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Remediation of Kalyani River water using plant-bacterial cell synergism

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ABSTRACT

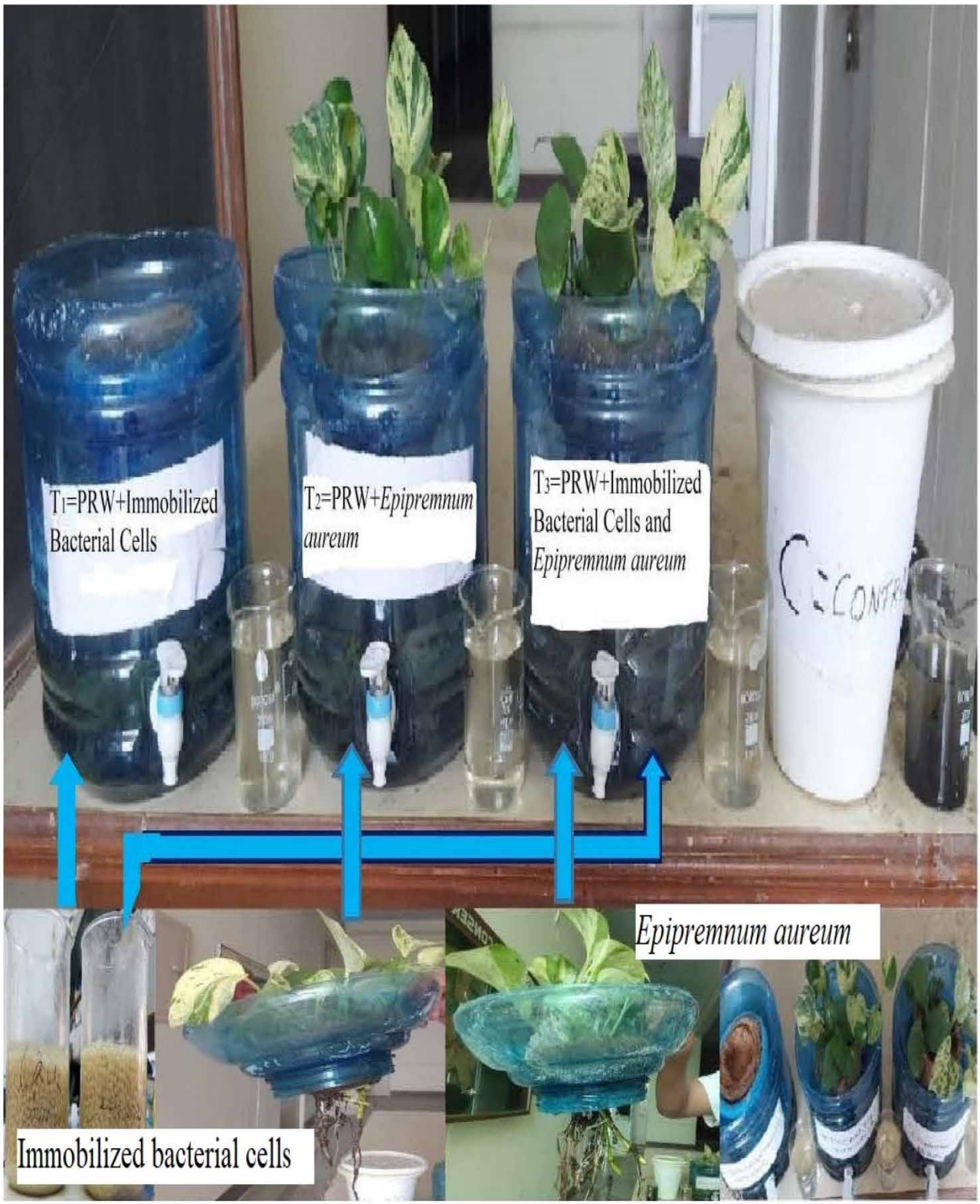
The purpose of this research was to evaluate the potential of plant-immobilized bacterial cells synergism for enhanced remediation of polluted river water. The polluted river water sample was collected from Kalyani river, Uttarakhand, India and characterized by high concentration of COD (1010 mg/l), BOD (230 mg/l), NO_3^--N (30 mg/l), PO_4^{3-}-P (48.9 mg/l), and Pb (1.028 mg/l). This water sample was treated on a lab scale with immobilized bacterial cells and *Epipremnum aureum* in various treatment setups. The treatment system 3 using a combination of immobilized bacterial cells and *Epipremnum aureum* had the highest pollutant removal efficiency of all the treatment setups tested. At 96 hours, the total COD, BOD, NO_3^--N, PO_4^{3-}-P and Pb contents of polluted river water sample were reduced to 60 mg/l, 20 mg/l, 2.4 mg/l, 11.7 mg/l, and 0.065 mg/l, respectively. Based on the findings, it is possible to conclude that utilizing plant-immobilized bacterial cell synergism is an environmentally friendly and cost-effective approach for enhanced remediation and rejuvenation of polluted river water. Furthermore, a field-scale application of plant-immobilized bacterial cell synergism via floating wetland construction for on-site treatment of contaminated water on the Kalyani river is recommended.

Key words: *Epipremnum aureum*, polluted river water, remediation, surface water resource

HIGHLIGHTS

- *Bacillus sp.* AK1 and AK3 were immobilized in sodium alginate and used for remediation of polluted river water.
- The combined use of immobilized bacteria and *Epipremnum aureum* resulted in a high pollutant removal efficiency.
- Inoculation of immobilized bacterial cells in the treatment system improved heavy metal uptake ability of *Epipremnum aureum*.

GRAPHICAL ABSTRACT



INTRODUCTION

Rivers play an essential role in human life in developing countries, providing various benefits such as shipping, electricity generation, water supply and agricultural production (Shahid *et al.* 2019). Despite this, rivers have also been used for cleaning and disposal. Massive amounts of wastewater from industry, domestic sewage, and intensive agriculture are discharged into the river, causing the water to become severely polluted and negatively impacting the aquatic environment, landscapes, and human health. The Kalyani River is a spring-fed river in Uttrakhand, India, that originates in the Tanda forest area of Nainital district. It passes through agricultural fields of Pantnagar followed by Integrated Industrial Estate, Pantnagar, before crossing the Rudarpur city from North-east to South-east. The disposal of industrial wastewater from industries in State Infrastructure and Industrial Development Corporation of Uttarakhand Ltd (SIIDCUL) coupled with municipal drains from residential area of the city, making Kalyani River highly polluted (Goyal & Gupta 2016).

There are various physicochemical conventional methods for elimination of pollutants from polluted rivers in order to minimize their environmental risks (Mahfooz *et al.* 2021). But there have been some serious drawbacks associated with these methods, such as production of toxic intermediates, high costs of treatment and high energy consumption (Shrivastava *et al.* 2020). Use of bioremediation technology for treatment of polluted river water provides various advantages such as less capital cost, no secondary pollution and less energy consumption compared to physiochemical methods. Bioremediation technology depends on the utilization of biological agents including bacteria, fungi, algae and plants to eliminate pollutants from contaminated environments.

Plants have capability for remediation of polluted river water. Currently, plant-assisted bioremediation, which exploits the synergistic action of plant root systems and natural microorganisms (bacteria and fungi) to remove pollutants from soils, sediments, or wastewater, is becoming a sustainable solution for contaminated environments due to its eco-friendly applications and cost effectiveness (Ancona *et al.* 2017). The plants contribute towards the pollutant removal process by entrapping pollutant particles in their roots. The plant roots also release a bioactive substance known as exudates which play a special role in pollutant removal. These substances serve as additional carbon sources that stimulate the bacteria to degrade pollutants (Chen *et al.* 2016).

Bacteria play a vital role in plant-bacteria synergism by degrading complex organic pollutants, assimilation of nitrogen and phosphorus, and reduction of nitrate, phosphate, and heavy metal concentrations in polluted water via metabolic and non-metabolic processes (Shahid *et al.* 2019). Plant Growth Promoting Bacteria (PGPB) are used to improve the remediation of polluted water and soil by plants through promoting plant development, which is otherwise inhibited due to toxic organic pollutant exposure (Khan *et al.* 2013). *Bacillus cereus* and *Bacillus safensis* are some of the bacterial strains that are commonly utilized for phytoremediation process enhancement (Saleem *et al.* 2014; Wu *et al.* 2019). However, the use of free bacterial cells for pollutant remediation is often not successful due to the low concentrations of active pollutant degrading bacteria especially in high toxicity industrial wastewater and high rate of bacterial loss. Similarly, substrate inhibition of microbial growth at high concentration of toxic chemicals and cell washout problem or separation difficulties as well as slow biodegradability or non- biodegradability of recalcitrant compounds are also some other drawbacks often associated with employing free bacterial cells in remediation of wastewater (Banerjee & Ghoshal 2011).

Recently, different researchers have suggested immobilized bacterial cell technology as a good strategy to overcome the problems associated with using free bacterial cells for bioremediation purposes and to improve bacterial cell degradation capability. *Bacillus cereus* immobilized in calcium alginate demonstrated excellent performance in the biodegradation of phenolic compounds in petroleum wastewater, reducing phenolic compound concentration and COD level by 95% (Banerjee & Ghoshal 2016). Bhatt *et al.* (2021) investigated the degradation of fipronil using immobilized bacterial cells of *Bacillus sp.* FA3 with sodium alginate and agar disc beads. The study revealed that immobilizing *Bacillus Sp.* FA3 cells with sodium alginate and an agar disc significantly increased fipronil degradation when compared to free or suspended culture. Yan *et al.* (2019) also used microbial immobilization technology for the remediation of nitrogen pollution from contaminated sediments of Shedu river in China, achieving 78.13% total nitrogen removal efficiency. Similarly, Fu *et al.* (2020) investigated the role of immobilized biologically activated beads for removal of pollutants present in the sediments of Shedu River, China, and immobilized activated beads showed the best pollutant removal performance for ammonium nitrogen (NH_4-N):76.3%, Total Nitrogen (TN): 93.3% and COD: 92.8%, respectively. Likewise, Sun *et al.* (2009) investigated the use of immobilized denitrifying bacteria to improve nitrogen removal in floating bed grown canna, achieving removal efficiencies of 72.1%, 100%, 75.8% and 94.6% for total nitrogen, ammonia nitrogen, nitrate nitrogen and COD respectively in 5 days.

The objective of this research work was to assess the potential of plant-immobilized bacterial synergism for remediation of polluted water of Kalyani River through use of immobilized *Bacillus sp.* AK1 and AK3 with Money plants (*Epipremnum aureum).* In this study, *Bacillus sp.* AK1 and AK3 were immobilized by using sodium alginate as carrier matrices and calcium chloride as a cross linking agent and used for treatment of polluted river water in combination with *Epipremnum aureum*. Then pollutant removal efficiency of combined use of immobilized bacterial cells and *Epipremnum aureum* was determined and compared to treatments using either immobilized bacterial cells or *Epipremnum aureum* separately.

MATERIAL AND METHODS

River water collection

The river water samples were collected in April 2021 from a SIIDCUL drain in Rudrapur city, where a large quantity of untreated or partially treated effluents from SIIDCUL industries joined the Kalyani River. Then the collected samples were transported to the laboratory and analysed for various physicochemical parameters such as pH, Total Dissolved Solids (TDS), Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), Nitrate-Nitrogen (NO_3^--N), Phosphate- Phosphorus (PO_4^{3-}-P) and heavy metals like Lead (Pb) and Copper (Cu) according to the standard methods (APHA 2012).

Selection and growth of plant

The *Epiprennum aureum* used in this study for the treatment of polluted river water was obtained from Pallavika Nursery, Rudrapur. This plant was selected based on its availability, resistance to harsh conditions, fast growing and high biomass production with minimal nutrient requirements (Kumari *et al.* 2015). Prior to the regular experiments, the selected plant was allowed to grow for a total period of 4 weeks in tap water. In each of the plastic containers, 5 L tap water was added with Hoagland's solution of the following composition (in ml/l): KNO_3 (3.0), Ca $(NO_3)2$ (3), $MgSO_4$ (1.0), KH_2PO_4 (1.0) and NaCl (1), H_3BO_3 (1), $MnCl_2$ (1), $ZnSO_4$ (0.38) and $CuSO_4$ (0.16) (Shyamala *et al.* 2019). Following the completion of the growth period, the plants were thoroughly rinsed in tap water to remove any sediments or other dirt elements that had become attached to them. Finally, the washed plants were placed in clean, transparent 10 litre cylindrical plastic containers for regular experiments (Figure 1).

Culture of bacterial cells

Bacillus sp. AK1 and AK3 bacteria strains were used in this research work. Previously isolated strains of the above-mentioned bacteria were obtained from National Environmental Engineering Research Institute (CSIR NEERI), New Delhi. The *Bacillus species* were chosen on the basis of their ability to degrade complex organic and inorganic pollutants in the wastewater (Mondal *et al.* 2019). Beside this, *Bacillus sp*. is also among a group of PGPB which enhances plant growth through production of siderophore, Indole Acetic Acid (IAA) and phosphorus solubilization (Sharma *et al.* 2021). Bacillus sp. AK1 and AK2 bacterial strains were cultivated in Luria-Bertani (LB) broth. Luria-Bertani medium is a nutritionally rich medium that has been used as a general-purpose bacterial culture medium for a wide range of facultative anaerobic organisms (MacWilliams & Liao 2006). After cultivation, bacterial cells were harvested by centrifugation and re-suspended in 0.2% (w/v) Phosphate Buffer Saline (PBS) solution.

Immobilized bacterial cells

The whole cell entrapment method by using sodium-alginate as the support carrier and calcium chloride as the crosslinking agent (Banerjee & Ghoshal 2011) was used to immobilize bacteria cells. The *Bacillus sp*. AK1 and AK3 that were grown separately in 1 L of Luria broth were centrifuged at 5,000 rpm for 10 minutes. After centrifugation, the bacterial cell pellet was separated by decanting the supernatant. The bacterial cell pellet was weighed and 3.15 g bacterial cell pellet was suspended in 80 ml of Phosphate Buffer Saline (PBS). A stock of 4% w/v sodium alginate solution was prepared in the nutrient broth medium and autoclaved at 121 °C for 15 minutes. Then, the suspended bacterial cells was added to 4% w/v sterilized sodium alginate solution in a large beaker and mixed by using a glass rod to disperse the cells well. The sodium alginate solution containing cell culture suspension was extruded drop wise into a cold sterilized 2% w/v $CaCl_2$ solution. The drops of sodium alginate-cell solution gelled to form a uniform and spherical shaped beads upon contact with calcium chloride solution. The immobilized cell beads were kept in calcium chloride solution overnight for hardening. The beads were then rinsed with distilled water to remove residual $CaCl_2$ and stored at 4 °c until further use for the polluted water treatment experiment.

Figure 1 | Setup of plant growth by using tap water for establishment of root system for further experiment.

Optimization of beads concentration experiment

Batch wise experiment was carried out in a one-litre conical flask containing polluted river water to determine the effects of bead concentration on wastewater treatment performance of immobilized cell system. Conical flasks containing polluted river water and beads at various concentrations were kept in an incubator at 37 °C for 48 hours. Then, the Chemical Oxygen Demand (COD) was analyzed for all treatments after 48 hours.

Experimental setups for remediation of polluted river water

These experiments were carried out in 10 L cylindrical plastic water container bottles, each of which contains 5 litres of tap water. Then *Epipremnum aureum* or money plants were grown with tap water for four weeks to establish their roots. After establishing the plant root system, the experiments were carried out by introducing 5 litres of polluted river water into each cylindrical plastic water container. To assess the effects of immobilized bacterial cells, plants and plant-immobilized bacterial cell synergism, plants and immobilized bacterial cells were applied in combination as well as separately in the cylindrical plastic container bottles containing the polluted river water (Figure 2). For immobilized bacterial cell beads inoculation, 125 g of immobilized bacterial cell beads were applied in combination with plants and separately into two separate 10 L cylindrical plastic water container bottles based on the result obtained from the batch experiment for optimization of beads concentration.

The following treatment setups were used:

T1: Polluted river water with immobilized bacterial cells only.
T2: Polluted river water with *Epipremnum aureum* only.
T3: Polluted river water with immobilized bacterial cells and *Epipremnum aureum*.
C: Polluted river water without immobilized bacterial cells and *Epipremnum aureum*.

Throughout the four-day experiment, 0.25 litre water samples were collected from each treatment system every 24 hours of Hydraulic Retention Time (HRT) and analysed for parameters such as COD, BOD, NO_3^--N and PO_4^{3-}-P according to the standard method (APHA 2012). The concentrations of lead (Pb) and copper (Cu) in water samples were determined using atomic absorption spectrophotometry (SensAA, DUAL, GBC Scientific Equipment, Australia).

Figure 2 | Experimental setups for treatment of polluted river water.

Plant samples preparation for heavy metal analysis

Plant samples (*Epipremnum aureum*) grown in polluted river water with and without immobilized bacterial cells inoculation were harvested from plastic container bottles where they were grown for a pollutant removal experiment. The plant samples were then thoroughly rinsed with distilled water before being separated into root and shoot portions. The plant's separated root and shoot parts were cut into small pieces with a clean knife and dried in a 100 °C oven for 24 hours. The dried plant samples (0.5 g each) were then digested with 10 ml concentrated HNO_3. After digestion, samples were allowed to cool, dissolved in 0.6% HNO_3, and the volume of each sample was maintained up to 15 ml (Rai *et al.* 2015). Following that, each sample was filtered with Whatman filter paper No. 42 before being aspirated into the atomic absorption spectroscopy and analysed for heavy metals (Cu and Pb) content of plant samples.

Bio-concentration factor (BCF): In this experiment, BCF was calculated using the formula reported by Soda *et al.* (2012), which indicates the metal uptake capability of shoot biomass.

$$\mathrm{BCF} = \frac{\mathrm{C.shoot}\ (mg/l)}{C.rw\ (mg/l)}$$

where, C.shoot is the heavy metal concentration in the shoot biomass (mg/l) and *C.rw* is the heavy metal concentration in the river water sample (mg/l).

Translocation factor (TF): The TF, which indicates the ability of a plant to translocate metal from root to shoot was calculated using the formula proposed by Rai *et al.* (2015), which indicates heavy metal translocation from root to shoot biomass.

$$\mathrm{TF} = \frac{C.shoot}{C.root}$$

where, *C.shoot* represents the metal concentration in above-ground tissue or shoot (mg/l) and *C.root* represents the metal concentration in roots (mg/l).

Data analysis

The statistical software SPSS V26 was used to analyse the data. The data collected from each treatment by three replicates was subjected to analysis of variance (ANOVA). The ANOVA test (level of significance $\alpha = 0.05$) was employed to compare means among different treatments.

RESULTS AND DISCUSSION

Physicochemical characteristics of Kalyani River water

Table 1 shows the physicochemical parameter values of Kalyani river water. The values of parameters such as COD, BOD, NO_3^--N, PO_4^{3-}-P and Pb were generally found to be higher than the permissible limits for discharge of wastewater into inland surface water.

The effects of bead concentration on immobilized bacterial cell system wastewater remediation potential

In wastewater treatment processes that use immobilized bacterial cells, the concentration of immobilized bacterial cell beads is critical (Khudhair & Ismail 2019). In a batch experiment, the effect of different concentrations of immobilized bacterial cell beads, i.e. (12.5 g/l, 25 g/l, and 50 g/l), on the COD removal efficiency of an immobilized bacterial cell system from polluted river water was tested.

Figure 3 depicts the results. Higher COD reduction was observed when 25 g/l immobilized bacterial cell beads were used.

Remediation of polluted river water

Reduction in COD and BOD after treatment

COD and BOD levels in water are important indicators of organic pollution (Ijaz *et al.* 2015). Throughout the experiment, there was a reduction in the COD and BOD content of polluted river water samples in all treatments (Table 2). T3 (combined use of immobilized cells and *Epipremnum aureum*) significantly reduced COD and BOD levels compared to T1 (use of immobilized bacterial cells only), T2 (use of *Epipremnum aureum* only) and C (without use of immobilized bacterial cells and *Epipremnum aureum*).

The reduction in COD and BOD is primarily determined by the oxygen concentration in the treated water, as decomposition of pollutants occurs through various oxidation reactions. In plant-bacteria synergism, the plant is anticipated to raise the concentration of oxygen in polluted water and, subsequently, the decomposition of organic matters by microorganisms as well (Ashraf *et al.* 2018). In this study, maximum COD and BOD reduction was observed in treatment system 3 using a combination of immobilized bacterial cells and *Epipremnum aureum*. The experimental results in Table 2 show that at 72 hours of HRT, the COD and BOD content were reduced to 110 and 28 mg/l, respectively, which is under the permissible limit

Table 1 | Physicochemical characteristics of river water collected from Kalyani River

Parameters	Concentration	Inland surface water discharge permissible limit of EPA Rules 1986
pH	6.3 ± 0.10	5.5–9
TDS (mg/l)	737 ± 7	2,100
COD (mg/l)	$1{,}010 \pm 2$	250
BOD (mg/l)	230 ± 2	30
NO_3^--N (mg/l)	30 ± 0.5	10
PO_4^{3-}-P (mg/l)	48.9 ± 0.2	5
Pb (mg/l)	1.028 ± 0.002	0.1
Cu (mg/l)	0.217 ± 0.001	3

EPA Rules, 1986. The environment (protection) rules, General standards for discharge of environmental pollutants proposed by Ministry of Environment and Forest, India. Standard deviation presented by $\pm$ sign.

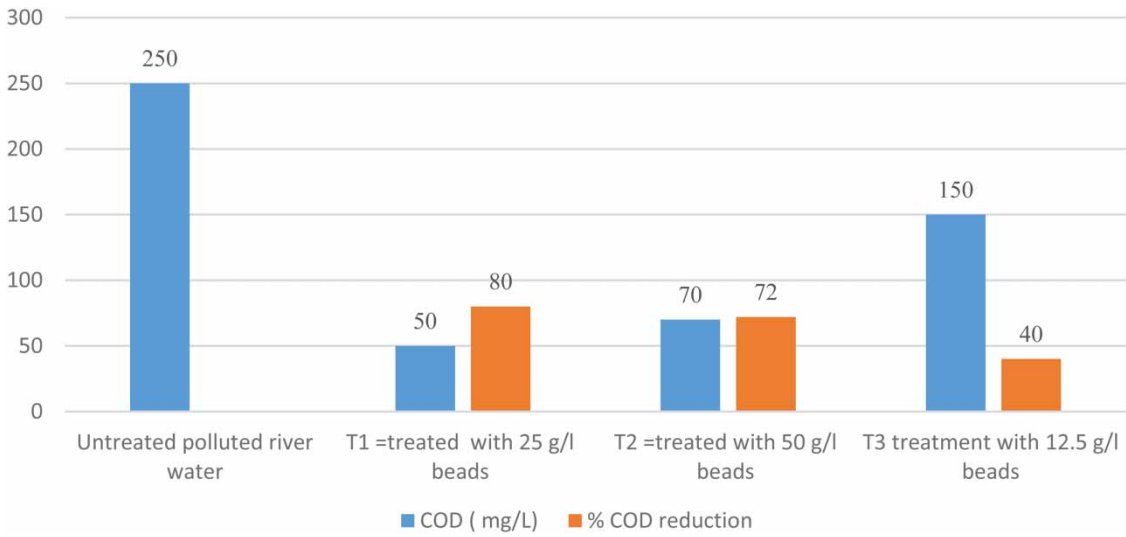


Figure 3 | COD concentrations and percentage of COD reduction for each treatment with different concentrations of immobilized bacterial beads after 48 hours of HRT.

Table 2 | Concentration of pollutants for different treatment setups with various HRT

		HRT					Statistical α-value	
Parameters	**Treatment setup**	**0 h**	**24 h**	**48 h**	**72 h**	**96 h**	**α- value**	**The mean difference at ($\alpha = 0.05$)**
COD (mg/l)	T1	$1010^{A} \pm 2$	$790^{C} \pm 1$	$590^{D} \pm 0.5$	$310^{G} \pm 0.4$	$260^{H} \pm 0.5$	0.000	Significant
	T2	$1010^{A} \pm 2$	$580^{D} \pm 1.5$	$330^{F} \pm 0.4$	$230^{I} \pm 0.2$	$210^{J} \pm 0.1$		
	T3	$1010^{A} \pm 2$	$490^{E} \pm 1$	$220^{IJ} \pm 0.3$	$110^{K} \pm 0.9$	$60^{L} \pm 0.2$		
	C	$1010^{A} \pm 2$	$985^{AB} \pm 1$	$970^{AB} \pm 0.1$	$950^{B} \pm 0.0$	$940^{B} \pm 0.0$		
BOD (mg/l)	T1	$230^{A} \pm 2$	$132^{D} \pm 0.1$	$72^{F} \pm 0.2$	$44^{I} \pm 0.1$	$30^{K} \pm 0.1$	0.000	Significant
	T2	$230^{A} \pm 2$	$144^{C} \pm 1.1$	$84^{E} \pm 0.1$	$50^{H} \pm 0.8$	$40^{J} \pm 0.2$		
	T3	$230^{A} \pm 2$	$126^{D} \pm 0.7$	$66^{G} \pm 0.2$	$28^{L} \pm 0.1$	$20^{M} \pm 0.1$		
	C	$230^{A} \pm 2$	$224^{A} \pm 0.3$	$220^{AB} \pm 0.4$	$216^{B} \pm 0.0$	$214^{B} \pm 0.00$		
NO_3^--N (mg/l)	T1	$30^{A} \pm 0.5$	$26.2^{C} \pm 0.1$	$18.4^{E} \pm 0.1$	$12.9^{H} \pm 0.1$	$11.8^{I} \pm 0.1$	0.000	Significant
	T2	$30^{A} \pm 0.5$	$25.4^{C} \pm 0.2$	$16.8^{F} \pm 0.1$	$6.2^{J} \pm 0.15$	$4.3^{K} \pm 0.05$		
	T3	$30^{A} \pm 0.5$	$23.6^{D} \pm 0.3$	$14.9^{G} \pm 0.1$	$3.8^{L} \pm 0.04$	$2.4^{M} \pm 0.05$		
	C	$30^{A} \pm 0.5$	$29.3^{AB} \pm 0.05$	$28.6^{AB} \pm 0.00$	$28.4^{B} \pm 0.00$	$28.3^{B} \pm 0.00$		
PO_4^{3-}-P (mg/l)	T1	$48.9^{A} \pm 0.2$	$44.1^{BC} \pm 0.1$	$30.7^{D} \pm 0.1$	$15.1^{E} \pm 0.1$	$12.6^{F} \pm 0.05$	0.001	Significant
	T2	$48.9^{A} \pm 0.2$	$44.3^{BC} \pm 0.1$	$31.4^{D} \pm 0.1$	$15.8^{E} \pm 0.05$	$13.2^{F} \pm 0.05$		
	T3	$48.9^{A} \pm 0.2$	$43.5^{C} \pm 0.2$	$30.3^{D} \pm 0.1$	$14.4^{E} \pm 0.1$	$11.7^{G} \pm 0.05$		
	C	$48.9^{A} \pm 0.2$	$47.5^{AB} \pm 0.1$	$46.9^{AB} \pm 0.1$	$46.5^{AB} \pm 0.00$	$46.2^{B} \pm 0.05$		

T1:Immobilized bacterial cells only, T2: *Epipremnum aureum* only, T3: Combination of immobilized bacterial cells and *Epipremnum aureum,* C: Without immobilized bacterial cells and *Epipremnum aureum*. Each value is the mean of three replicates, and the mean difference between the treatments is significant at **(α=0.05)** where the value is less than 0.05. The letters labelled on values represent the statistically significant/non-significant difference between/within treatments. Values with the same letters are not significantly different from each other, and vice versa; for example, a value labelled with 'AB' is not significantly different from values labelled with A or B, but it is significantly different from a value labelled with 'DE'/D or E. The standard deviations are represented by $\pm$.

of inland surface water discharge. Furthermore, at HRT of 96 hours, COD and BOD concentrations were reduced by 94.06% and 91.3% (Figure 4) and reached a value of 60 mg/l and 20 mg/l respectively.

The study results essentially revealed that treatment system 3 (using a combination of immobilized bacterial cells and *Epipremnum aureum*) successfully removed the high concentrations of COD and BOD. This rapid and significant reduction in COD and BOD is likely due to enhanced organic matter degradation by immobilized bacterial cells via dissolved oxygen

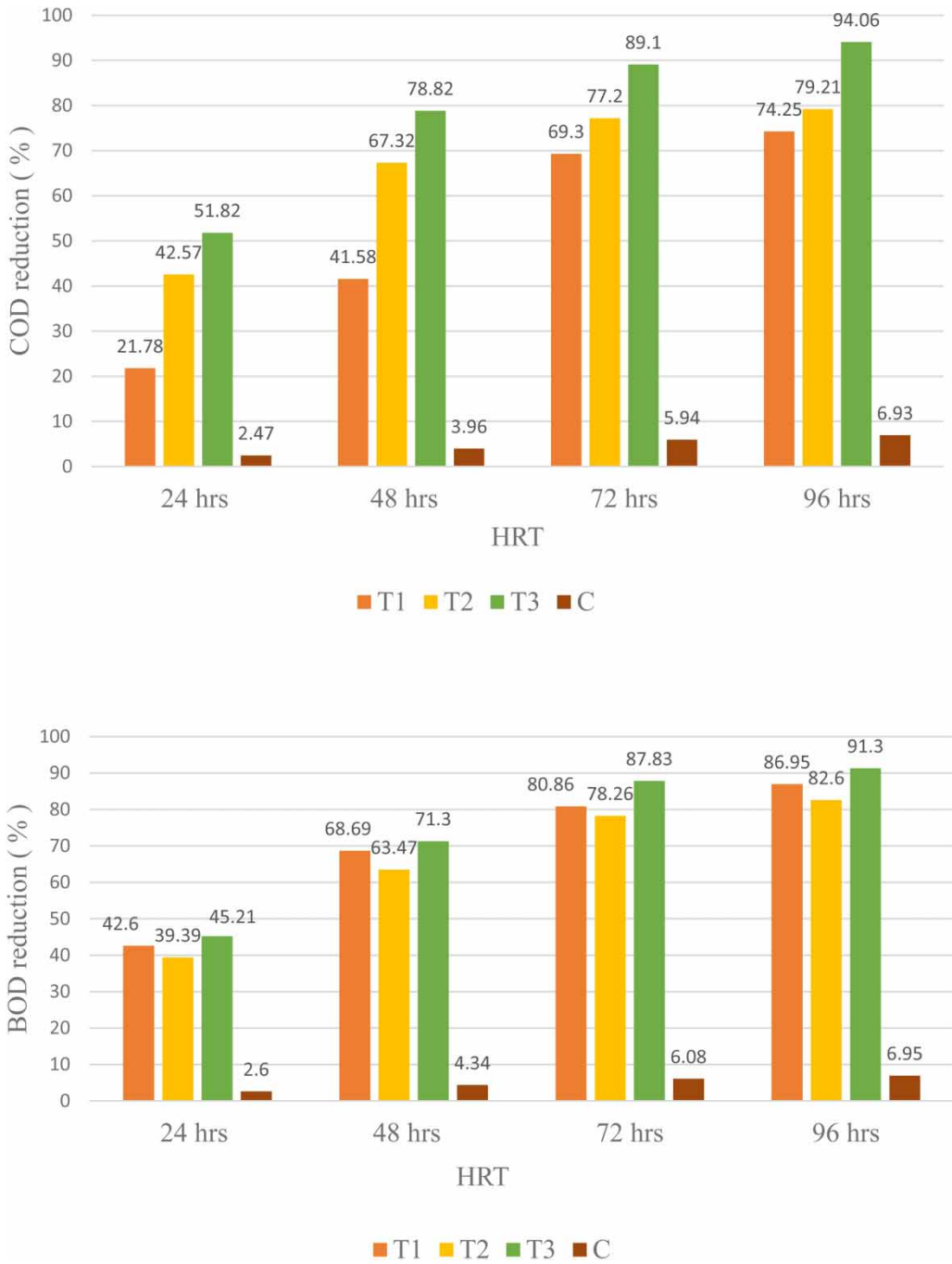


Figure 4 | The percentage of COD and BOD removed from polluted river water by various treatments. T1: polluted river water with immobilized bacterial cells only, T2: polluted river water with *Epipremnum aureum* only, T3: polluted river water with both immobilized bacterial cells and *Epipremnum aureum* and C: polluted river water without immobilized bacterial cells and *Epipremnum aureum*.

and exudate release by the plant root system. Furthermore, entrapment or physical filtering of suspended particles by plant roots may contribute to COD and BOD reduction (Shahid *et al.* 2019).

Reduction in nitrate-nitrogen (NO_3^--N) and phosphate-phosphorus (PO_4^{3-}-P)

The removal of nitrate-nitrogen (NO_3^--N) and phosphate-phosphorus (PO_4^{3-}-P) from rivers is critical because a high concentration of nitrate-nitrogen combined with phosphate-phosphorus causes eutrophication of surface water bodies. In this study,

treatment system 3, which used a combination of immobilized bacterial cells and *Epipremnum aureum*, demonstrated a significant reduction in NO_3^--N and PO_4^{3-}-P in polluted river water when compared to all other treatment systems (see Table 2).The experimental results in Figure 5 and Table 2 show that after 96 hours of HRT, the concentrations of NO_3^--N and PO_4^{3-}-P were reduced by 92 and 76.07% and reached a value of 2.4 and 11.7 mg/l respectively.

The study result basically revealed that treatment system 3, which used a combination of immobilized bacterial cells and *Epipremnum aureum*, successfully reduced the NO_3^--N content below the maximum permissible limit of inland surface water discharge. This might be attributed to the combined effect of NO_3^--N uptake by the plant root as it is exposed to water only and assimilation of nitrogen by immobilized bacterial cells. Similarly, treatment 3 had a higher efficiency of PO_4^{3-}-P removal than treatment systems 1 and 2, which could be attributed to the enhanced activity of immobilized bacterial cells.

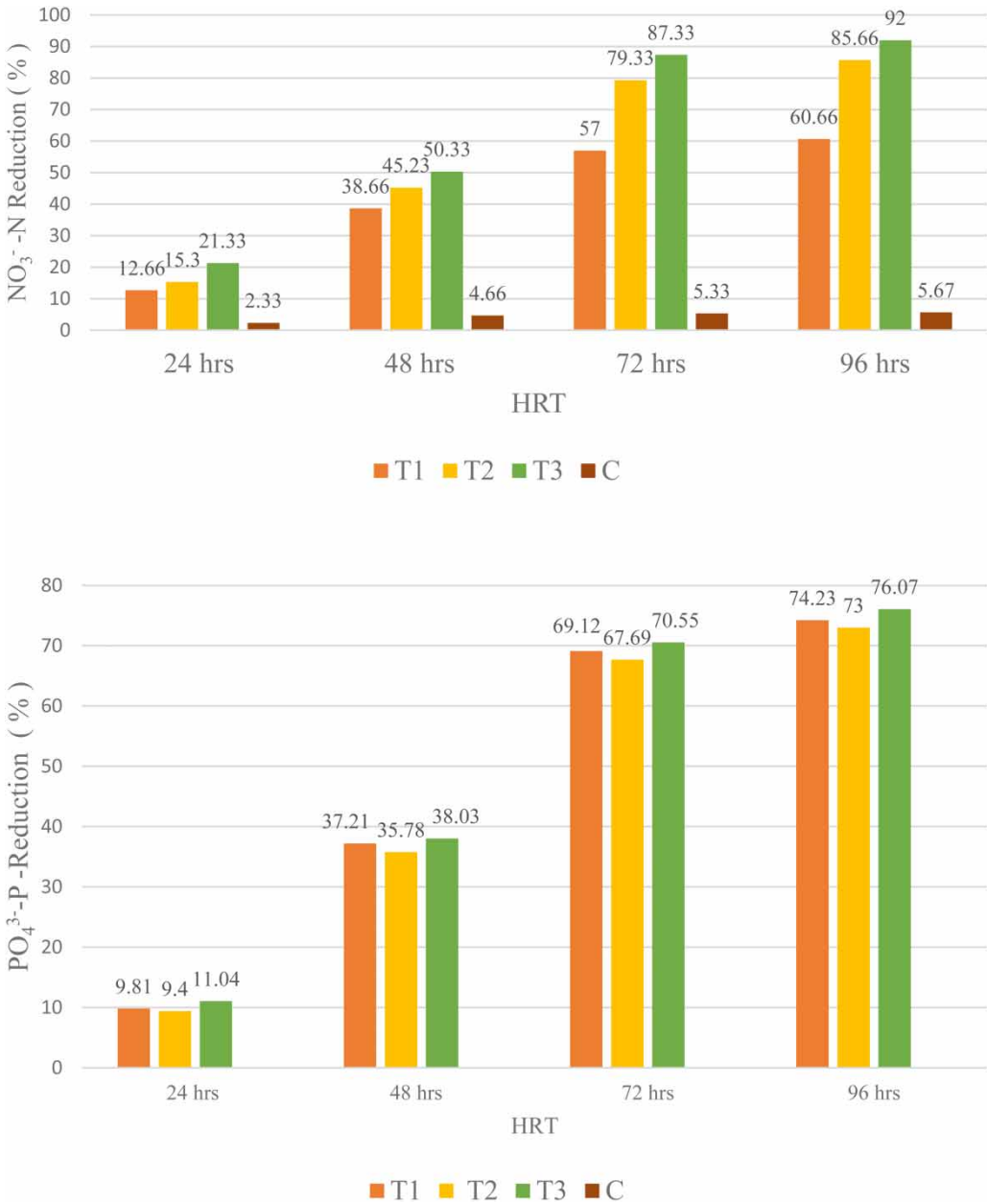


Figure 5 | The percentage of NO_3^--N and PO_4^{3-}-P removed from polluted river water by various treatments. T1: polluted river water with immobilized bacterial cells only, T2: polluted river water with *Epipremnum aureum* only, T3: polluted river water with both immobilized bacterial cells and *Epipremnum aureum* and C: polluted river water without immobilized bacterial cells and *Epipremnum aureum*.

Reduction and translocation heavy metal concentration after treatment

T3 (a combination of immobilized bacterial cells and *Epipremnum aureum*) resulted in a significantly higher reduction in heavy metal concentrations in polluted river water samples than T1, T2, and C (see Table 3).

Pb and Cu concentrations were reduced from 1.028 mg/l to 0.065 and 0.217 to 0.014 mg/l in treatment system 3 (using a combination of immobilized bacterial cells and *Epipremnum aureum*) respectively, which is below the maximum permissible limit of inland surface water discharge after 96 hours of HRT.

The two major processes involved in heavy metal removal are direct uptake by plants and bio-sorption of immobilized bacterial cells. 'Inoculation of immobilized bacterial cells increased heavy metal uptake by improving bioavailability, sorbing metallic ions on bacterial cell walls, or uptake of bioavailable heavy metals by plant roots' (Ashraf *et al.* 2018).

The heavy metals concentration analysis of plant samples (*Epipremnum aureum*) grown in polluted river water with and without inoculation of immobilized bacterial cells was done to evaluate heavy metal uptake and accumulation capacity of *Epipremnum aureum*, as well as the effects of inoculation of immobilized bacterial cells on the *Epipremnum aureum* heavy metal accumulation potential. The study results are shown in Table 4. The *Epipremnum aureum* grown with inoculation of immobilized bacterial cells (T3) accumulated more Pb and Cu in the root and shoot parts, respectively.

The accumulation of Pb in the root (0.312 mg/l) of *Epipremnum aureum* was greater than that in the shoot (0. 230 mg/l). While, the concentration of Copper (Cu) was higher in the shoot (0.229) than in the root (0.146 mg/l) of *Epipremnum aureum*.

The potential of plants for phytoremediation purposes can be evaluated by Bio-concentration Factors (BCF) and Translocation Factors (TF) (Galal *et al.* 2018). The BCF of Pb and Cu for *Epipremnum aureum* grown in polluted river without immobilized bacterial cell inoculation was 0.1 and 0.97, respectively, while the BCF of Pb and Cu for *Epipremnum aureum* grown in polluted river water with inoculation of immobilized bacterial cells was 0.22 and 1.05, respectively. Pb and Cu translocation factors (TF) were 0.39 and 1.52 for *Epipremnum aureum* grown in polluted river water without immobilized bacterial cell inoculation, respectively, and 0.74 and 2.05 for *Epipremnum aureum* grown in polluted river water with immobilized bacterial cell inoculation.

The study results show that the accumulation of Pb in *Epipremnum aureum* roots and shoots is greater in T3 (*Epipremnum aureum* grown in polluted river water with inoculation of immobilized bacterial cells than in T2 (*Epipremnum aureum* grown in polluted river water without inoculation of immobilized bacterial cells), but the accumulation in the root is greater than in

Table 3 | Reduction in heavy metals by different treatment setups after 96 h

Heavy metals	Treatment setup	Initial concentration (mg/l)	Final concentration (mg/l)	Removal efficiency (%)
Pb	T1	1.028	0.326	68.29
	T2	1.028	0.255	75.19
	T3	1.028	0.065	93.68
Cu	T1	0.217	0.032	85.25
	T2	0.217	0.032	85.25
	T3	0.217	0.014	93.56

Initial concentration (IC) = value of parameters before treatment; final concentration (FC) = value of parameters after treatment; reduction rate = $IC - FC/IC \times 100\%$.

Table 4 | Heavy metals (Pb and Cu) uptake of *Epipremnum aureum* grown using polluted river water with and without inoculation of immobilized bacterial cells

		Concentration of heavy metal (mg/l)			BCF and T F	
Treatment setup	Heavy metals	River water	Root	Shoot	BCF	TF
T2	Pb	1.028	0.263	0.102	0.10	0.39
	Cu	0.217	0.139	0.211	0.97	1.52
T3	Pb	1.028	0.312	0. 230	0.22	0.74
	Cu	0.217	0.146	0.229	1.05	2.05

T2: polluted river water with *Epipremnum aureum* only, T3: polluted river water with combination of immobilized bacterial cells and *Epipremnum aureum,* BCF: Bio-concentration Factors, TF: Translocation Factors.

the shoot. Thus, the ability of *Epipremnum aureum* to tolerate and accumulate Pb in its roots may be a useful mechanism for removal of lead (Pb) as rhizofiltration. 'Rhizofiltration refers to the use of plant roots to absorb, concentrate, and precipitate toxic metals from contaminated water' (Galal *et al.* 2018). Therefore, rhizofiltration capability of *Epipremnum aureum* for Pb could be improved by inoculation of immobilized bacterial cells.

Copper (Cu) accumulation in the root and shoot improved in T3 (using immobilized bacterial cells and *Epipremnum aureum*), but it is higher in the shoot than the root. This implies an increase in Translocation Factors (TF) of Cu with immobilized bacterial cell inoculation, indicating a high amount of copper (Cu) translocation from roots to shoots of *Epipremnum aureum*. Thus, the combined use of immobilized bacterial cells and *Epipremnum aureum* in the treatment system improved the phytoextraction ability of *Epipremnum aureum* for copper.

CONCLUSION

The result of the present research work reveals that the combined use of immobilized bacterial cells and plant (*Epipremnum auruem*) in a treatment system for remediation of polluted river water shows high reduction in COD, BOD, NO_3^-N, PO_4^{3-}-P and Pb compared to either use of immobilized bacterial cells or *Epipremnum aureum* separately. The result of the study indicates that the combined use of plant and immobilized bacterial cells is ecofriendly technology for enhanced remediation of polluted river water. Based on the study result, we recommend the field application of plant-immobilized bacterial synergism through floating wetlands on wastewater collection drains to alleviate their pollutant load prior to disposing into river water or other surface water bodies. Hence immobilized bacterial cells and their use in combination with plants is unexplored, and additional research work is needed for the practical application of this technology for in-situ remediation of polluted river water.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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