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Phytoremediation of Composite Industrial Effluent Using Sacred Lotus (*Nelumbo nucifera* Gaertn): A Lab-Scale Experimental Investigation

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Abstract: This study investigates the phytoremediation of composite industrial effluent (CIE) released from multiple industries within the SIIDCUL cluster, Haridwar, India, using the sacred lotus (*Nelumbo nucifera* Gaertn) plant. Batch-mode phytoremediation experiments were conducted using three selected concentrations (0%: borewell water as control, 50%, and 100%) of CIE for 45 days. Results show that the *N. nucifera* plant significantly reduced loads of physicochemical and heavy metal pollutants of CIE. In particular, the maximal removal of total dissolved solids (TDS: 89.56%), biochemical oxygen demand (BOD: 78.20%), chemical oxygen demand (COD: 79.41%), total Kjeldahl's nitrogen (TKN: 86.48%), phosphorus (P: 76.37%), cadmium (Cd: 70.37%), copper (Cu: 85.82%), chromium (Cr: 68.61%), iron (Fe: 72.86%), lead (Pb: 76.92%), and zinc (Zn: 74.51%) pollutants was noted in the 50% CIE concentration treatment. Heavy metal bioaccumulation and translocation factor values (>1) for root and leaf parts show that the *N. nucifera* plant was a hyperaccumulator. However, the contents of heavy metals were higher in the root than the leaf part of the *N. nucifera* plant. Moreover, the selected plant growth attributes such as fresh plant biomass (760.70 ± 8.77 g/plant; without flowers), chlorophyll content (4.30 ± 0.22 mg/g fwt.), plant height (154.05 ± 4.55 cm), root length (70.35 ± 2.42 cm), leaf spread (41.58 ± 0.26 cm), number of leaves (10.00 ± 1.00 per plant), and number of flowers (16.00 ± 2.00) were also maximal in the 50% CIE concentration. This study provides a sustainable approach towards the effective biotreatment of noxious mixed effluent using plant-based green technology.

Keywords: effluent management; environmental pollution; heavy metals; industrial wastes; *Nelumbo nucifera*; phytoremediation

1. Introduction

Recent developments in human civilization have contributed to drastically increasing the global population, thereby creating new settlements. New industries are being established to create needful articles for humans, which are continuously increasing day by day [1,2]. However, increasing amounts of solid, semisolid, gaseous, and liquid waste are being released from these industrial activities, rendering them the greatest threat to our environment. Such industries include pharmaceuticals, food and beverages, paper and pulp manufacturing, paper mill, distillery, petrochemicals, oil, and refineries [3]. Wastes released from the industrial sectors contain high loads of organic and inorganic pollutants such as excessive nutrients, heavy metals, radionuclides, polyaromatic hydrocarbons (PAHs), plastics, and volatile organic compounds (VOCs) [4]. Additionally, heavy metals are conservatively well-defined as elements with metallic properties (ductility, conductivity, stability as cations, ligand specificity, etc.). The most common heavy metals responsible for environmental degradation are cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni), and zinc (Zn). These pollutants create disturbances in ecological functioning, and affect both living and nonliving components [5]. Despite recent advancements in industrial effluent disposal strategies, it is estimated that, out of the total generated effluent, only 20% is adequately or partially treated before open disposal [6]. In particular, low- or middle-income countries show higher socioeconomic inequalities in terms of industrial effluent management plans and policies than those of developed and high-income countries [7]. Therefore, the proper treatment and disposal of these effluents are challenging and in high demand, especially in developing countries such as India.

For the rapid development and fulfillment of human needs, governments are creating clustered industrial sectors containing several operational units located in a specified area. In India, major clustered industrial hubs include Mumbai-Pune, the Hugli, Bangalore, Gujarat, Chotanagpur, Vishakhapatnam-Guntur, Gurgaon-Delhi-Meerut, Kollam-Thiruvananthapuram, and Haridwar-Dehradun, which contain thousands of operational industrial units [8]. The management of huge effluent generated from such hubs is challenging due to their varied physicochemical characteristics. The State Infrastructure and Industrial Development Corporation of Uttarakhand Limited (SIIDCUL) in the Haridwar district occupies an area of about 2034 acres with nearly 700 industries in operation. The effluent produced from SIIDCUL industrial cluster is released into a passing channel (seasonal canal) before it is partially treated inside the common effluent treatment facility. Due to the huge volume of produced composite industrial effluent (CIE), a major portion is discharged without adequate treatment, thereby causing a serious environmental menace in the vicinity [9]. Therefore, the efficient treatment of CIE in the Haridwar district has become a matter of serious concern. Out of the several techniques used for industrial wastewater treatment, phytoremediation technology has appeared as an emerging technology of environmental restoration that uses plants to remediate harmful pollutants from contaminated soil, air, and water [10,11]. Several aquatic plant species such as *Azolla pinnata*, *Eichhornia crassipes*, *Pistia stratiotes*, *Trapa natans*, *Chrysopogon zizanioides*, *Lemna* sp., *Typha latifolia*, and *Nelumbo nucifera* have the potential for wastewater treatment due to their genetic compatibility toward certain toxic pollutants. These plants are capable of growing in contaminated water bodies having high pollutant loads, thus facilitating their natural cleanup [12,13]. *N. nucifera* Gaertn. is an aquatic macrophyte belonging to the Nelumbonaceae family. It is commonly known as the sacred lotus or Indian lotus, and is found in ponds and lakes with excessive nutrient load. It is native to north–central India up to an altitude of 1400 m. *N. nucifera* is widely grown in artificial ponds for aesthetic purposes, and also for its flowers, seeds, stems, and submerged roots [14]. The flowers and seeds of *N. nucifera* have several beneficial and biochemical constituents, and are thereby sold to medicine manufacturers [15]. *N. nucifera* has remarkable potential to grow in temperate regions (30–35 °C), and may, therefore, help in cleaning the contaminated water bodies of these regions. Several varieties of the lotus are available for cultivation such as seed lotus, flower lotus, pips, stems, and rhizomes. Several studies have also reported that

N. nucifera is an ideal candidate for the phytoremediation of different types of wastewater, including domestic, industrial, and synthetic, rendering it an ideal candidate for phytoremediation purposes [16]. Till now, no study has reported the utilization of the sacred lotus (*N. nucifera*) plant for the phytoremediation of CIE. Considering the aforementioned, the present study explores the use of the *N. nucifera* plant in the batch-mode phytoremediation of CIE released from the SIIDCUL industrial cluster of Haridwar, India. Moreover, the reduction in and accumulation of various physicochemical and heavy metal pollutants of CIE were investigated to study the concentration-dependent performance of *N. nucifera*.

2. Materials and Methods

2.1. Collection of Experimental Materials

For the present study, juvenile plants of sacred lotus (*Nelumbo nucifera* Gaertn) were collected from a local pond of Jamalpur Kalan Village, Haridwar, India (29°54′05.4″ N and 78°07′20.6″ E). Farmers were asked to collect *N. nucifera* plants that had a healthy root system (Figure 1). On the other hand, the composite industrial effluent (CIE) of the State Infrastructure and Industrial Development Corporation of Uttarakhand (SIIDCUL) was collected from the effluent discharge channel near Bharat Heavy Electric Limited (BHEL) township, Haridwar, Uttarakhand, India (29°56′19.3″ N and 78°05′03.4″ E). The CIE samples were collected in polyvinyl chloride (PVC) containers of 50 L capacity and immediately transported to the laboratory for further experimentation.

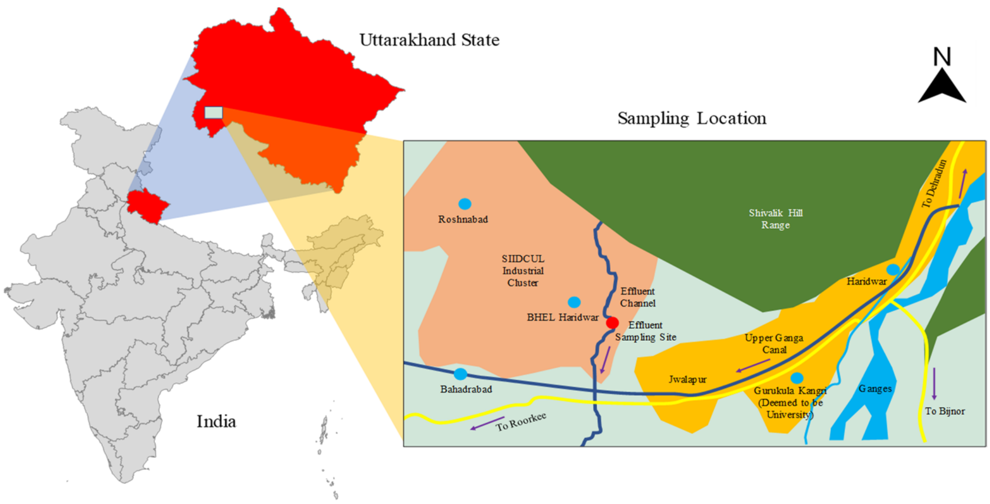

Figure 1. Map showing the sampling site for the collection of composite industrial effluent (CIE) from the SIIDCUL industrial cluster of Haridwar, India (map not in scale).

2.2. Experimental Design for Phytoremediation Experiments

Before initiating the phytoremediation experiments, the *N. nucifera* plants were carefully acclimatized in the aquatic macrophytes pond of the Multipurpose Experimental Area (MEA) of the Department of Zoology and Environmental Science, Gurukula Kangri (Deemed to be University), Haridwar, India (29°55′10.2″ N and 78°07′08.8″ E). Plastic tubs of 25 L capacity were filled with a total of 20 L of the working volume of CIE. Three different treatments of CIE *viz.*, Control (20 L borewell water), 50% (10 L CIE + 10 L borewell water), and 100% (20 L absolute CIE), were used to conduct the phytoremediation experiments. The tub was prefilled with 2 kg of garden soil, and the root of one healthy *N. nucifera* plant was submerged completely in it. Afterward, the tub was filled with 20 L borewell water and left for 24 h until the water became clear. Lastly, the borewell water was carefully drained, and tubs were filled carefully with different treatments of CIE without disturbing the soil surface. The phytoremediation experiments lasted for 45 days until the growth of the plants had become stationary. Figure 2 shows different experimental steps adopted in the current study.

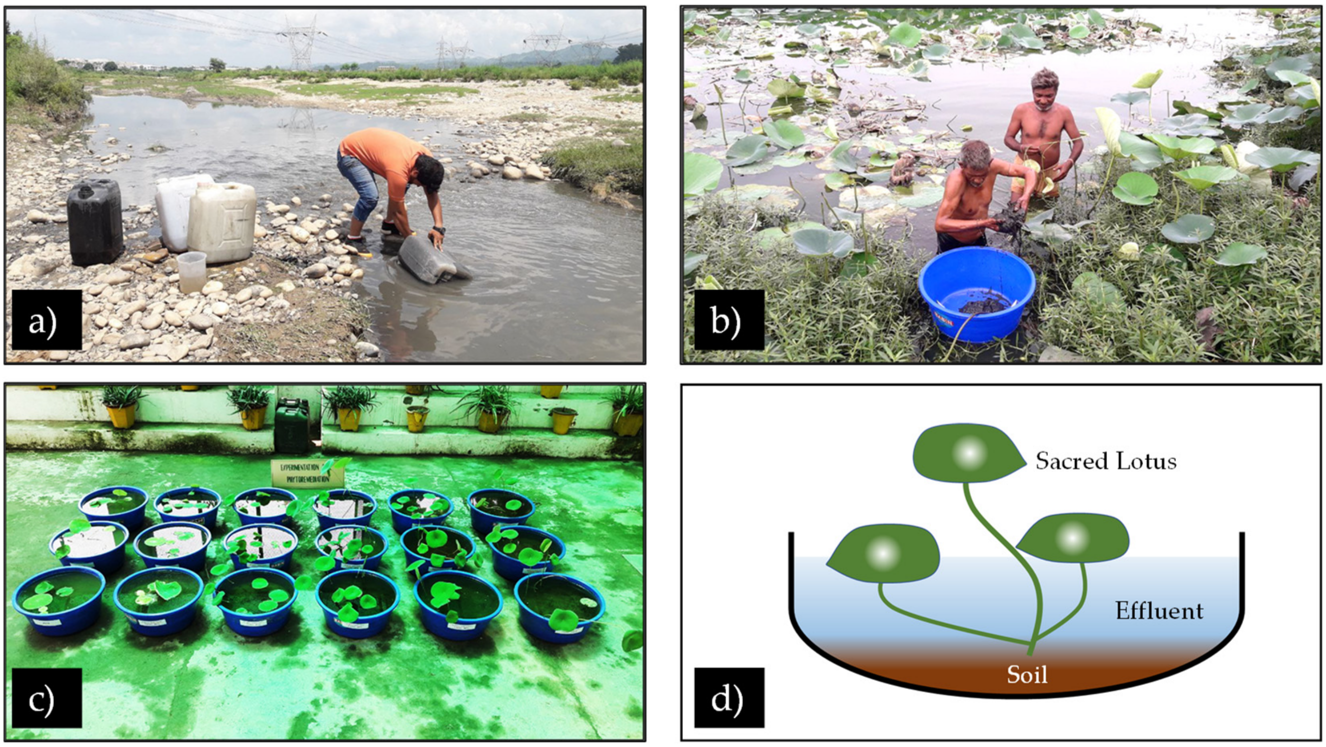

Figure 2. Various experimental activities during the current study. (**a**) Collection of composite industrial effluent; (**b**) collection of sacred lotus (*N. nucifera*) plant from the submerged field; (**c**) view of phytoremediation step; (**d**) illustration of a tub used for phytoremediation.

2.3. Chemical and Instrumental Analyses

In this study, the borewell water and CIE were analyzed for the selected physicochemical and heavy metal parameters, namely, pH, electrical conductivity (EC: dS/m), total dissolved solids (TDS: mg/L), biochemical oxygen demand (BOD: mg/L), chemical oxygen demand (COD: mg/L), total Kjeldahl's nitrogen (TKN: mg/L), phosphorus (P: mg/L), cadmium (Cd: mg/L), copper (Cu: mg/L), chromium (Cr: mg/L), iron (Fe: mg/L), lead (Pb: mg/L), and zinc (Zn: mg/L). For this purpose, standard methodologies recommended by APHA [17] and AOAC [18] were adopted. The *N. nucifera* plant biomass was also analyzed for the selected heavy metals (Cd, Cu, Cr, Fe, Pb, and Zn) and biochemical parameters (chlorophyll content: mg/g fresh weight basis). Specifically, pH, EC, and TDS were measured using a pre-calibrated meter (1611, ESICO, Parwanoo, India). The BOD_5 was determined using Winkler's dissolved oxygen method, while COD was determined by using open reflux-$K_2Cr_2O_7$ digestion followed by spectroscopic measurement (60 Cary, Agilent Technologies, Santa Clara, CA, USA) [19]. Similarly, TKN was determined by following Kjeldahl's acid digestion and distillation method as described in a previous study [20]. The contents of heavy metals were determined using atomic absorption spectroscopy (AAS: A-Analyst 800, PerkinElmer, Waltham, MA, USA) after digesting 10 mL of water or CIE sample in a diacid mixture (HNO_3:$HClO_4$; 3:1) placed on a hot plate for 1 h. On the other hand, 1 g of plant biomass was predigested (12 h) using 5 mL of the diacid mixture followed by hot-plate digestion. The heavy metal analysis of plant biomass was performed in the same way as in the case of water samples. The contents of chlorophyll in the fresh leaves of the *N. nucifera* plant were also determined by following 80% acetone extraction followed by spectroscopic measurement [21]. The other plant growth and morphological parameters were also determined by using calibrated digital weighing and measuring scales.

2.4. Data Analysis and Software

The efficiency of the *N. nucifera* plants in terms of removal of pollutants from CIE was depicted using the removal efficiency index. The index represents the aggregated difference between the initial and final parameter values [20]. The percentage removal efficiency was calculated using Equation (1):

$$\text{Removal efficiency } (\%) = \left(\frac{\text{I} - \text{F}}{\text{I}}\right) \times 100 \quad (1)$$

where I and F represent the initial and final pollutant concentrations in the CIE, respectively. The heavy metal uptake efficiency of the *N. nucifera* plants was also exhibited using bioaccumulation and translocation factors. Bioaccumulation factor (BCF) is the proportion of the concentration of a chemical in the plant to the chemical concentration medium, and expressed as liter units per kilogram (ratio of mg of chemical to mg of chemical per kg of plant per liter of effluent medium). The BCF was calculated by using Equation (2) [22]:

$$\text{Bioaccumulation factor (Bf)} = \frac{C_{\text{plant}}}{C_{\text{effluent}}} \quad (2)$$

where C_{plant} is the metal concentration in the plant sample (mg/kg; dry weight basis), while $C_{effluent}$ is the metal concentration in the CIE medium (mg/L). Similarly, the translocation factor helps in screening the hyperaccumulator plant species for suitability in the phytoextraction of heavy metals. Generally, TF values greater than 1 indicate the strong translocation of heavy metals to the aerial parts of the plant. To evaluate the potential of phytoextraction, the translocation factor (TF) was calculated by using Equation (3) [23].

$$\text{Translocation factor } (T_f) = \frac{C_a}{C_r} \quad (3)$$

where C_a is the heavy metal concentration in the aerial parts of the plant, and C_r is the heavy metal concentration in the root parts of the plant as mg/kg (dry weight basis).

All the experiments were conducted as six replicates, and values are presented as mean followed by standard deviation. The significant difference between the treatment groups and treatment days was evaluated on the basis of a single-tailed (unpaired) Student's *t*-test, one-way analysis of variance (ANOVA), Pearson correlation, and principal component analysis (PCA) tests. The level of statistical significance was adjusted to a 95% confident interval ($p < 0.05$) for all tests. Microsoft Excel (Version 2019, Microsoft Corp., Redmond, DC, USA) and OriginPro (Version 2022a, Student edition, OriginLab Corp., Northampton, MA, USA) software packages were used for data analysis and visualization.

3. Results and Discussion

3.1. Pollution Load of Composite Industrial Effluent Used in This Study

The single-tailed Student's *t*-test showed a significant ($p < 0.05$) difference between all the tested physicochemical characteristics of borewell water and CIE, as shown in Table 1. It was also evidenced that most of the parameters of CIE were beyond the standard limit of surface water discharge except for TKN, Cd, Cu, and Pb, as prescribed by the Central Pollution Control Board (CPCB) of India. Thus, CIE has a higher pollution load and should not be disposed of directly into the effluent channel without proper treatment. The higher pollution load of CIE was likely due to the cluster of diverse industries operating collectively in this region. The presence of different heavy metals in CIE might be due to the operation of different manufacturing units such as electroplating, agrochemical, food processing, pharmaceuticals, food, and beverages, cosmetics, commercial complexes, and workshops, which frequently release their mixed-type effluents in the common effluent channel of the SIIDCUL.

Table 1. Average characteristics (mean ± SD; $n = 3$) of borewell water and composite industrial effluent (CIE) used for the phytoremediation experiments.

Parameters	Borewell Water	Composite Industrial Effluent (CIE)	Student's *t*-Test ^		CPCB Standard for Surface Discharge
			t-Value	*p*-Value	
pH	7.71 ± 0.08	8.85 ± 0.09	16.397	<0.001	5.5–9.0
EC (dS/m)	0.58 ± 0.01	1.40 ± 0.02	63.516	<0.001	*na*
TDS (mg/L)	487.06 ± 24.49	1071.84 ± 85.25	11.419	<0.001	*na*
BOD (mg/L)	4.10 ± 0.19	374.49 ± 20.29	31.616	<0.001	30
COD (mg/L)	11.14 ± 2.20	1118.11 ± 58.10	32.976	<0.001	250
TKN (mg/L)	13.11 ± 2.31	76.55 ± 9.35	11.409	<0.001	100
P (mg/L)	6.85 ± 1.55	43.67 ± 8.18	7.660	<0.001	5.0
Cd (mg/L)	*Bdl*	1.92 ± 0.07	47.507	<0.001	2.0
Cu (mg/L)	0.03 ± 0.01	2.16 ± 0.07	52.174	<0.001	3.0
Cr (mg/L)	*Bdl*	2.14 ± 0.12	30.888	<0.001	*na*
Fe (mg/L)	1.09 ± 0.06	8.36 ± 0.26	47.190	<0.001	3.0
Pb (mg/L)	*Bdl*	0.22 ± 0.02	19.052	<0.001	0.1
Zn (mg/L)	0.43 ± 0.05	9.84 ± 0.31	51.905	<0.001	5.0

^: Statistically significant from each other if $p < 0.05$; *Bdl*: below detectable limits; *na*: not available; CPCB: Central Pollution Control Board of India.

These findings were supported by the results of Shalini et al. [24], who evaluated the different parameters of CIE and noted values in the range of 2.42–7.81 for pH, 0.45 mS/m for EC, 1.00–602 mg/L for TSS, 280–6950 mg/L for TDS, 281–7035 mg/L for TS, 0.03–1.17 mg/L for Pb, 0.06–0.13 mg/L for Cr, 0.04 mg/L for Fe, and 0.11–1.65 mg/L for Mn. Moreover, Kumar and Thakur [25] analyzed various parameters of the CIE and reported that the values of heavy metals were in the range of 1.50–1.65 mg/L for Cr, 2.71–2.84 mg/L for Cd, 1.42–1.56 mg/L for Cu, 2.04–2.45 mg/L for Fe, 1.02–1.26 mg/L for Mn, 0.42–0.50 mg/L for Zn. Thus, previous studies have shown that the CIE had a considerable load of different pollutants that need to be treated before its disposal in the effluent channel.

3.2. Reduction of Pollution Load by Sacred Lotus (N. nucifera)

After the phytoremediation experiments, a significant ($p < 0.05$) reduction in the pollution load of CIE was achieved, as shown in Table 2. However, the highest removal of all parameters was observed using 50% CIE concentration, followed by 100% and control CIE treatments. Figure 3 shows the assessment of pollutant removal efficiencies among the selected experimental treatments. In particular, the maximal removal efficiency of the selected physicochemical parameters was noted as TDS (89.56%), BOD (78.20%), COD (79.41%), TKN (86.48%), and P (76.37%), respectively. Similarly, the contents of the removal of heavy metals also significantly ($p < 0.05$) declined after the termination of phytoremediation experiments, where maximal removal efficiency was achieved as Cd (70.37%), Cu (85.82%), Cr (68.61%), Fe (72.86%), Pb (76.92%), and Zn (74.51%), respectively. The maximal removal of these pollutants in 50% CIE concentration might have been because absolute (100%) CIE had a high pollution load, which may not be feasible for *N. nucifera* plant growth. Similarly, the control treatment showed minimal removal due to the lesser availability of nutrients, which led to slower plant growth. Hyperaccumulator macrophytes such as *N. nucifera* are well-known for their capabilities to recycle nutrients within the aquatic bodies [26]. The physiological and metabolic adaptations of these plants allow for them to capture certain organic and inorganic pollutants that act as their primary nutrients, thereby cleaning the aquatic bodies through natural pathways [27]. These exceptional properties of aquatic macrophytes render them ideal candidates for the phytoremediation of industrial effluents such as CIE.

Table 2. Reduction in the pollution load of composite industrial effluent (CIE) by sacred lotus (*N. nucifera*) before and after phytoremediation experiments.

Parameters	Treatments	Concentration	
		Initial	Final
pH	Control	7.78 ± 0.04 a	6.21 ± 0.02 b
	50%	8.59 ± 0.03 a	6.03 ± 0.02 b
	100%	8.94 ± 0.03 a	6.88 ± 0.04 b
EC (dS/m)	Control	0.58 ± 0.01 a	0.28 ± 0.07 b
	50%	1.08 ± 0.05 a	0.40 ± 0.05 b
	100%	1.42 ± 0.07 a	0.94 ± 0.04 b
TDS (mg/L)	Control	513.42 ± 6.77 a	187.25 ± 5.37 b
	50%	902.71 ± 5.10 a	94.28 ± 5.02 b
	100%	1162.49 ± 5.04 a	547.27 ± 6.13 b
BOD (mg/L)	Control	4.27 ± 0.05 a	1.60 ± 0.06 b
	50%	235.60 ± 4.06 a	51.35 ± 5.08 b
	100%	392.50 ± 4.36 a	147.78 ± 5.78 b
COD (mg/L)	Control	13.20 ± 0.96 a	5.85 ± 1.02 b
	50%	708.89 ± 6.07 a	145.95 ± 2.52 b
	100%	1171.20 ± 3.60 a	445.57 ± 5.87 b
TKN (mg/L)	Control	15.72 ± 2.05 a	4.83 ± 1.73 b
	50%	61.24 ± 2.07 a	8.28 ± 1.22 b
	100%	86.74 ± 2.97 a	24.50 ± 2.10 b
P (mg/L)	Control	8.10 ± 0.98 a	3.31 ± 0.23 b
	50%	34.74 ± 2.02 a	8.21 ± 0.07 b
	100%	52.50 ± 1.74 a	18.48 ± 1.90 b
Cd (mg/L)	Control	*Bdl*	*Bdl*
	50%	1.18 ± 0.01 a	0.35 ± 0.08 b
	100%	1.98 ± 0.04 a	1.14 ± 0.01 b
Cu (mg/L)	Control	0.03 ± 0.01 a	0.01 ± 0.02 b
	50%	1.34 ± 0.03 a	0.19 ± 0.07 b
	100%	2.22 ± 0.04 a	1.16 ± 0.06 b
Cr (mg/L)	Control	*Bdl*	*Bdl*
	50%	1.37 ± 0.02 a	0.43 ± 0.05 b
	100%	2.26 ± 0.02 a	1.062 ± 0.07 b
Fe (mg/L)	Control	1.14 ± 0.01 a	0.50 ± 0.01 b
	50%	5.60 ± 0.02 a	1.52 ± 0.02 b
	100%	8.57 ± 0.02 a	3.25 ± 0.02 b
Pb (mg/L)	Control	*Bdl*	*Bdl*
	50%	0.13 ± 0.02 a	0.03 ± 0.07 b
	100%	0.23 ± 0.08 a	0.11 ± 0.02 b
Zn (mg/L)	Control	0.47 ± 0.04 a	0.16 ± 0.02 b
	50%	6.08 ± 0.09 a	1.55 ± 0.04 b
	100%	10.14 ± 0.03 a	4.92 ± 0.02 b

Values are mean ± SD of six replicates; The same letters (a, b) indicate no significant difference between the initial and final parameter values at $p < 0.05$; *Bdl*: below detectable limits.

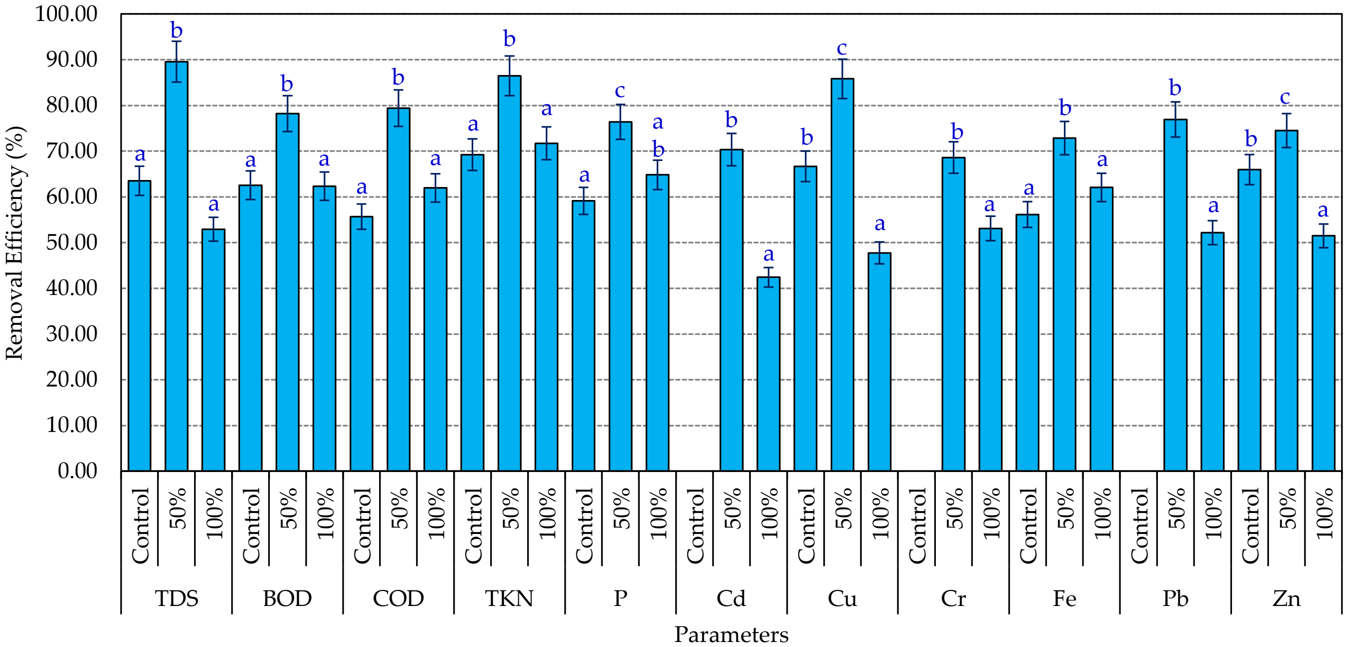

Figure 3. Removal efficiency (%) of different physicochemical and heavy metal parameters of composite industrial effluent using sacred lotus (*N. nucifera*) plant (the same letters (a, b, c) indicate no significant difference between the initial and final parameter values at $p < 0.05$).

Although the *N. nucifera* plant is widely investigated for its phytoremediation potential in previous studies, it has not been explored for the biotreatment of mixed-type effluents such as CIE. In their study, Thongtha et al. [28] performed phytoremediation experiments for the biotreatment of domestic wastewater using the *N. nucifera* plant, and found that the loads of pH, EC, TDS, BOD, COD, and TP were significantly reduced after five consecutive cycles of 25 days. Similarly, Mishra [29] also investigated the Cd and Cu reduction efficiency of *N. nucifera* from a synthetically prepared solution, and reported about 87–96% Cu and 65–85% Cd reduction in the 7 days of the incubation period. Moreover, a study by Kumar et al. [26] investigated the heavy metal removal potential of *N. nucifera* plants found in Pariyej Community Reserve Lake in Gujarat, India. They reported that *N. nucifera* had the potential to eradicate the contents of Cd, Cu, Co, Ni, Pb, and Zn. Abd Rasid et al. [30] also investigated the efficiency of *N. nucifera* for the treatment of domestic wastewater, and found that BOD, COD, and turbidity were reported as 97.10%, 55.00%, and 88.80%, respectively. Hence, the findings reported in these studies are in line with those obtained in the current study on reducing the pollution load of CIE using the *N. nucifera* plant.

3.3. Bioaccumulation of Heavy Metals by Sacred Lotus (N. nucifera)

Table 3 depicts the results of heavy metal concentration in *N. nucifera* plant tissues (roots and leaves) before and after phytoremediation. The contents of the selected heavy metals in the *N. nucifera* plant were significantly ($p < 0.05$) increased after CIE treatment. The decreasing order of heavy metal accumulation was Fe > Zn > Cu > Pb > Cr > Cd for both the root and leaf parts of the *N. nucifera* plant. In particular, the maximal contents of the selected heavy metals in the root and leaf parts of *N. nucifera* were Cd (0.73 ± 0.06 and 0.75 ± 0.02 mg/kg), Cu (4.95 ± 0.04 and 5.14 ± 0.01 mg/kg), Cr (4.42 ± 0.96 and 4.69 ± 0.11 mg/kg), Fe (28.00 ± 0.99 and 29.96 ± 0.43 mg/kg), Pb (4.47 ± 0.01 and 4.85 ± 0.02 mg/kg), and Zn (16.13 ± 0.47 and 17.34 ± 1.06 mg/kg), respectively. The accumulation patterns were in line with the pollution removal capacity where 50% CIE treatment showed the maximal accumulation of heavy metals. Comparatively, the root parts of the *N. nucifera* exhibited a greater accumulation of Fe and Cr, while Zn, Cu, Cd, and Pb were mostly accumulated in the leaf part of the plant. This might have been due to the induced toxicity of Zn, Cu, Cd, and Pb in the root of aquatic macrophytes, which damages the plant root system by altering the plant physiology and disrupting the biochemical mechanism [9,11]. BCF values for all heavy metals were also >1, indicating the strong bioaccumulation properties of the *N. nucifera* plant. However, the leaf parts of the *N. nucifera* showed high BCF values compared to those of the leaf part. Similarly, TF (>1) values also suggested that the plant had a good affinity for translocating the heavy metals from its root part to the leaf part. The highest accumulation of heavy metals from 50% CIE treatment might have been due to their favorable concentration within the medium that allowed for efficient uptake by the *N. nucifera* plant.

Table 3. Accumulation of selected heavy metals by the root and leaf parts of sacred lotus (*N. nucifera*) before and after phytoremediation of composite industrial effluent (CIE).

Heavy Metals	Treatments	Before Phytoremediation (mg/kg)		After Phytoremediation (mg/kg)		BCF		TF
		Roots	Leaves	Roots	Leaves	Roots	Leaves	
Cd	Control	0.34 ± 0.09 a	0.37 ± 0.04 a	0.34 ± 0.09 a	0.37 ± 0.04 a	*na*	*na*	*na*
	50%			0.73 ± 0.06 b	0.75 ± 0.02 b	2.10	2.14	1.02
	100%			0.72 ± 0.06 b	0.70 ± 0.10 b	0.64	0.62	0.97
Cu	Control	4.37 ± 0.05 a	4.43 ± 0.08 a	4.38 ± 0.02 a	4.38 ± 0.04 a	273.80	273.90	1.00
	50%			4.77 ± 0.03 b	4.99 ± 0.02 b	11.99	12.55	1.05
	100%			4.95 ± 0.04 b	5.14 ± 0.01 b	4.25	4.42	1.04
Cr	Control	3.04 ± 0.08 a	3.90 ± 0.08 a	3.70 ± 0.05 b	3.90 ± 0.08 b	*na*	*na*	*na*
	50%			4.42 ± 0.96 b	4.69 ± 0.11 b	10.20	10.81	1.06
	100%			4.22 ± 1.06 b	4.33 ± 0.14 b	3.98	4.08	1.02
Fe	Control	26.25 ± 3.83 a	27.24 ± 4.00 a	26.54 ± 6.90 a	27.16 ± 3.90 a	53.31	54.55	1.02
	50%			28.00 ± 0.99 b	29.96 ± 0.43 a	18.43	19.71	1.07
	100%			28.68 ± 1.62 a	28.79 ± 1.75 a	8.83	8.86	1.00
Pb	Control	4.37 ± 0.06 a	4.81 ± 0.03 a	4.37 ± 0.06 a	4.81 ± 0.03 a	*na*	*na*	*na*
	50%			4.41 ± 0.07 b	4.89 ± 0.04 a	125.30	139.08	1.11
	100%			4.47 ± 0.01 b	4.85 ± 0.02 a	38.95	42.19	1.08
Zn	Control	14.10 ± 0.05 a	15.79 ± 2.83 a	14.41 ± 0.04 b	15.90 ± 0.31 a	85.97	94.82	1.10
	50%			15.51 ± 0.12 b	17.81 ± 1.14 a	10.01	11.49	1.15
	100%			16.13 ± 0.47 b	17.34 ± 1.06 a	3.28	3.53	1.07

Values are mean ± SD of six replicates; The same letters (a, b) indicate no significant difference between the initial and final parameter values at $p < 0.05$; BCF: bioaccumulation factor; TF: translocation factor (root to leaves); *na*: not applicable.

In this, Cu, Fe, Cr, and Mn are trace elements essentially taken by higher plant root systems for efficient growth, whereas Cd and Pb might also be absorbed as a substitute for other elements. However, Cd and Pb are highly toxic metals that negatively affect plant growth and that are still widely accumulated to create a chemical equilibrium for efficient survival in metal-stressed environments. Therefore, Cd and Pb contents present in CIE were accumulated by *N. nucifera* plants in the current study while their actual functions were unknown. Very limited studies are available on the phytoaccumulation of heavy metals by *N. nucifera* in wastewater treatment [31]. Mishra [29] found that the *N. nucifera* plant accumulated up to 1.10 and 0.41 mg/g of Cd, and 1.70 and 0.41 mg/g of Cu in its root and leaf parts, respectively, when grown in aqueous solutions. Moreover, Ashraf et al. [32] found that the sacred lotus had good accumulation properties, particularly for Pb, Cu, and Zn elements. Similarly, Ashraf et al. [33] also studied the heavy metal uptake potential of *N. nucifera* from selected lake sites of Bestari Jaya, Malaysia. They reported that *N. nucifera* had a significant accumulation of Pb, Cu, and Zn, where BCF values reached 0.30, 0.33, and 0.24, respectively.

3.4. Changes in Plant Growth Attributes of Sacred Lotus (*N. nucifera*)

In this study, CIE had various nutrients, including macro and micronutrients, which played an important role in the growth of *N. nucifera* plants. After the phytoremediation experiments, significant ($p < 0.05$) improvement was observed in the selected plant growth attributes of *N. nucifera* (Table 4). Since the utmost factor altering the growth and development of the plants is the accessibility of the nutrients (in the form of contaminations) within its growing medium, 50% CIE treatment showed the maximal increment in fresh plant biomass (760.70 ± 8.77 g/plant; without flowers), chlorophyll content (4.30 ± 0.22 mg/g fwt.), plant height (154.05 ± 4.55 cm), root length (70.35 ± 2.42 cm), leaf spread (41.58 ± 0.26 cm), number of leaves (10.00 ± 1.00 per plant), and number of flowers (16.00 ± 2.00). Additionally, the flower yield in the 50% treatment was more than twofold that of the control treatment. Though the plant growth attributes increased from control to 50% CIE treatment, absolute CIE loading showed a decline parallel to the pollutant removal efficiency. On the other hand, Pearson correlation studies revealed that CIE loading had a significant positive relationship with all growth traits of *N. nucifera*, as given in Figure 4a. Similarly, the PCA biplot (Figure 4b) also showed that CIE had the highest positive influence on fresh plant biomass followed by leaf spread, the number of flowers, and plant height. Plants respond to external nutrient stress, which might be a reason behind the lesser growth of sacred lotus in 100% CIE treatment. The plants were able to adapt efficiently in the 50% treatment, which resulted in efficient chlorophyll activities and thereby high biomass production. Previous studies have shown that *N. nucifera* has high growth rates and can spread over water bodies within a short period [34]. However, the attributed growth largely depended on the nutrient bioavailability.

Table 4. Changes in plant growth attributes of sacred lotus (*N. nucifera*) grown in selected concentrations of composite industrial effluent (CIE).

Parameters	Treatments	Changes	
		Initial	Final
Fresh Plant Biomass (g/plant; without flowers)	Control	465.24 ± 7.25 a	660.64 ± 4.71 b
	50%	475.77 ± 8.63 a	760.70 ± 8.77 b
	100%	470.84 ± 9.80 a	670.40 ± 17.89 b
Chlorophyll content (mg/g fwt.)	Control	2.90 ± 0.05 a	3.77 ± 0.47 b
	50%	2.88 ± 0.07 a	4.30 ± 0.22 b
	100%	2.92 ± 0.05 a	3.95 ± 0.06 b
Plant Height (cm)	Control	82.60 ± 1.30 a	113.54 ± 3.18 b
	50%	82.80 ± 0.98 a	154.05 ± 4.55 b
	100%	83.50 ± 2.04 a	140.22 ± 2.81 b
Root Length (cm)	Control	27.47 ± 0.25 a	50.92 ± 1.50 b
	50%	26.82 ± 0.70 a	70.35 ± 2.42 b
	100%	25.30 ± 0.46 a	65.20 ± 1.74 b
Leaf Spread (cm)	Control	16.10 ± 0.12 a	28.50 ± 0.35 b
	50%	15.20 ± 0.15 a	41.58 ± 0.26 b
	100%	14.39 ± 0.20 a	32.14 ± 0.41 b
Number of Leaves (per plant)	Control	3.00 ± 0.00 a	6.00 ± 1.00 b
	50%	3.00 ± 0.00 a	10.00 ± 1.00 b
	100%	3.00 ± 0.00 a	7.00 ± 2.00 b
Number of Flowers	Control	*na*	7.00 ± 1.00
	50%	*na*	16.00 ± 2.00
	100%	*na*	13.00 ± 1.00

Values are mean ± SD of six replicates; data presented as mean followed by the standard deviation of six replicates. The same letters (a, b) indicate no significant difference between the initial and final parameter values at $p < 0.05$; *na*: not applicable.

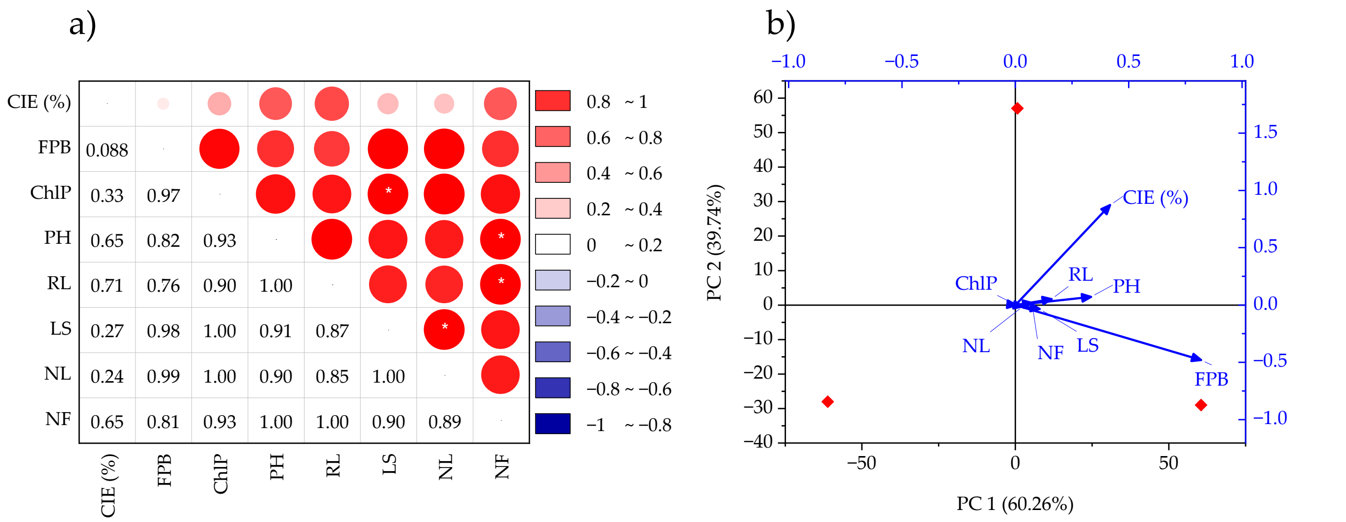

Figure 4. (**a**) Pearson correlation and (**b**) principal component (PC) analysis biplot showing interactions between composite industrial effluent concentration (CIE) and various plant growth attributes of sacred lotus (*N. nucifera*) (FPB: fresh plant biomass; ChlP: chlorophyll content; PH: plant height; RL: root length; LS: leaf spread; NL: number of leaves; NF: number of flowers; *: level of significance i.e., $p < 0.05$).

Previous studies showed that many varieties of *N. nucifera* can be grown for agribusiness. The plant may achieve a height of 190 cm, where the numbers of flowers and fruits were in the ranges of 10–15 and 30–52, respectively [35]. A report by Wang et al. [16] showed that oxidative stress induced by Cd disrupted the defense system of the *N. nucifera*, which resulted in lesser chlorophyll production compared to the control treatment. They found that the relative leaf area was also significantly reduced under high Cd stress, which supported the results of the current study regarding lower values of leaf spread. Similarly, in a study conducted by Obando [36], *N. nucifera* showed decreased plant growth response under high Mn load in terms of lower seedling biomass and chlorophyll contents. Therefore, CIE loading had a significant effect on plant growth and biochemical attributes of *N. nucifera*, where 50% concentration was identified as the best treatment for phytoremediation purposes.

4. Conclusions

The findings of this study showed that composite industrial effluent (CIE) of the SIIDCUL complex (Haridwar, India) had significant loads of physicochemical and heavy metal pollutants. After the termination of phytoremediation experiments, the sacred lotus (*Nelumbo nucifera*) plant was able to reduce these pollutants most efficiently by using a 50% concentration treatment of CIE. Moreover, heavy metal accumulation studies showed that *N. nucifera* was identified as a hyperaccumulator macrophyte as revealed by bioaccumulation and translocation factor values (>1). Therefore, considering the problem of mixed-type industrial effluents like CIE in Haridwar, *N. nucifera* might be an ideal candidate for its sustainable management. Further studies on the phytochemical characterization and management of the harvested biomass of *N. nucifera* plant are highly recommended.

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