



# Assessment of Growth Promoting Ability of Three Cyanobacterial Isolates Under Sewage Water Irrigation

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## ABSTRACT

Heavy metal pollution from the increased use of sewage effluent for irrigation is posing a hazard to agricultural ecosystems. Thus, a reliable and simple method of reducing the impact of heavy metals on plant growth is required. In the present study, cyanobacterial species were isolated from the sewage water irrigated soil of Rohtak city, Haryana, India, and characterized by various biochemical parameters. The cyanobacterial filtrates were then used to analyze their effects on the growth performance of rice seedlings under various concentrations of sewage wastewater. The results revealed a statistically significant increase in biomass, photosynthetic pigments, nitrate reductase activity, plumule, and radical length of rice seedlings by application of cyanobacterial filtrates. The antioxidant system (peroxidase enzyme and catalase enzyme activity) was also found to be stimulated. As compared to introduced species, the extracts of isolated species had a more favorable, statistically significant effect on rice seedlings. Our study indicated that these isolates have a high tolerance against heavy metals and are potentially useful as biofertilizers for the crops in sewage water irrigated agroecosystems.

## INTRODUCTION

In India, sewage wastewater is being extensively used for irrigation purposes. Increasing urbanization and industrial activities contribute to the increase in heavy metals in sewage wastewater which affects both agricultural and aquatic ecosystems (Mohod & Dhote 2013). The sewage water irrigation negatively affects the plant's cellular and physiological processes resulting in the reduced growth of the plant. The general toxicity of heavy metals, as well as their inhibition of water intake, has an impact on seed germination (Kranter & Colville 2011). However, at lower concentrations, some of the constituents of sewage water are beneficial to plant growth.

The accumulation of heavy metals in the soil necessitates the development of cost-effective, simple-to-use techniques for the bioremediation of sewage-irrigated soil. The native microbes are suitable candidates to be used for soil bioremediation as they can change their morphology and physiology after coming in contact with various toxicants and heavy metals. These changes may indicate their tolerance towards heavy metals. Among the various microbes, cyanobacteria are known to have protective mechanisms against different types of stresses. Their abundance, growth, and biomass reflect the characteristics of the specific habitat in which they live and can be linked to environmental impacts. The photosynthetic behavior of cyanobacterial species shows the presence of

heavy metals. When used for bioremediation, cyanobacteria promoted the establishment of microbial population, increased organic matter, and also soil stability (Alvarez et al. 2021). They fix atmospheric nitrogen and produce growth-promoting substances, vitamins, auxins, amino acids, and sugars (Suresh et al. 2019) which are helpful in plant growth. Stabilization of disturbed ecosystems like sewage irrigated soil is dependent upon the successful establishment of the most effective cyanobacterial community.

In this study, comparative effects of selected, isolated strains of cyanobacteria were examined on various growth parameters, nitrate reductase activity, and antioxidant system responses in rice seedlings under different concentrations of sewage water, using Petri-culture experiments

## MATERIALS AND METHODS

### Isolation and Culture of Cyanobacterial Species

Cyanobacterial species were isolated from sewage water irrigated soil (sandy loam) of Rohtak city, Haryana, India, by agar plate spreading and serial dilution techniques (Andersen & Kawachi 2005) using BG-11 medium (Stanier et al. 1971) and were identified as *Oscillatoria* sp., *Lyngbya* sp., and *Phormidium* sp. with the help of keys given by Desikachary (1959). Purified cyanobacterial strains were maintained at  $28 \pm 3^\circ\text{C}$  and illuminated under a 16:8 hr light-dark cycle

using cool fluorescent tubes. The introduced cyanobacterial species were obtained from the Centre for Conservation of Blue-Green Algae, I.A.R.I. New Delhi

The chlorophyll content in cyanobacteria was estimated by the standard methods of Mckinney (1941) and carotenoid pigments by Jensen (1970). Phyco-biliproteins were estimated by the cold extraction method by Siegelman & Kycia (1978). Total proteins were estimated by the method of Herbert et al. (1971). Total carbohydrates were estimated by the anthrone reagent method (Spiro 1966).

The 15-day old cultures of both isolated and introduced species of *Oscillatoria*, *Lyngbya*, and *Phormidium* were taken and centrifuged at 10000 rpm for 20 minutes. The supernatant was collected and concentrated to 10% of its original volume.

### Petriculture Experiments

The twenty surface-sterilized rice seeds (variety P-1121) in each Petri plate were kept at 32°C temperature inside the BOD incubator and moistened with 10 mL of sewage wastewater regularly. The concentrations of the sewage wastewater used were 25%, 50%, 75%, and 100%. After three days, 5 mL of concentrated supernatants of both native and introduced species of *Oscillatoria*, *Lyngbya*, and *Phormidium* were added to the germinating rice seedlings and incubated for a further period of five days. The rice seedlings without cyanobacterial amendments were used for comparison purposes. The growth parameters of rice seedlings were measured on the ninth day.

Photosynthetic pigments estimation was done following the formula given by Gupta (2000). The biomass of rice

seedlings was calculated according to the procedure of Allen et al. (1974). For catalase estimation, the decomposition of H<sub>2</sub>O<sub>2</sub> was measured by recording the decline in absorbance at 240 nm for 3 min. following the method of Aebi (1984). Peroxidase was assayed as described by Pundir et al. (1999). Nitrate reductase activity was assayed by following the method of Srivastava (1975). All experiments were performed in triplicates.

The data obtained were subjected to mean and standard deviation. Analysis of variance tests was used to evaluate the differences among the various treatments.

## RESULTS AND DISCUSSION

The preliminary study showed that the presence of heavy metals (Cu, Zn, Ni, Cd) affects the chlorophyll content and protein synthesis in isolated cyanobacterial species in the order Ni>Cu>Zn>Cd (Dhankhar & Rana 2016). Generally, all heavy metals slow down the growth performance of cyanobacterial species. Table 1 shows the decreased concentrations of protein, chlorophyll, and phycobilin pigment content in cyanobacteria isolated from sewage water irrigated soil as compared with the corresponding values in the introduced cyanobacterial species. The reduction in protein and pigment synthesis is caused by the long exposure to toxicants and heavy metals present in sewage water irrigated soil. The amount of secondary metabolites products i.e. sugars were found to be increased under stress conditions (Hana et al. 2008). Among the isolated species, *Oscillatoria* sp. has the highest chlorophyll content followed by *Lyngbya* sp. and *Phormidium* sp. The maximum contents of phycobiliproteins (Phycocyanin, allophycocyanin, and phycoerythrin

Table 1: Biochemical constituents of isolated strains of cyanobacteria from sewage irrigated soil.

Biochemical constituents	Isolated <i>Oscillatoria</i> sp.	Introduced <i>Oscillatoria</i> sp.	Isolated <i>Lyngbya</i> sp.	Introduced <i>Lyngbya</i> sp.	Isolated <i>Phormidium</i> sp.	Introduced <i>Phormidium</i> sp.
Protein	2.75	4.15	1.8	4.8	4.1	4.62
Sugars	2.96	2.4	3.05	2.34	3.02	2.13
Chl.	1.42	1.58	0.928	1.23	1.07	1.39
Chl.a	0.125	0.0973	0.0887	0.135	0.072	0.079
Phycocyanin	1.6	1.06	0.88	1.26	1.82	1.07
Phycocyanin	0.114	1	1.04	1.31	1.19	1.35
Phycoerythrin	4.78	6.29	4.59	8.09	8.79	12.19

Table 2: Effect of cyanobacterial extracts of native and introduced cyanobacterial species on shoot length of rice seedlings under various sewage water concentrations.

Sewage water conc.	Isolated <i>Lyngbya</i> sp.	Isolated <i>Oscillatoria</i> sp.	Isolated <i>Phormidium</i> sp.	Introduced <i>Lyngbya</i> sp.	Introduced <i>Oscillatoria</i> sp.	Introduced <i>Phormidium</i> sp.	Control
25	5.1±0.650	3.83±0.080	3.82±0.875	4.84±0.40	2.37±0.802	2.46±0.645	3.67±0.33
50	6.95±0.57	4.76±0.780	5.37±0.615	6.12±0.51	2.85±0.600	3.38±1.20	5.02±0.377
75	3.92±0.45	4.17±0.665	3.85±0.597	4.04±0.20	2.17±0.270	1.87±1.03	3.8±0.581
100	1.82±0.62	3.28±0.786	2.92±0.790	3.67±0.56	1.72±0.875	1.78±0.575	2.9±0.270

Table 3: Effect of cyanobacterial extracts of native and introduced cyanobacterial species on root length of rice seedlings under various sewage water concentrations.

Sewage water conc.	Isolated <i>Lyngbya</i> sp.	Isolated <i>Oscillatoria</i> sp.	Isolated <i>Phormidium</i> sp.	Introduced <i>Lyngbya</i> sp.	Introduced <i>Oscillatoria</i> sp.	Introduced <i>Phormidium</i> sp.	Control
25	4.84±0.142	4.18±0.185	3.4±0.148	2.75±0.55	0.75±0.218	1.58±0.156	3.41±0.285
50	6.12±0.062	4.2±0.638	4.2±0.236	6.2±0.451	2.02±0.715	3.12±2.04	3.1±0.596
75	4.04±0.601	5.15±0.795	2.55±0.530	3.52±0.65	4±0.332	3.02±0.610	2.2±0.730
100	3.67±0.775	3.06±0.517	3.02±0.625	2.26±0.89	2.1±1.50	2.46±0.465	2±0.497

Table 4: Effect of cyanobacterial extracts of native and introduced cyanobacterial species on the biomass of rice seedlings under various sewage water concentrations.

Sewage water conc.	Isolated <i>Lyngbya</i> sp.	Isolated <i>Oscillatoria</i> sp.	Isolated <i>Phormidium</i> sp.	Introduced <i>Lyngbya</i> sp.	Introduced <i>Oscillatoria</i> sp.	Introduced <i>Phormidium</i> sp.	Control
25	26.0±0.44	32.2±0.975	20.56±0.262	13.2±0.68	16.21±0.555	19.1±0.652	28.7±0.551
50	29.6±0.45	33.87±0.366	21.31±0.32	16.7±0.60	18.84±0.565	22.3±0.71	29.6±0.147
75	24.2±0.327	20.91±0.59	18.44±0.459	12±0.521	17.68±0.446	16.9±0.621	24.7±0.489
100	20.6±0.803	18.67±0.305	16.85±0.852	11.6±0.24	13.84±0.838	14.3±0.587	22.8±0.753

Table 5: Effect of cyanobacterial extracts of native and introduced cyanobacterial species on total chlorophyll content of rice seedlings under various sewage water concentrations.

Sewage water conc.	Isolated <i>Lyngbya</i> sp.	Isolated <i>Oscillatoria</i> sp.	Isolated <i>Phormidium</i> sp.	Introduced <i>Lyngbya</i> sp.	Introduced <i>Oscillatoria</i> sp.	Introduced <i>Phormidium</i> sp.	Control
25	1.79±0.015	1.85±0.011	1.83±0.007	1.66±0.04	1±0.125	0.57±0.037	1.8±0.011
50	2.141±0.05	2±0.0642	2.409±0.02	1.54±0.04	1.59±0.024	0.861±0.003	1.418±0.015
75	1.67±0.02	2.28±0.072	2.1±0.141	1.3±0.03	0.873±0.004	0.775±0.005	1.12±0.045
100	0.817±0.009	1.76±0.02	1.527±0.032	1±0.077	0.734±0.006	0.762±0.022	0.792±0.023

are protein complexes from the phycobiliprotein family) were found in *Phormidium* sp. The variations observed in pigment content of the isolated species may be caused by environmental and genetic factors (Ismaiel et al. 2014).

The exogenous cyanobacterial treatments alleviated the inhibitions caused by sewage wastewater on the plumule and radical length of rice seedlings, as is evident from Tables 2 and 3. All the culture filtrates cause a significant increase ( $p \leq 0.05$ ) in the seedling growth under various sewage water concentrations. This might be due to the production of active compounds like hormones and vitamins from cyanobacteria, that inhibit the growth of pathogenic bacteria and fungi and increase plant growth (Grzesik & Duda 2014, Mohan & Kumar 2015, Suresh et al. 2019). The biomass content also showed a statistical increase (Table 4) in all the treatments. The increase in root length and biomass of rice by cyanobacterial treatments was also reported (Zhou et al. 2020). The extracts of native cyanobacteria had a more positive effect on the growth of rice seedlings at fifty percent sewage water concentrations than introduced cyanobacterial exudates. Further marked reductions were observed with

higher concentrations of sewage water. This may be due to the suppression of growth by a high amount of toxic ions and total dissolved solids and heavy metals in the sewage wastewater (Li et al. 2007).

The chlorophyll and carotenoid content had statistically significant values ( $p < 0.05$ ) as native cyanobacterial inoculation showed positive effects under low sewage water concentrations (Tables 5 and 6). The increased carotenoid content may be attributed to the plant defense strategy to overcome the heavy metal stress, as these are non-enzymatic antioxidants. There was an increase of 30 to 56.7%, 7.2 to 26.6%, 14.2 to 47.8% in chlorophyll content of rice seedlings supplemented with the filtrate of the native *Oscillatoria* sp., *Lyngbya* sp., and *Phormidium* sp. respectively. The increase in chlorophyll content in rice seedlings upon cyanobacterial inoculation was also reported (Khushwaha & Banerjee 2015, Padhy et al. 2016). In contrast to the increase, a reduction in photosynthetic pigments was observed at a hundred percent concentration of sewage wastewater. It could be due to the presence of the high amount of heavy metals and salts which potentiates a retarding effect by interference with structural

Table 6: Effect of cyanobacterial extracts of native and introduced cyanobacterial species on carotenoid content of rice seedlings under various sewage water concentrations.

Sewage water conc.	Isolated <i>Lyngbya</i> sp.	Isolated <i>Oscillatoria</i> sp.	Isolated <i>Phormidium</i> sp.	Introduced <i>Lyngbya</i> sp.	Introduced <i>Oscillatoria</i> sp.	Introduced <i>Phormidium</i> sp.	Control
25	0.14±0.006	0.168±0.002	0.176±0.003	0.19±0.006	0.212±0.006	0.204±0.005	0.204±0.001
50	0.184±0.004	0.232±0.005	0.352±0.009	0.284±0.009	0.368±0.003	0.184±0.004	0.14±0.0076
75	0.152±0.004	0.128±0.008	0.32±0.068	0.264±0.004	0.272±0.005	0.164±0.009	0.128±0.004
100	0.128±0.005	0.112±0.009	0.248±0.007	0.112±0.012	0.224±0.005	0.156±0.005	0.112±0.004

Table 7: Effect of cyanobacterial extracts of native and introduced cyanobacterial species on peroxidase activity (H<sub>2</sub>O<sub>2</sub> decomposed per minute/gram fresh weight) of rice seedlings under various sewage water concentrations.

Sewage water conc.	Isolated <i>Lyngbya</i> sp.	Isolated <i>Oscillatoria</i> sp.	Isolated <i>Phormidium</i> sp.	Introduced <i>Lyngbya</i> sp.	Introduced <i>Oscillatoria</i> sp.	Introduced <i>Phormidium</i> sp.	Control
25	5.9±0.023	5.15±0.193	5.83±0.015	5.9±0.040	5.83±0.025	5.6±0.0152	6.82±0.06
50	5.9±0.069	6.2±0.076	6±0.095	6.4±0.037	6.4±0.1	6.17±0.0435	7.2±0.034
75	13.2±0.17	11.81±0.04	6.88±0.028	17±0.115	13.5±0.36	6.8±0.07	7.7±0.05
100	14.4±0.4	17.4±0.368	9.38±0.158	17.8±0.3	17.6±0.463	9.6±0.30	8.1±0.20

Table 8: Effect of cyanobacterial extracts of native and introduced cyanobacterial species on catalase activity (H<sub>2</sub>O<sub>2</sub> decomposed per minute/gram fresh weight) of rice seedlings under various sewage water concentrations.

Sewage water conc.	Isolated <i>Lyngbya</i> sp.	Isolated <i>Oscillatoria</i> sp.	Isolated <i>Phormidium</i> sp.	Introduced <i>Lyngbya</i> sp.	Introduced <i>Oscillatoria</i> sp.	Introduced <i>Phormidium</i> sp.	Control
25	315±0.0	630.4±0.0	157.6±0.0	367.73±0.0	630.4±0.0	694.1±0.0	367.73±0.0
50	472.8±0.0	655.7±0.0	420.2±0.0	525.3±0.0	741.6±0.0	753.6±0.0	472.79±0.0
75	577.8±0.0	712.6±0.0	515.7±0.0	630.3±0.0	795±0.0	786.4±0.0	735.46±0.0
100	877±0.0	772.8±0.0	888.2±0.0	893±0.0	840±0.0	815.8±0.0	840.5±0.0

Table 9: Effect of cyanobacterial extracts of native and introduced cyanobacterial species on nitrate reductase activity (Micro mol. NO per gram fresh wt./hour) of rice seedlings under various sewage water concentrations.

Sewage water conc.	Isolated <i>Lyngbya</i> sp.	Isolated <i>Oscillatoria</i> sp.	Isolated <i>Phormidium</i> sp.	Introduced <i>Lyngbya</i> sp.	Introduced <i>Oscillatoria</i> sp.	Introduced <i>Phormidium</i> sp.	Control
25	5.33±0.152	6.16±0.057	7.3±0.1	4.63±0.60	6.3±0.035	6.39±0.026	5.19±0.035
50	5.79±0.175	11.33±0.115	5.5±0.0814	7.54±0.12	10.16±0.144	7.25±0.09	5.5±0.0450
75	4.9±0.173	6.79±0.0458	4.3±0.0737	6.2±0.055	8.14±0.045	6.21±0.070	4.28±0.051
100	4.84±0.07	6.76±0.041	4.0±0.332	5.41±0.08	7.45±0.0360	6.48±0.416	3.99±0.345

components of chloroplast (Thind & Malik 1988). As a result of the application of cyanobacterial extracts, the level of chlorophyll pigment in rice seedlings irrigated with up to 50% concentration of sewage wastewater was observed to be restored.

It can be seen from Tables 7 and 8 that the enzymatic (catalase and peroxidase) activities of rice seedlings significantly ( $p < 0.05$ ) increased in all the treatments. The increased levels suggest the activation of oxidative stress defense mechanisms which may be an adaptive response of rice seedlings against sewage water stress. The application of cyanobacteria extracts might have resulted in an increased antioxidant defense

system for rice seedlings. This is supported by the previous findings (Essa et al. 2015, Hanaa et al. 2008).

The extracts of native and introduced cyanobacterial species significantly increased ( $p < 0.05$ ) the nitrate reductase activity (Table 9) at various studied sewage water concentrations except at a hundred percent sewage water concentrations. Raghuram & Sopory (1995) suggested that nitrate reductase activity depends upon active photosynthesis and requires photosynthetically generated reductant (NADH) and energy. Hence reduction in nitrate reductase activity could be due to reduced chlorophyll biosynthesis at high sewage water concentrations, thus, supplying lower levels

of photosynthates. Analysis of variance indicated significant differences in growth and enzyme activity among various cyanobacterial treatments and sewage water concentrations studied.

## CONCLUSION

All the studied cyanobacterial isolates revealed a potent ability to alleviate the damage caused by the sewage wastewater on rice seedlings. The fifty percent sewage water concentration with cyanobacterial extracts proved to be the best optimal combination for rice seed germination.

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