2.41 ±0.85	1466±1	3.53 ±0.065	904±2	3.62 ±0.41	21.4 ± 0.04	60.8 ± 0.06	32 ± 0.12	62 ± 0.12
	Nostoc muscorum	8.2 ± 0.03	1.2 ± 0.006	1450± 2	3.33 ± 0.07	906± 2	3.74 ± 0.57	27.9 ± 0.05	48.9 ± 0.07	35 ± 0.15	58.4 ± 0.16

On the contrary, the boxplot showed the reduction percentages of various physical parameters over different incubation times (Fig., 1). The most significant reductions are observed in BOD and COD, especially after 3 weeks of incubation. *N. muscorum* achieved high values after 2 weeks of removal percentage (17.72%) whereas *A. platensis* (65.56%), had the highest one after 3 weeks. Generally, cluster analysis of the tested Cyanobacteria species (Fig., 2) indicated that *N. muscorum* was present in one group and was separated with a high dissimilarity factor and it was the lowest effective removal % in physical parameters than *A. platensis* and *O. simplicissima* which were grouped closely together in one sub-group and they have a higher degree of similarity to each other.

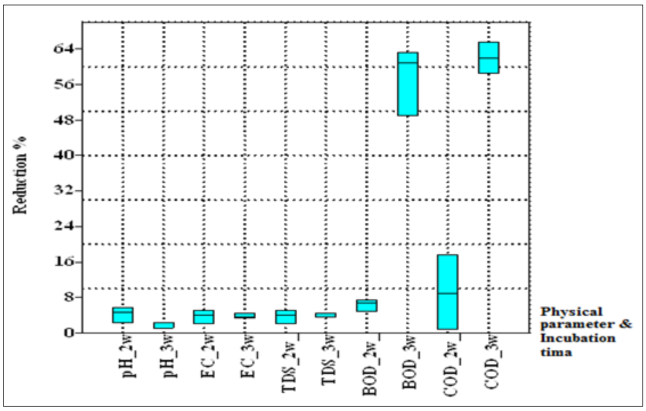

Figure 1 Boxplot of the reduction % of physical parameters of wastewater after 2 and 3 weeks by the tested Cyanobacteria species

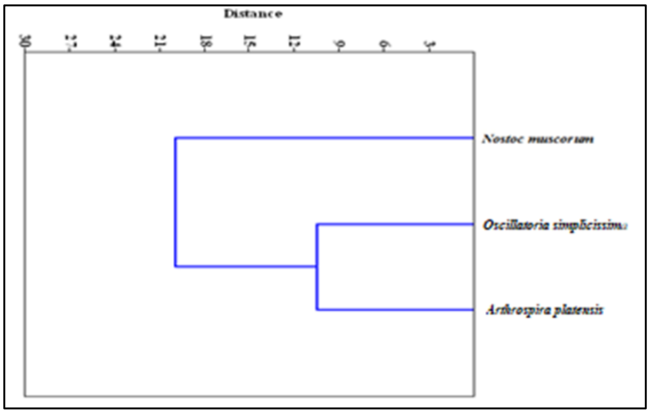

Figure 2 Cluster analysis of the tested Cyanobacteria showing the effect on the reduction % of physical parameters of wastewater

Table (2) clarified the concentration and removal % of the chemical parameters from wastewater by the tested Cyanobacteria species over two different incubation times, 2 weeks and 3 weeks. All the tested cyanobacteria species were effective in reducing NH3 over a longer treatment time in wastewater. NH_3 reduction was generally higher after three weeks of treatment compared to two weeks. *Nostoc muscorum* showed the highest NH_3 reduction efficiency, achieving over 96% reduction (98.33±0.01) in three weeks. The three cyanobacteria species were effective in reducing NO2 concentration in the wastewater. At 2 weeks, there was no significant NO2 reduction observed in any of the cyanobacteria treatments compared to the control. After 3 weeks, all three cyanobacteria species achieved a NO_2 reduction of over 90%. The tested cyanobacteria species were effective in removing nitrates NO_3 from wastewater.

The removal % by *A. platensis* was the highest in NO_3 (96.67%). The percentage of total nitrogen decreased after treatment with the selected cyanobacteria for two and three weeks. There was a significant reduction in TN after both two and three weeks of treatment with all three cyanobacteria species. *Arthrospira platensis* achieved a reduction % to reach 87.06±0.72 after 3 weeks. All three cyanobacteria species were effective in reducing TP concentration compared to the control. The TP reduction appears to be greater for longer treatment times. After two weeks of treatment, the TP reduction ranged from 24% to 28%. After three weeks of treatment, the TP reduction was even greater, ranging from 43% to 69%. *N. muscorum* recorded the maximum removal % after 2 and 3 weeks.

Boxplot of the chemical parameters of wastewater (Fig., 3) showed that *A. platensis* after 2 weeks achieved a low removal % to reach the minimum one (0%) with NO_2 but the removal % increased after 3 weeks to 96.67%. In general, cluster analysis of the selected Cyanobacteria species (Fig.,4) of physical parameters reduction % designated that *A. platensis* was present in one group and it was separated with a high dissimilarity factor since it was the lowest effective removal % in chemical parameters of wastewater than *O. simplicissima* and *N. muscorum* which were sharing in one sub-group.

Table 2 The effect of the tested Cyanobacteria species on the values (±SD) and reduction percentage (%) (±SD) of chemical parameters of wastewater after 2 and 3 weeks of incubation

Chemical parameters of wastewater (±SD)		**NH_3 conc.**	**NH_3 reduction %**	**No_2 conc.**	**No_2 reduction %**	**No_3 conc.**	**No_3 reduction %**	**TN conc.**	**TN reduction %**	**TP conc.**	**TP reduction %**
control / Cyanobacterial species		60 ± 0.3		0.4 ± 0.01		2.6 ±0.02		63.9±0.003		5 ± 0.1	
2 weeks	*A. platensis*	56± 0.2	6.66± 0.14	0.4± 0.01	0	1.9± 0.02	26.92± 0.2	58.2±0.12	7.61±0.38	3.8± 0.05	24± 0.52
	O. simplicissima	44± 0.2	26.66±0.04	0.1± 0.01	75.03±1.86	2± 0.2	23.11± 7.1	46.2±0.56	26.66±0.52	3.7± 0.04	26± 0.68
	N. muscorum	54± 0.6	10± 0. 55	0.1± 0.01	75.03±1.86	1.4± 0.02	46.15±0.35	55.5±0.63	11.9±0.53	3.6± 0.01	28± 1.24
control / Cyanobacterial species		6± 0.2		3± 0.1		3.2± 0.02		12.2± 0.32		3.9 ± 0.04	
3 weeks	*A. platensis*	0.48±0.02	96.67±0.23	0.1± 0.01	96.67±0.23	1± 0.1	96.67±0.23	1.58±0.13	87.06±0.72	2.2± 0.01	43.59±0.33
	O. simplicissima	0.72±0.01	90±0.33	0.3± 0.2	90 ±0.33	1.5± 0.01	53.12±0.02	2.52±0.04	79.34±0.21	1.9± 0.04	51.28±0.53
	N. muscorum	0.92±0.01	98±.33±0.01	0.05±0.01	98.33±0.01	2.5± 0.01	21.87±0.18	3.47±0.02	71.54±0.56	1.2± 0.03	69.23±0.46

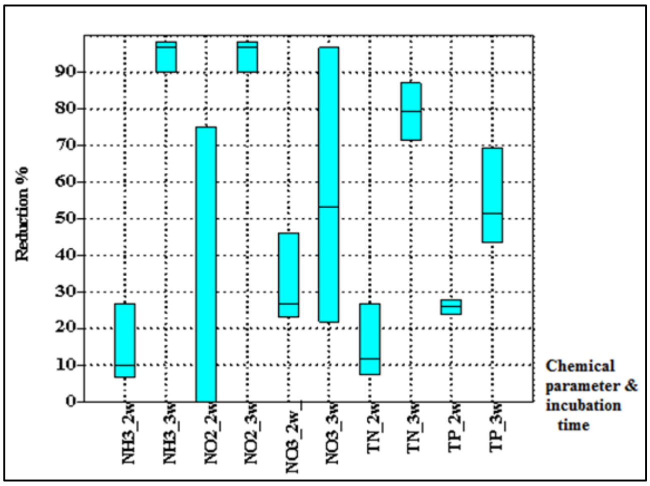

Figure 3 Boxplot of the reduction % of chemical parameters of wastewater after 2 and 3 weeks by the tested Cyanobacteria species

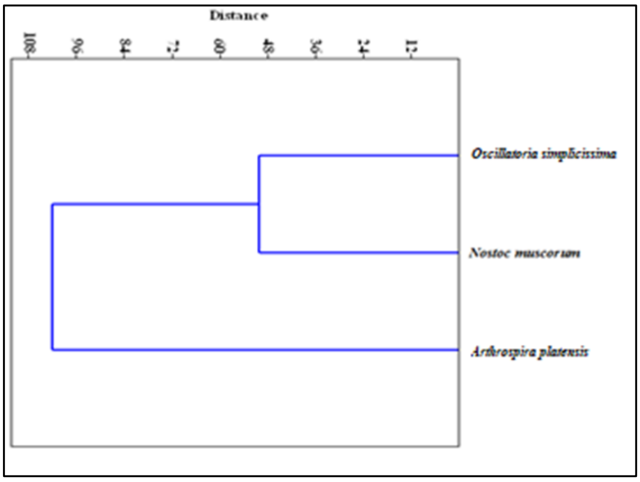

Figure 4 Cluster analysis of the tested Cyanobacteria showing the effect on the reduction % of chemical parameters of wastewater

3.2. Impact of cyanobacteria on nitrogen and phosphorus removal

The N/P ratio was calculated after the growth of the Cyanobacteria species for 2 and 3 weeks of incubation in comparison with a blank of crude wastewater as shown in Table (3) and Fig. (5). Overall, the tested Cyanobacteria species after 2 weeks recorded increasing of removal % than after 3 weeks. The data (±SD) showed that the removal % of the N/P ratio increased after 2 weeks of incubation as follows *N. muscorum* (15.41±0.13) and *A. platensis* (15.31±0.1) > *O. simplicissima* (12.48±0.025) as for the blank (12.59±0.185). After 3 weeks, the removal % of the N/P ratio decreased than the blank (3.12±0.05) as *N. muscorum* (2.88±0.055)> *O. simplicissima* (1.32±0.01)> *A. platensis* (0.71±0.055). Cluster analysis of the tested Cyanobacteria species on the reduction % of N/P ratio (Fig., 6) showed that *N. muscorum* and *A. platensis* were the most effective removal % which was related in one sub-group where *O. simplicissima* was the lowest efficient removal % and separated with high dissimilarity factor than the other selected species.

Table 3 The effect of the tested Cyanobacteria species on the N/P ratio (±SD) in wastewater after 2 and 3 weeks

N/P (ppm) (±SD)	**N/P (ppm) 2W**	**N/P (ppm) 3W**
control / Cyanobacterial species	12.59±0.185	3.12±0.05
Arthrospira platensis	15.31±0.1	0.71±0.055
Oscillatoria simplicissima	12.48±0.025	1.32±0.01
Nostoc muscorum	15.41±0.13	2.88±0.055

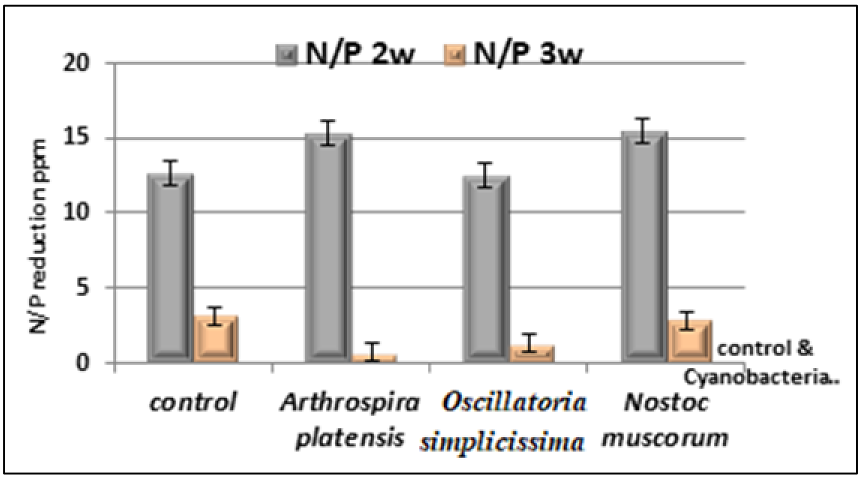

Figure 5 The effect of the tested Cyanobacteria species on the N/P ratio after a 2 and 3-week incubation period

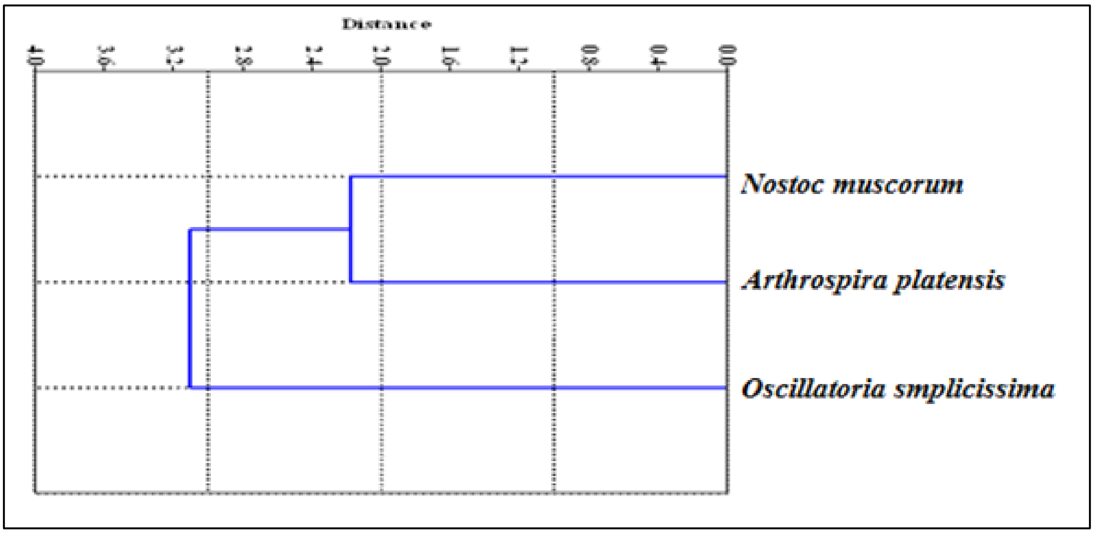

Figure 6 Cluster analysis of the tested Cyanobacteria species on the reduction % of N/P ratio of wastewater

3.3. Effect of cyanobacteria on removal of heavy metals

The concentration of various heavy metals in wastewater treated with different cyanobacteria species after 2 and 3 weeks of incubation was listed in Table (4). The concentrations of all heavy metals are generally higher in the control compared to those treated with cyanobacteria. *A. platensis* appeared to be more effective at reducing lead (Pb) compared to the other species. The concentration of some metals, such as Nickel (Ni), appears to decrease over time (from 2 weeks to 3 weeks) in all treatment groups, including the control.

The efficiency of the tested Cyanobacteria species to removal (%) of Ag, Zn, Cd, Pb, Cu, Mn, Fe, and Ni from wastewater after 2 and 3 weeks of incubation is summarized in Table (5) and box plot Fig. (7). All three blue-green algae species were able to remove some amount of heavy metals from the wastewater. In general, the percentage reduction increased with treatment duration (from 2 weeks to 3 weeks). *Nostoc muscorum* showed the highest overall removal efficiency for most metals, particularly for cadmium, lead, and nickel, achieving nearly 100% reduction after 3 weeks. All the tested Cyanobacteria species recorded the highest removal % of Mn after 2 weeks as follows: *O. simplicissima* (99.3%)> *N. muscorum* (97.9%)> *A. platensis* (96.7%) to reach 100% after 3 weeks. *A. platensis* demonstrated the strongest removal capability for five heavy metals as Zn (6.3%), Pb (46.8%), Cu (72.4%), Mn (100%) and Fe (50.9%). *N. muscorum* effectively reduced four heavy metals as Ag (57.5%), Cd (28.6%), Pb (47.7%), and Mn (100%). Finally, *O. simplicissima* achieved 100% removal for Manganese and 54.0% reduction for Nickel.

Table 4 The concentration of heavy metals and the control of the tested Cyanobacteria species from wastewater after 2 and 3 weeks of incubation

Parameters of wastewater (±SD)		Ag	Zn	Cd	Pb	Cu	Mn	Fe	Ni
control / Cyanobacteria species		0.0055 ± 0.0005	0.137 ± 0.002	0.0096 ±0.0002	0.102 ± 0.004	0.013 ±0.02	0.581±0.004	0.399 ± 0.003	0.065 ± 0.003
2 weeks	*A, platensis*	0.0035 ± 0.0005	0.12 ± 0.003	0.0083 ±0.0002	0.098 ±0.002	0.011±0.002	0.019±0.003	0.308 ±0.002	0.032±0.003
	O. Simplicissima	0.0035 ± 0.0015	0.134 ± 0.004	0.009 ± 0.0002	0.065 ±0.002	0.008±0.0005	0.004±0.003	0.302 ±0.004	0.045±0.003
	N. muscorum	0.0015 ± 0.0005	0.114 ± 0.001	0.0095 ±0.0002	0.046 ±0.002	0.011±0.001	0.014±0.004	0.245 ±0.003	0.052±0.002
control / Cyanobacteria species		0.005 ± 0.001	0.1025±0.0005	0.0084 ±0.0001	0.092 ± 0.003	0.014 ± 0.002	0.14 ±0.001	0.45 ±0.005	0.063 ±0.002
3 weeks	*A. platensis*	0.005 ± 0.001	0.096 ± 0.004	0.0079 ±0.0001	0.049 ± 0.002	0.004±0.002	0	0.221 ±0.004	0.05±0.002
	O. simplicissima	0.005 ± 0.001	0.102 ± 0.0002	0.0072 ±0.0003	0.087 ±0.002	0.007±0.002	0	0.382 ±0.003	0.029±0.003
	N. muscorum	0.002 ± 0.0005	0.1 ± 0.0007	0.006 ± 0.0001	0.048 ±0.002	0.01±0.002	0	0.389 ±0.003	0.057±0.004

Table 5 The effect of the tested Cyanobacteria species on the removal (%) of heavy metals from wastewater after 2 and 3 weeks of incubation

Parameters of wastewater (±SD)		Ag reduction %	Zn reduction %	Cd reduction %	Pb reduction %	Cu reduction %	Mn reduction %	Fe reduction %	Ni reduction %
2 weeks	*Arthrospira platensis*	36.56 ± 3.33	12.41 ± 0.003	13.54 ± 0.28	3.87 ±1.81	15.63±2.43	96.73±0.495	23.06 ±0.17	50.84±2.35
	Oscillatoria simplicissima	37.67 ± 21.69	2.2 ± 1.49	9.32 ± 3.97	36.15 ±4.47	38.87±5.77	99.31±0.515	24.31 ±0.435	30.81±1.42
	Nostoc muscorum	73.13 ± 6.67	16.78 ± 0.48	1.04 ± 0.02	54.80 ±3.73	14.82±5.47	97.92±1.21	38.60 ±0.29	19.98±0.62
3 weeks	*Arthrospira platensis*	0	6.32 ± 4.36	5.93 ± 2.31	46.75 ± 0.43	72.42±10.44	100.00	50.89 ±0.345	20.64±0.65
	Oscillatoria simplicissima	0	0.293 ± 0.195	14.3 ± 2.55	5.41 ± 0.91	50.69±7.31	100.00	15.10 ±0.275	54.04±3.3
	Nostoc muscorum	57.5 ± 18.87	2.44 ± 0.5	28.57 ± 0.34	47.74 ± 3.88	28.96±4.17	100.00	13.55 ±0.295	9.59±3.48

In general, cluster analysis of the tested Cyanobacteria species showed that *N. muscorum* was the most distinct removal % of HMs which separated with a higher dissimilarity factor than the other selected species *O. simplicissima* and *A. platensis* which were related in one sub-group due to their closer similarity (Fig; 8).

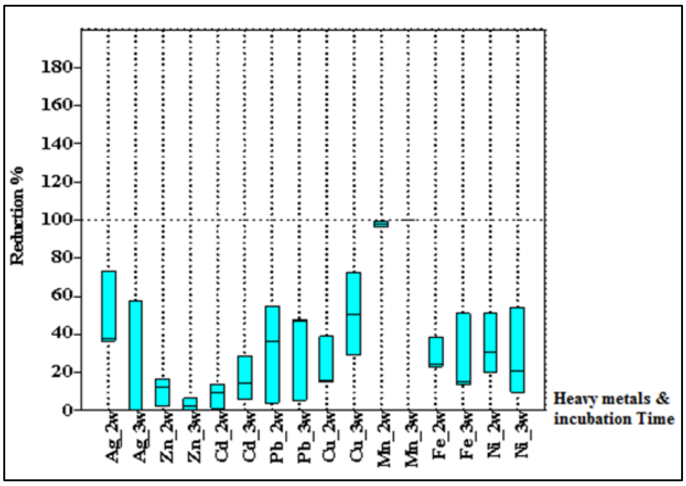

Figure 7 Boxplot of the removal% of heavy metals of wastewater after 2 and 3 weeks by the tested Cyanobacteria species

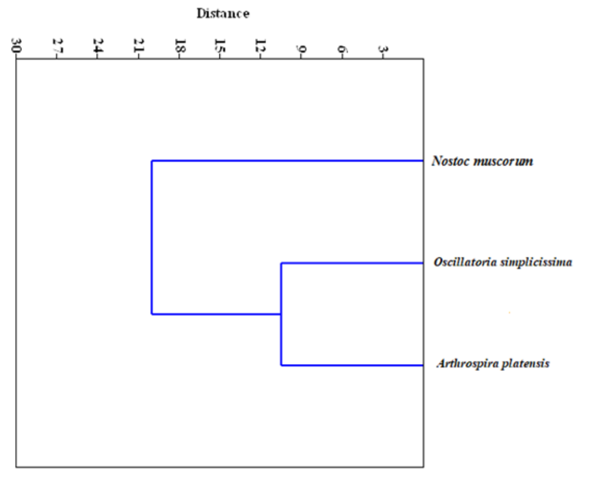

Figure 8 Cluster analysis of the tested Cyanobacteria species on the reduction % of heavy metals in wastewater

4. Discussion

Phycoremediation by Cyanobacteria is considered an effective agent for HM biosorption of wastewater treatment. Phycoremediation depends principally on the biosorption and bioaccumulation abilities of algae, with biosorption leading to the bioremediation process [37] and [38]. Cyanobacteria possess a remarkable ability to absorb and accumulate HMs from water mostly from wastewater by either ionic or covalent bonding [16]. Their fast growth rates and large surface area-to-volume ratio make them efficient at capturing these pollutants [5].

The physical parameters of wastewater recorded a high reduction % by the tested Cyanobacteria species after 2 weeks of incubation then decreased after 3 weeks. pH, EC, and TDS indicated slight changes or minimal reduction in these physical parameters during the period of study. TDS appears to be a major indicator of wastewater quality, with lower TDS indicating better quality. This is attributed to these parameters being less affected by the incubation process and strongly influencing the sorption capacity of HMs; however, the process can occur within a wide pH range [39]. [40] also revealed that low pH affects the ability of microalgae to absorb nutrients and reduce the activity of enzymes implicated in photosynthesis. Significant reduction in BOD and COD after 3 weeks compared to 2 weeks recognized to enhance reduction over time. The longer treatment time allowed for more BOD and COD to be removed from the wastewater. [41] also recorded the highest removal efficiency of COD and BOD in the mixotrophic growth with *Scenedesmus parvus*. *N. muscorum* had the lowest effective removal % in physical parameters than *A. platensis* and *O. simplicissima*. The efficiency of the incubation process on the different physical parameters, meaning these parameters were more significantly reduced over time.

There was a noticeable increase in the effectiveness of the reduction % with longer incubation times for most chemical parameters. The reduction of ammonium (NH_4) and nitrate (NO_3) showed significant improvement with an extended incubation time. The nitrite (NO_2) reduction showed a wide range for both incubation times but with the excess of a long period. Total nitrogen (TN) and total phosphorus (TP) demonstrated moderate progress in reduction % with elongating the incubation times. So, prolonged treatment or incubation times commonly lead to higher reduction percentages of these chemicals. *A. platensis* had the lowest effective removal % in chemical parameters of wastewater than *O. simplicissima* and *N. muscorum.*

The tested Cyanobacteria species recorded increasing in N/P ratio removal % after 2 weeks more than after 3 weeks except for the control. *N. muscorum* and *A. platensis* were the most effective removal % whereas *O. simplicissima* was the lowest efficient removal %. [42] showed that inorganic nutrients are necessary for the growth of algae, particularly phosphate and nitrogen. Cyanobacteria can not only remove heavy metals but also help treat wastewater by consuming excess nitrogen and phosphorus. This contributes to a cleaner overall effluent [43].

Different species of Cyanobacteria utilize various mechanisms to link with HMs which include biosorption i.e. binding to cell walls. In our study, the reduction percentages varied significantly according to the incubation times. Furthermore, the efficiency of Cyanobacteria can fluctuate depending on the type of HMs. The effectiveness of the tested Cyanobacteria species recorded a high removal % of Mn after 2 weeks as follows: *O. simplicissima* > *N. muscorum* > *A. platensis* to reach 100% after 3 weeks by all the selected species. [44] mentioned that manganese, a nutrient that is essential for microalgae, prevents algal growth. Significant amounts of Mn (III/IV) oxides were found in both intracellular and extracellular, resulting from Mn (II) oxidation. This suggests that photosynthetic algae may modify the Mn cycle by converting soluble Mn (II) to intracellular bound Mn and subsequently to solid-state Mn (III/IV) oxides. Through indirect oxidation, microalgae may also accelerate Mn (II) oxidation by raising the pH of the solution and producing more dissolved oxygen as they grow.

The removal % values of Cu, Pb, and Zn ranged as 38.9 - 50.7, 36.1 - 5.4, and 2.2- 0.3% respectively during 2 - 3 weeks of incubation *O. simplicissima*. [9] mentioned that *Oscillatoria quadripunctulata* showed HMs removal capacity of 37-50 % for copper, 35-100% for lead, and 32-100% in the case of zinc from the sewage and petrochemical industry effluent. Over three weeks, different cyanobacteria species demonstrated varying abilities to remove heavy metals (HMs) from the wastewater. After two weeks, *N. muscorum* significantly reduced high levels of five heavy metals (Ag, Zn, Pb, Mn, and Fe). Following this, *A. platensis* reduced three heavy metals (Cd, Mn, and Ni), then *O. simplicissima* reduced two heavy metals (Cu and Mn). After three weeks, *A. platensis* achieved the highest removal efficiency for five heavy metals (Zn, Pb, Cu, Mn, and Fe). Next, *N. muscorum* removed four heavy metals (Ag, Cd, Pb, and Mn) and finally, *O. simplicissima* maintained its ability to remove two HMs (Mn and Ni). The study also revealed that some metals, such as Pb and Cu, showed higher reduction rates with longer incubation times. In contrast, other metals, such as Ni and Zn, did not exhibit a clear tendency. [4] stated that the rate of adsorption is rapid at the beginning which gets slow with time due to the non-availability of unoccupied binding sites.

This study recorded that Cu^{2+} was removed regularly 15.6 and 72.4% after 2 and 3 weeks respectively by *A. platensis.* [45] and [6] proved that *A. platensis* removed 91 % of Cu2+ after cultivation in municipal wastewater. Generally, *N. muscorum* was the most distinct removal % of HMs than *O. simplicissima* and *A. platensis*. [46] also confirmed that Cyanobacteria *Nostoc* sp. possess a high capacity for HMs removal from aqueous solution. [47] reported that microalgae have proven to be excellent biosorbent substances and to be very successful at clearing pollutants out of a variety of water environments. This is pointer agreement with many studies as [48]; [49]; and [50] who suggest that several Cyanobacteria genera, such as *Anabaena, Cyanobium, Nostoc, Cyanothece, Arthrospira, Microcystis, Synechocystis*, and *Leptolyngbya*, have shown promising results on Cu, Cd, Zn, Cr, Pb, Ni, Co or Hg removal.

The biomass produced from cyanobacteria and grown in polluted water, has accumulated within it harmful heavy metals, some of which may affect plants and at the same time human health, except their use in growing plants that are not suitable for food or feeder and used as ornamental plants, for example biofuel production or fertilizer creation [4] and [23]. Overall, Cyanobacteria offer a promising and eco-friendly approach to HMs and nutrient removal from wastewater.

5. Conclusion

Certain cyanobacterial species as *Arthrospira platensis*, *Oscillatoria simplicissima*, and *Nostoc muscorum* were utilized to remove the heavy metals from wastewater. The effectiveness of phycoremediation in this study depends on various factors, including physicochemical parameters, Nutrients, incubation period, specific cyanobacterial species, wastewater composition, and high metal adsorption capacity. Cyanobacteria can employ nutrients like nitrogen (TN) and phosphorus (TP) present in wastewater for their growth, promoting their remediation potential. The ability of the tested cyanobacteria species to absorb heavy metals from wastewater can offer a sustainable and eco-friendly process to cooperate and purify the wastewater.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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