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Assessment of Heavy Metal Removal from Industrial Effluents by *Spirogyra* Species under Controlled Laboratory Conditions: A Case Study from Hayatabad Industrial State, Peshawar

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Abstract

Industrial wastewater often contains toxic heavy metals (HMs) such as arsenic (As), cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn), which pose serious risks to human health and the environment. This study evaluated the potential of two *Spirogyra* species, *S. aequinotilalis* and *S. pratensis*, for the removal of HMs from wastewater collected from the Hayatabad Industrial State (HIS), Peshawar, Pakistan, under controlled laboratory conditions. Batch experiments were conducted at varying metal concentrations (5-50 mg/L), pH levels (5-8), and contact times (0-120 minutes). The results demonstrated that *S. aequinotilalis* achieved maximum removal efficiencies of 82% for Pb, 76% for Cd, 69% for Cu, 64% for Zn, and 58% for As, while *S. pratensis* removed 78% of Pb, 71% of Cd, 65% of Cu, 60% of Zn, and 52% of As under optimal conditions (pH 7, 100 mg/L biomass, 90 minutes contact time). These findings highlight the efficient and low-cost potential of locally isolated *Spirogyra* species for HM remediation and provide insights for sustainable wastewater treatment in industrial areas.

Keywords

Spirogyra aequinotilalis, *Spirogyra pratensis*, Heavy metals, Wastewater treatment, Bioremediation

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1. Introduction

The rapid growth of industrialization and human activities has resulted in the production of large volumes of wastewater (WW), which often flows into adjacent aquatic ecosystems such as streams, ponds, lakes, rivers, and oceans without adequate treatment or decontamination [1,2]. This WW commonly contains toxic heavy metals (HMs) such as arsenic (As), cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn), most of which are non-degradable in nature [3]. Various industries, including ore mining, marble processing, plastic manufacturing, petroleum refining, metal plating, and silver-plating, are major sources of water contamination due to the release of potentially toxic HMs, posing risks to human health and the environment [4]. In addition, household wastes, leachates from dumping sites, and agricultural runoff contribute further toxic HMs to WW [5].

HMs tend to bioaccumulate in living organisms, and the process of biomagnification can increase their concentration to levels that cause toxic effects, with many metals being potentially carcinogenic [6,7]. Since HMs are non-biodegradable, highly toxic, and stable, their removal from the environment is essential [8]. Industrial WW contains chemical compounds in various forms and concentrations, and discharge without proper treatment can lead to substantial surface and groundwater pollution [9]. Different manufacturing sectors release various toxic HMs into the environment [10]. Crude oil refineries and metallurgical industries discharge metals such as Cd, As, Cr, Cu, Pb, Hg, and Zn, while chemical and petrochemical industries primarily release Cd, As, Cu, and Pb. Chemical fertilizer industries emit Cd, As, Cr, Cu, Pb, Hg, and Zn. Paper industries contribute Cd, As, Cu, and Pb, whereas ceramics and glass industries release Cd and Cr. Leather processing and textile industries mainly discharge Cd [10]. It has been reported that exposure to approximately 40 toxic HMs can adversely affect all biota, including humans, at various life stages, causing long-term environmental and health impacts [11]. Industrial emissions containing toxic HMs create serious ecological and human health problems [12]. These metals can enter the food chain, producing long-term effects on living organisms [13]. Many countries have established standards to regulate industrial wastewater discharge and ensure that HM concentrations remain within permissible limits, highlighting the urgent need for proper WW management.

In recent years, advanced materials have garnered significant attention for their potential in water treatment due to their high surface area, tunable properties, and capacity for selective pollutant removal. Materials such as nanoparticles, metal-organic frameworks (MOFs), and composite adsorbents have shown promise in enhancing the efficiency of water purification processes [14]. These materials can be engineered to target specific contaminants and operate under varying environmental conditions, making them versatile tools for water remediation. However, despite the progress in advanced material development, the cost and complexity of their synthesis often hinder their widespread application. In contrast, biological systems like algae offer a more sustainable, low-cost alternative that could complement or even replace these advanced materials in certain settings. Adsorption is an effective and widely used method for removing pollutants from water due to its simplicity, low cost, and high efficiency. Various adsorbents, including natural and advanced materials, have been explored to enhance removal of heavy metals and other contaminants, making adsorption a key strategy for water treatment [15].

In response to these challenges, biosorption and bioremediation have emerged as low-cost, effective strategies for HM removal [16]. Biosorption refers to the passive uptake of toxic metals by living or dead biomass, in contrast to bioaccumulation, which occurs only in living organisms and depends on metabolic activity. Using dead biomass for HM removal can reduce potential toxicity issues while retaining high efficiency [16]. Previous studies have demonstrated the potential of various microorganisms including bacteria, plants, fungi, and algae for the bioremediation and phytoremediation of industrial WW [17,18]. Algal species, in particular, have been extensively studied for their ability to remove toxic HMs from polluted water, including household and industrial effluents [19]. Species such as *Chlorella vulgaris*, *Spirogyra*, and *Chlorella fusca* have shown promising HM removal capabilities [20].

Globally, the treatment of HMs from WW has attracted considerable attention as a cost-effective and environmentally sustainable approach. While conventional methods such as nanotechnology, biochar, activated carbon, ion exchange, and chemical precipitation are effective, they are often costly and have practical limitations [21-24]. Recently, algae-based bioremediation has emerged as a low-cost, efficient method for removing toxic HMs from water systems [25]. Algae leverage photosynthesis to convert sunlight into biomass while uptaking metals, making them a promising green technology [26]. The efficiency of HM removal depends on algal species as well as environmental factors such as pH, metal concentration, and temperature [27]. Biological treatments offer advantages over physical and chemical methods, emphasizing the need for sustainable approaches [28]. In this study, *Spirogyra aequinotilalis* and *Spirogyra pratensis* were evaluated for their ability to remove toxic HMs from the WW of the Hayatabad Industrial State (HIS), Peshawar, Khyber Pakhtunkhwa, Pakistan.

2. Material and Methods

2.1 Study Area

Hayatabad is the residential and industrialized region of District, Peshawar and situated at 33°59'5.99" N 71°27'14.99" E (Figure 1). Hayatabad Industrial State (HIS) was established by the Department of Industries, Government of Pakistan in 1963. HIS is located in the western zone of district Peshawar near to Jamroad Road. Warsak Canal running in west side of HIS, while the southern-sides are bordered by dry land. The HIS contains different kinds of manufacturing industries, like plastic, cement, paints, paper, wood furniture, steel factories, beverages, foodstuff, oil, fabric, laser printing, marble, pharmacological companies, fibre and cotton, Polyvinyl chloride (PVC), tiles, pottery and many others. Waste run-offs from these mentioned industries comes through the distinct pipes and fall into Malankandher Nala (the central channel) and then goes into Kabul River, thus polluting the entire ecosystem.

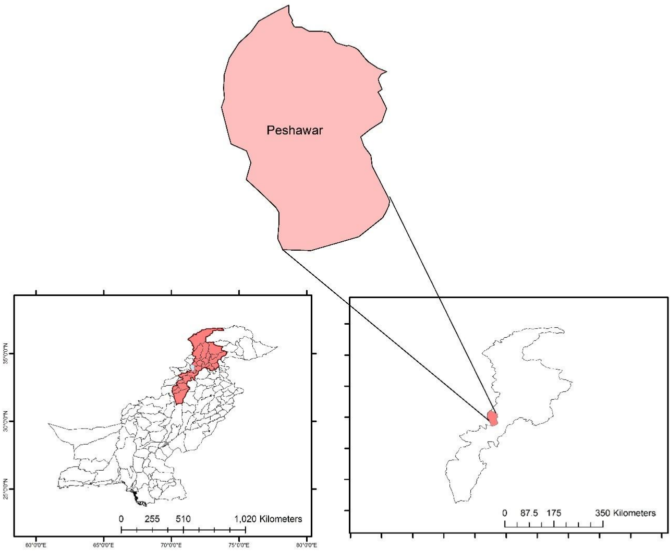

Figure 1. Study area map showing Hayatabad Industrial State (HIS) in District Peshawar, Pakistan.

2.2 Selection, Identification and Growing of Algae Species

S. aequinotilalis (Figure 2) and *S. pratensis* (Figure 3) were collected from the local fresh water pond in Amirabad village, District Charsadda, Khyber Pakhtunkhwa, Pakistan. The collected species were cleaned carefully with fresh water to remove the particles of soil and then rinsed with deionized water. The above stated species of algae were observed under electron microscope (JEM-2100, JEOL Ltd. Akishima, Tokyo, Japan) and identified taxonomically, later it was verified by a taxonomist in the Department of Microbiology, Abdul Wali Khan University, Mardan, Pakistan. These species were then grown in purified water for almost 3 to 4 weeks under the sun light with room temperature and then applied for the following research work.

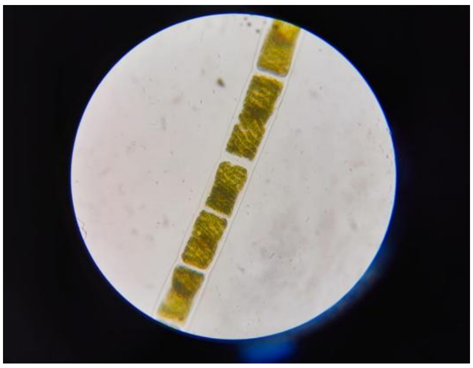

Figure 2. *Spirogyra aequinotilalis.*

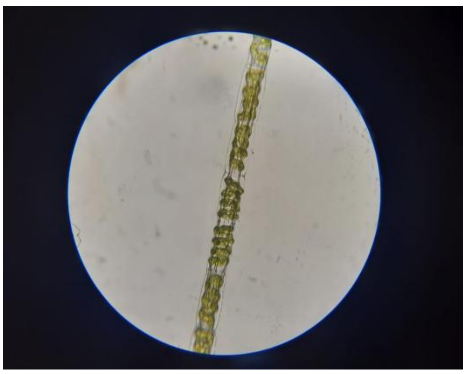

Figure 3. *Spirogyra pratensis.*

2.3 Morphological Analysis of Algal Biomass

The surface morphology and structural characteristics of the freshwater algae *S. aequinotilalis* and *S. pratensis* were analyzed using Atomic Force Microscopy (AFM) and Scanning Electron Microscopy (SEM). AFM (Dimension Icon, Bruker Corporation, Billerica, Massachusetts, United States) was used to examine the surface topology of algal cells under ambient conditions, providing high-resolution three-dimensional surface images. SEM (FEI Quanta 250, Thermo Fisher Scientific, Hillsboro, Oregon, United States) was employed to observe detailed cell morphology and structural changes after exposure to heavy metals. Samples were prepared by washing the algal biomass with distilled water, air-drying, and coating with a thin layer of gold prior to SEM imaging. All imaging procedures were conducted following standard protocols to ensure reproducibility.

2.4 Preparation of Standard Solutions

Standard solutions of HMs were synthesized using Cr $(NO_3)_2$, Cd $(NO_3)_2$, Ni $(NO_3)_2$ and Pb $(NO_3)_2$ (analytical grade) in distilled water (Milli-Q grade). The initial amount of Cr, Cd, Ni and Pb ranging from 0.5-10 mg/L, the cultural medium for species was adapted in deionized water. In advance, the culture medium for algae was sanitized by autoclave (FEI Quanta 250, Thermo Fisher Scientific, Hillsboro, Oregon, United States) at 125 °C for almost 10 minutes, while the pH was kept in the range of 6.7 to 6.8 with 1 M of sodium hydroxide (NaOH) [29].

2.5 Trial-Based Experimental Setup

A series of preliminary trials were conducted to evaluate the effectiveness of *S.aequinotilalis* and *S. pratensis* in removing selected heavy metals from aqueous solutions under controlled laboratory conditions. All tests were conducted in Volumetric flasks (250 mL) including 150 mL of Gorham's medium and added 20 ml of each Cr, Cd, Ni and Pb standard solutions of desirable amount 0.5, 1, 5 and 10 mg/L with pH value 6.7-6.8. For the avoidance of HMs contamination, all Volumetric flasks and Glass wares were saturated in 10 % Nitric acid (HNO_3) for 24 hours, rinsed with deionized water and then dried out at 100 °C in oven (LABQ 315, LabEquip, Chatswood, New South Wales, Australia) in order to applied. Alive species of algae (3 g of each) such as *S. equinoctials* and *S. pratensis* have been added to all flasks. Different experiments were carried out under control environment and each test was carried out in triplicate. Throughout the cultivation phase of two week, the average temperature was kept at 20 °C, whereas light and dark time period was 12:12 hours in organized and hygienic setting with constant ventilation and light amount was constantly produced by tungsten tube light.

2.6 Experimental Setup for Industrial Wastewater

Preliminary experiments were conducted to evaluate the effectiveness of *S. aequinotilalis* and *S. pratensis* in removing heavy metals from industrial WW under controlled laboratory conditions. The treatment experiments of HMs and fresh water algae were done in different plastic containers each containing 1 L of industrial waste water. The containers were cleaned with Nitric acid (HNO_3) to clear the metal ions and after rinsed with washed with deionized water. 3 g of fresh water algae of both *S. equinoctials* and *S. pratensis* were added to each plastic container. The overall test was conducted for nine days in three replicates under sun light and dark 12:12 h at average temperature of 20 ±1 °C.

2.7 Analysis of Heavy Metals

Heavy metal concentrations in the WW before and after treatment were measured using Atomic Absorption Spectrophotometry (Analyst 700 Perken Elmer, Waltham, Massachusetts, USA). After completion of nine days cultivation, samples of algae were extracted from each plastic container, and then cleaned five times with 5mM of Ethylenediaminetetraacetic acid (EDTA) then with distilled water to eliminate rapidly the attached HMs. The collected samples were then dried up in oven at 100 °C for 50 minutes. Concisely, 2 g of processed powder algae was added to beaker having 15 mL solution of nitric acid (HNO_3) (70 %) and hydrogen peroxide (H_2O_2) (30 %). After beakers were kept on a hot dish at 100 °C. Then solution was filtered over Millipore filter paper 0.30-0.40 µm into volumetric flask of 100 mL and then the absolute solution was prepared by deionized water.

2.8 Data Analysis

All laboratory experiments were conducted in triplicate (n = 3), and the results for heavy metal concentrations are presented as the mean value ± standard deviation. The removal efficiency for each heavy metal, expressed as a percentage, was calculated based on the change in concentration before and after treatment using the standard formula: Removal Efficiency (%) = $[(C_0 - C_e) / C_0] \times 100$, where C_0 represents the initial metal concentration (mg/L) and C_e represents the equilibrium or final concentration (mg/L) after the treatment period. Graphical representations of the results were generated using OriginPro software (OriginLab Corp., Pottstown State, Pennsylvania, USA).

3. Results and Discussion

3.1 Results of Water Sample Analyses

The physicochemical characteristics of the water samples were analyzed to evaluate the effectiveness of *S. aequinotilalis* and *S. pratensis* in removing contaminants. Samples were collected at the beginning and end of the experiment, and parameters including pH, Electrical Conductivity (EC), Total Dissolved Solids (TDS), Total Suspended Solids (TSS), Biochemical Oxygen Demand (BOD), and Chemical Oxygen Demand (COD) were measured (Table 1 and Table 2). The pH values ranged from 6.20-6.88 initially and 6.34-7.14 at the end, indicating slightly alkaline conditions. EC decreased from 2.20-5.1 mS/cm to 0.30-0.45 mS/cm, suggesting that heavy metals were present in a form accessible for removal. TDS was reduced from 0.5-3.0 mg/L to 0.1-0.8 mg/L, while TSS decreased from 3.0-4.0 mg/L to 2.0-3.0 mg/L, showing effective treatment. BOD values dropped from 50-340 mg/L to 19-198 mg/L, and COD decreased from 100-650 mg/L to 50-352 mg/L, confirming substantial improvement in water quality. Overall, the results demonstrate that both algal species effectively reduced all measured physicochemical parameters, highlighting their potential for industrial WW treatment.

Table 1. Physicochemical characteristics of industrial wastewater before and after treatment with *Spirogyra* species.

Parameters		Sample 1		Sample 2	
		Average	Efficiency %	Average	Efficiency %
pH	Initial	6.20		6.88	
	Final	6.34	74	7.14	84
EC (mS/cm)	Initial	2.20		5.1	
	Final	0.30	87.20	0.45	92.12
TSS (mg/L)	Initial	3.0		4.0	
	Final	2.0	33.33	3.0	25
TDS (mg/L)	Initial	0.5		3.0	
	Final	0.1	80	0.8	74.44
BOD (mg/L)	Initial	50		340	
	Final	19	62	198	48.23
COD (mg/L)	Initial	100		650	
	Final	50	50	352	46.85

Table 2. Physicochemical characteristics of industrial wastewater before and after treatment with *Spirogyra* species.

Parameters		Sample 3		Sample 4	
		Average	Efficiency %	Average	Efficiency %
pH	Initial	6.30		6.34	
	Final	6.62	54	7.08	67
EC (mS/cm)	Initial	2.40		4.8	
	Final	0.50	80.23	0.31	94.65
TSS (mg/L)	Initial	4.0		3.5	
	Final	2.5	38.50	2.5	30
TDS (mg/L)	Initial	0.4		2.5	
	Final	0.05	88.50	0.10	96
BOD (mg/L)	Initial	70		330	
	Final	25	65.71	188	43.04
COD (mg/L)	Initial	150		620	
	Final	86	43.33	325	47.59

3.2 Trial base Experiment

Table 3 and Figure 4 reviews the removal of Cr, Cd, and Pb by *S. aequinotilalis* at various amounts of 0.5-10 mg/L. By applying *S.aequinotilalis*, the Cr amount was reduced to 0.03 mg/L from 0.5 mg/L, 0.20 mg/L from 1.0 mg/L, 1.72 mg/L from 5.0 mg/L and 3.74 mg/L from 10 mg/L respectively in 2 weeks with mean 6.7 pH at 20 ^{o}C. The uptake efficiency for Cd was reported as 0.5 mg/L reduced to 0.04 mg/L, 1.0 mg/L reduced to 0.38 mg/L, 5.0 mg/L reduced to 2.12mg/L and 10 mg/L reduced to 3.74 mg/L. As well as Pb concentration also reduced effectively by following Cr and Cd. 0.5 mg/L reduced to 0.025 mg/L, 1.0 mg/L reduced to 0.40 mg/L, 5.0 mg/L reduced to 1.92 mg/L and 10 mg/L reduced to 4.44 mg/L. The removal trends in the order of Cr > Cd > Pb from water samples when the amount of these HMs were less but generally *S. aequinotilalis* shows good efficiency in the uptake of these selected HMs while related to other species of algae.

Table 3. Heavy metal uptake by *Spirogyra aequinotilalis* from synthetic standard solutions (mg/L).

HMs		S1	%	S2	%	S3	%	S4	%
Cr	Initial	0.5		1		5		10	
	Final	0.03	96	0.20	80	1.72	66.6	3.74	62.6
Cd	Initial	0.5		1		5		10	
	Final	0.04	92	0.38	62	2.12	57.6	4.20	58
Ni	Initial	0.5		1		5		10	
	Final	0.03	64	0.41	57	1.61	74	4.87	79
Pb	Initial	0.5		1		5		10	
	Final	0.025	95	0.40	60	1.92	61.6	4.44	55.6
Zn	Initial	0.5		1		5		10	
	Final	0.3	40	0.60	40	3.32	34.6	6.40	46
Cu	Initial	0.5		1		5		10	
	Final	0.32	36	0.67	33	3.35	33	6.50	35

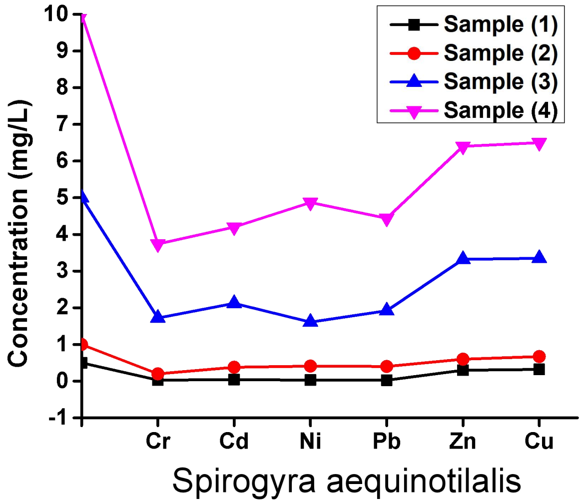

Figure 4. Heavy metal removal efficiency of *Spirogyra aequinotilalis* from synthetic aqueous solutions.

As well as Table 4 and Figure 5 shows the uptake efficiency of selected HMs through *Spirogyra pratensis* at different amounts of 0.5-10 mg/L. The amount of Cr, Cd and Pb reduced efficiently by applying *Spirogyra pratensis*, the Cr concentrations reduced to 0.06, 0.50, 2.92 and 4.22 mg/L from 0.5, 1.0, 5.0 and 10 mg/L respectively. As well as the amount of Cd decreased to 0.07, 0.58, 3.20 and 4.88 mg/L from 0.5, 1.0, 5.0 and 10 mg/L. The Pb quantity reduced to 0.02, 0.60, 2.31 and 5.02 mg/L from 0.5, 1.0, 5.0 and 10 mg/L by applying 3 g of *Spirogyra pratensis*.

Table 4. Heavy metal uptake by Spirogyra pratensis from synthetic standard solutions (mg/L).

HMs		S1	%	S2	%	S3	%	S4	%
Cr	Initial	0.5		1		5		10	
	Final	0.06	88	0.50	50	2.92	41.6	4.22	68
Cd	Initial	0.5		1		5		10	
	Final	0.07	86	0.58	42	3.20	36	4.88	62
Ni	Initial	0.5		1		5		10	
	Final	0.02	67	0.31	63	1.12	78	4.97	81
Pb	Initial	0.5		1		5		10	
	Final	0.02	96	0.60	40	2.31	53.8	5.02	55.6
Zn	Initial	0.5		1		5		10	
	Final	0.33	44	0.64	46	3.34	33	6.55	35
Cu	Initial	0.5		1		5		10	
	Final	0.36	33	0.7	30	3.38	32	6.63	34

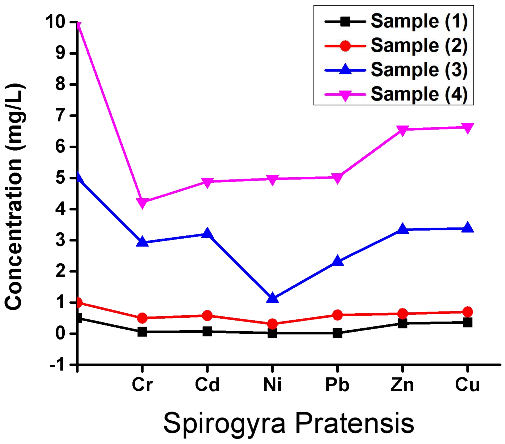

Figure 5. Heavy metal removal efficiency of *Spirogyra pratensis* from synthetic aqueous solutions.

3.3 Physiochemical Parameters of Industrial Wastewater of HIS, Peshawar, Pakistan

Physiochemical parameters of industrial waste water samples are reviewed in Table 5. The samples were collected at the different points of HIS. The collected samples were then tested for Electrical conductivity (EC), Total suspended solid (TSS), Total dissolved solid TDS, Biochemical oxygen demand (BOD), Chemical oxygen demand (COD) and pH values as well as Cr, Cd and Pb after final filtration. The waste water is released in to the adjacent water canals containing different types of HMs. HMs presence in water bodies is one of biggest environmental issue in world especially in developing nations such as Pakistan. This waste water full of HMs is released in to the water sources without any proper treatment in Pakistan. Results show that the concentration of selected HMs was detected above the permissible limits of Pakistan-National Environmental Quality Standards (NEQs). The detected amount for Pb was 2.82 mg/L, Cr 1.83 mg/L while Cd was found in lesser amount of 1.62 mg/L as compared to As and Pb.

Table 5. Physicochemical parameters of industrial wastewater from Hayatabad Industrial State (HIS), Peshawar, Khyber Pakhtunkhwa.

Parameters	Values	NEQs (mg/L)
pH	4.96±0.3	6-10
EC (mS/cm)	892±20	NA
TSS (mg/L)	43±5.2	NA
TDS (mg/L)	488±25	3500
BOD (mg/L)	30±5.2	80
COD (mg/L)	58±6.2	150
Cr (mg/L)	1.85	0.1
Cd (mg/L)	1.68	0.1
Ni (mg/L)	2.41	4.0
Pb (mg/L)	2.82	0.5
Zn (mg/L)	2.40	5.0
Cu (mg/L)	1.80	1.0

3.4 Removal of Heavy Metals by *Spirogyra equinoctials*

The primary amount of Cr, Cd, Ni, Pb, Zn and Cu was identified in industrial waste water gathered from the HIS, Peshawar, KP, Pakistan see Table 5. The detected concentration of these HMs such as Cr, Cd, Pb and Cu was above the standard limits regulated by Pakistan National Environmental Quality Standards (NEQs) see Table 5. The industrial waste water was treated with the specie of fresh water *S. aequinotilalis*. The results indicate that *S.equinoctials* are cultivated very fine and simply cultivated in industrial WW under the physiochemical parameters discussed in Figure 6 and Table 5. The *S. aequinotilalis* indicates the substantial removal for the particular HMs like Cr, Cd and Pb that reported in Table 3.

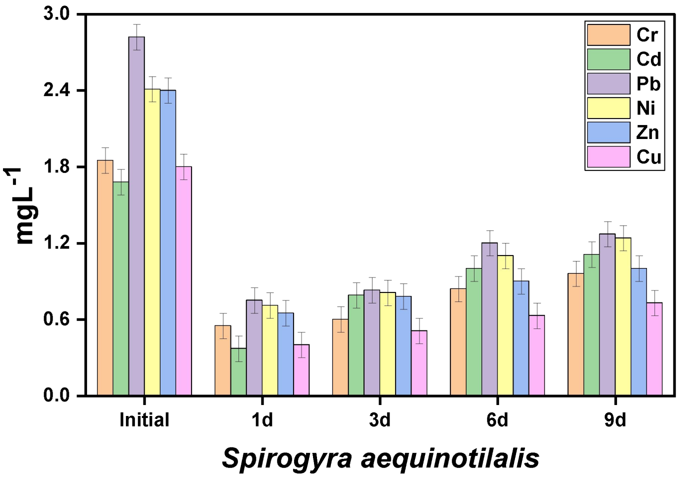

Figure 6. Heavy metal removal efficiency of *Spirogyra aequinotilalis* from industrial wastewater over a 9-day treatment period.

The bioaccumulation effectiveness of *S. aequinotilalis* were a little reduced after nine days. The samples were collected after 1 d, 3 d, 6 d and 9 d at 3 d intervals (Figure 6). After 1 d the *S. aequinotilalis* indicates more removal capacity for Cr that was 30.2 % while the Cd and Pb elimination proficiency was 22.6-26.4 % respectively reported in Table 6. After 3 d *S. aequinotilalis* reveals great treatment capability for Cd. The removal capacity for Cd was reported 47 % while Cr and Pb removal was reported 32.1-29.8% respectively. After 6 d the removal effectiveness of *S. aequinotilalis* for Cr, Cd and Pb was 45.3, 60.2 and 41.8 % respectively. The removal efficiency after 6 d for the certain HMs was reported in order of Cd > Cr > Pb. After 9 d the removal competence of *S. aequinotilalis* was a little reduced for the particular HMs. The Cd and Cr was bio accumulated 67.6 and 51.5 % respectively by *S.aequinotilalis* while Pb removal efficiency was 45.3 after completion of 9 d. The removal efficiency of *S. aequinotilalis* for Zn was reported 27.6, 32.5, 36.6 and 41.6 % at d 1, 3, 6 and 9 respectively while for Cu it was reported 22.2, 28, 35 and 40.5 % after d 1, 3, 6 and 9 respectively.

Table 6. Heavy metal removal from industrial wastewater by *Spirogyra* species over a 9-day treatment period.

Algae Species	Heavy Metal	Initial HM (mg/L)	Day 1		Day 3		Day 6		Day 9	
			Conc. (mg/L)	Eff. (%)	Conc. (mg/L)	Eff. (%)	Conc. (mg/L)	Eff. (%)	Conc. (mg/L)	Eff. (%)
S. aequinotilalis	Cr	1.85	0.55±0.03	30.2	0.60±0.02	32.1	0.84±0.03	45.3	0.96±0.01	51.9
	Cd	1.68	0.37±0.02	22.6	0.79±0.03	47	1.00±0.02	60.2	1.11±0.01	66
	Pb	2.82	0.75±0.01	26.4	0.83±0.02	29.8	1.20±0.02	41.8	1.27±0.03	45
	Ni	2.41	0.71±0.02	29.5	0.81±0.02	33.6	1.10±0.02	45.6	1.24±0.02	51.4
	Zn	2.4	0.65±0.02	27.6	0.78±0.02	32.5	0.90±0.02	36.6	1.00±0.02	41.6
	Cu	1.8	0.40±0.02	22.2	0.51±0.02	28	0.63±0.02	35	0.73±0.02	40.5
S. pratensis	Cr	1.85	0.47±0.01	25.4	0.56±0.02	30.7	0.71±0.02	38.6	0.76±0.02	41
	Cd	1.68	0.35±0.02	20.8	0.68±0.01	40.4	0.82±0.02	48.8	0.96±0.02	57.1
	Pb	2.82	0.50±0.02	17.7	0.80±0.03	28.3	1.06±0.01	37.5	1.20±0.03	42.5
	Ni	2.41	0.55±0.02	22.8	0.89±0.02	36.9	0.98±0.02	40.6	1.10±0.02	45.6
	Zn	2.4	0.56±0.02	23.3	0.64±0.02	26.6	0.76±0.02	31.6	0.97±0.02	40.4
	Cu	1.8	0.37±0.02	20.5	0.46±0.02	25.5	0.55±0.02	30.5	0.62±0.02	34.4

*Note: Values represent mean ± standard deviation (n=3). Conc. = Concentration; Eff. = Removal efficiency.

3.5 Removal of Heavy Metals by *Spirogyra pratensis*

The Table 6 reviews the removal capability of *S. pratensis* for the designated HMs in industrial waste water. The *S. pratensis* removal efficiency is fewer as compared with *S. aequinotilalis* but good algae for the elimination of certain HMs. The treatment efficiency of *S.pratensis* after 1 d was reported 20.8, 25.4 and 31.2 % for Cd, Cr and Pb respectively. The proposed HMs after 3 d by *S. pratensis* considerably treat Cd from industrial waste water and removal efficacy was 40.4 %. The Cr and Pb removal efficiency were 30.7-38.5 % respectively after 3 d. After 6 d *S. pratensis* indicates great removal for the Cd 62.3 % followed by Cr and Pb, 38.6 and 42.4 % correspondingly and the removal capability were in order of Cd> Pb > Cr. After 9 d the removal efficiency of *S. pratensis* was reduced slightly as compared with 6 d. After 9 d *S. pratensis* reduced Cd, Pb and Cr 58.9 %, 35.8% and 44.6.

The removal efficiency of *S. pratensis* for Zn was reported 23.3, 26.6, 31.6 and 40.4 % at d 1, 3, 6 and 9 respectively while for Cu it was reported 20.5, 25.5, 30.5 and 34.4 % after d 1, 3, 6 and 9 respectively (Figure 7). The *S.pratensis* are simply obtainable and cultured. The results show that *S.pratensis* are eco-friendly in treatment of industrial WW gathered from HIS.

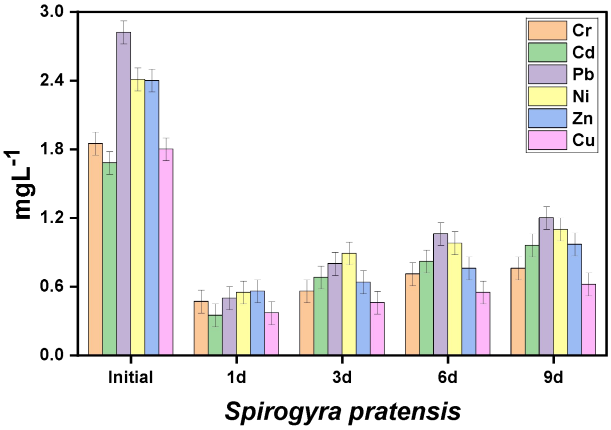

Figure 7. Heavy metal removal efficiency of *Spirogyra pratensis* from industrial wastewater over a 9-day treatment period.

4. Discussion

The industrial waste water is released in to the nearby water bodies containing of toxic HMs such Cr, Cd, Pb, Zn Cu and Cr. Because of its toxicity and persistency this problem is one of biggest ecological issue all over the world especially in developing nations such as Pakistan, China, India and Bangladesh. This industrial waste water full of heavy metal and releasing into water bodies without any proper treatment in Asian countries such as Pakistan, different chemical ways are utilized to treat toxic HMs from these from industrial waste water [30]. Such traditional approaches have several defects, for example, great chemical supplies, unstable HMs treatment and producing poisonous sludge as a byproduct [31].

Currently, bioremediation has developed an alternative method to such conventional biochemical techniques [32]. Bioremediation is low-cost, non-damaging and limited effluence leftovers [33]. Bioremediation have been applied to treat toxic HMs from polluted industrial waste water on a commercial level [33]. Bio treatment of these HMs by fresh water algae has emerged one of the top efficient techniques for bioremediation of industrial waste water. HMs resilient fresh water algae have been cultured in waste water and HMs contaminated ecosystems [34].

In this current study the results reviewed that *S. aequinotilalis* and *S. pratensis* clearly removed much Cr, Cd and Pb from the industrial WW. The removal efficacy various since of the primary number of nominated HMs existed in the industrial waste water. The *S. aequinotilalis* removal effectiveness for Cd after 6 and 9 d reported to 60.2 and 67.6 % individually while its primary amount in the industrial waste water was 1.68 mg/L. The As removal efficacy reported to 45.3 % and Pb removal efficacy reported to 41.8 % at pH 6.8 while the primary amount of Cr and Pb was 1.85 and 2.82 mg/ L respectively in the industrial WW. After completion of 9 d the *S. aequinotilalis* treated 67.6, 51.5 and 45.3 % of the Cd, Cr and Pb from industrial waste water. *S. aequinotilalis* eliminate the designated HMs within the permissible limits of Pakistan NEQs.

The *Spirogyra pratensis* also indicates great removal capability for the Cd, Cr and Pb. After 9 d *S. pratensis* reduced Cd, Pb and Cr were reported 58.9 %, 35.8% and 44.6%. The treatment efficacy was reported less as compared to *S. aequinotilalis*. The *S. aequinotilalis* and *S. pratensis* can cultivate healthy in the industrial WW because no dead and yellow part of these Algae appeared after completion of 9 d growing in the industrial waste water and display great removal capacity for certain HMs. The results of the current study show that *S. aequinotilalis* and *Spirogyra pratensis* species are eco-friendly in the treatment of industrial waste water due to its simply growing, healthy development throughout 9 d testing time period, simply obtainability, and cost-effective method. The Cd, Cr and Pb amount detected in industrial waste water are above the standard limits reported in Table 5. These HMs cause cancer to humans and animals as well as shows toxicity to plants [35]. Government of Pakistan made permissible limits (NEQs) of 0.1, 0.1 and 0.5 mg/L for Cd, Cr and Pb respectively to protect human health and environment (Table 5). In fresh water algae species, the cell wall is the core site for HMs sorption [36].

Our study indicates that the *S. aequinotilalis* and *S. pratensis* has the utmost removal capability for Cd, Cr and Pb while the amount is great in the industrial waste water. Maximum research reports are available on the treatment efficacy by applying dead algae biomass. Deng et al. [37] investigated dead biomass of *Cladophora sp.* to remove the Cd and Pb and he revealed that dead biomass of *Cladophora sp.* can treat much amount of Cd and Pb. Our research obviously indicates great removal efficacy of particular HMs by applying *S. aequinotilalis* and *S. pratensis* algae. The removal effectiveness of the studied heavy metals by the algae followed the order *S. aequinotilalis* > *S. pratensis*, indicating that *S. aequinotilalis* was generally more efficient in metal uptake. While dead algal biomass may slowly settle in canals and streams, contributing to sediment accumulation, living freshwater algae offer significant advantages for WW treatment. Living algae are low-cost, easy to cultivate, and maintain high removal efficiency, making them a practical and environmentally friendly option for the remediation of heavy metals from industrial WW. Their continuous metabolic activity ensures effective metal uptake over their lifespan, and their in-situ applicability reduces the need for additional processing or transport.

5. Conclusions

In conclusion, the freshwater algae species *Spirogyra aequinotilalis* and *Spirogyra pratensis* demonstrate significant potential for the bioremediation of heavy metals from contaminated water. The algae showed effective removal rates for Cr, Cd, Ni, Zn, Cu, and Pb, with the highest efficiencies observed for Cr and Cd at elevated metal concentrations. Despite their limited lifespan of approximately one week, both algae species maintained their ability to effectively uptake heavy metals throughout their duration. These findings underscore the feasibility of utilizing *Spirogyra* species as a low-cost, environmentally friendly, and sustainable solution for heavy metal removal from industrial WW. Future research could further optimize cultivation conditions and explore the molecular mechanisms behind their metal uptake processes.

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Conflict of Interest

The authors confirm that there are no known financial or personal conflicts of interest that could have influenced the findings or interpretations presented in this study.

Generative AI statement

The author declares that no Gen AI was used in the creation of this manuscript.

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