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Bioremediation of Heavy Metals from Industrial Effluents Using *Bacillus pakistanensis* and *Lysinibacillus composti*

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Abstract: Aquatic pollution is one of the main problems due to rapid development in industrialization. The remediation of industrial wastewater (IWW) by microorganisms is an environmentally friendly technique. This study was conducted to assess pollution load in IWW and to use *Bacillus pakistanensis* and *Lysinibacillus composti* individually and in a consortium for bioremediation. The IWW was obtained from Hayatabad Industrial Estate and evaluated for physicochemical parameters and metal concentration. The pH, color, electrical conductivity (EC), turbidity, temperature, sulfide, fluoride, chloride, biological oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), total dissolved solids (TDS), calcium hardness, magnesium hardness, and total hardness were noted as 6.82, 440 TCU, 1.195 mS/cm, 54.65 mg/L, 26.8 °C, 5.60 mg/L, 3.6 mg/L, 162 mg/L, 85.5 mg/L, 921 mg/L, 232 mg/L, 794 mg/L, 590 mg/L, 395 mg/L, and 985 mg/L, respectively. The metals such as manganese, copper, chromium, cadmium, cobalt, silver, nickel, calcium, magnesium, and lead were also analyzed as 1.23 mg/L, 0.81 mg/L, 2.12 mg/L, 0.18 mg/L, 0.151 mg/L, 0.24 mg/L, 1.12 mg/L, 0.113 mg/L, 14.5 mg/L, and 0.19 mg/L, respectively. A pot experiment was performed for two weeks to evaluate the efficiency of the selected species. The IWW and tap water (control) were treated with selected species, individually and in a consortium. After treatment, a considerable reduction was noted in the color 87.3%, EC 46.5%, turbidity 84.1%, sulfide 87.5%, fluoride 25.0%, chloride 91.3%, BOD 96.4%, COD 86.5%, TSS 90%, TDS 45.0%, Ca hardness 42.3%, Mg hardness 77.2%, and total hardness 52.2%. After the experiment, samples of water were also analyzed for metal concentrations by atomic absorption spectrophotometry. The selected species removed 99.3% of Mn, 99.6% of Cu, 97.8% of Cr, 94.4% of Cd, 46.3% of Co, 85.1% of Ag, 88.4% of Ni, 98.8% of Ca, 91.5% of Mg, and 90.5% of Pb. The *t*-test analysis showed that the treatment with the selected species significantly decreased the metal concentrations in the IWW ($p \leq 0.05$).

Keywords: bioremediation; bacteria; physicochemical parameters; heavy metals; bioremoval efficiency

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1. Introduction

Anthropogenic activities such as industrialization and urbanization release large amounts of wastewater (WW) into nearby aquatic environments without any treatment. Different industries such as photographic processing, metal plating, petroleum refining, and mining are the main causes of water contamination by releasing potentially heavy metals

(HMs) [1], fertilizers, and other organic and inorganic contaminants [2]. Approximately two million tons of effluents and sewage are exhausted into the water daily. While waterborne diseases affect about 1.5 million children each year. According to a United Nations report (2010), these conditions are the most horrible in underdeveloped nations, where raw sewage (90%) and industrial wastes (70%) are drained into the surface water [3]. Therefore, access to clean water and sanitation is insufficient and is the main problem affecting human health [4].

A large amount of water pollutants cause a raise in turbidity, total suspended solids (TSS), coloration, total dissolved solids (TDS), biological oxygen demand (BOD), and chemical oxygen demand (COD), while the contents, including fluoride, chloride, and sulfide make the water unsafe for drinking and irrigation purposes. Urbanization and industrialization supplemented large amounts of WW to the natural aquatic ecosystem, consisting of HMs. The HMs in the natural ecosystem are of greater importance due to their nonbiodegradability, persistency, and toxicity [1]. Some HMs are essential for the organism in trace levels for growth, such as iron (Fe), selenium (Si), zinc (Zn), cobalt (Co), and copper (Cu), although larger amounts of each can cause toxic effects that are teratogenic/mutagenic, synergistic, acute, and chronic [5].

About 40 HMs, even at trace levels, are considered potentially toxic for living organisms and humans due to their increased assimilation in the food chain [6] and, therefore, they are becoming an enduring burden on the environment [1]. Heavy metals discharged in ecosystems possess a severe risk [7]. Whenever the HMs accumulate in the food chain via living organisms, it leads to cytotoxic, mutagenic, and carcinogenic impacts on humans and wildlife [8,9]. The physiological function of the aquatic organisms' reproductive system, kidneys, and liver can be badly affected through direct HM contact. Alterations in the physicochemical characteristics of the aquatic ecosystem can affect the diversity and activity of aquatic bacteria, zooplankton, and plants [10,11]. Various HMs raise the acidity of the blood, resulting in the extraction of Ca (calcium) from bones for the pH restoration of blood, leading to osteoporosis. This is generally found in children and aged people [12]. The major environmental problem emerges from HM emissions from Zn, Hg, Cu, Cr, and Cd industries. Consequently, the removal of HMs from industrial effluents is necessary [12].

There are several methods for HMs removal from the water, such as reverse osmosis, chemical precipitation, ion exchange, biochar, adsorption on activated carbon, and membrane filtration [13]. Though, the achievability of these costly methods and various technological factors may limit the execution of these techniques [14]. All of the above traditional methods for HM removal have some limitations despite their ability to eliminate metals, up to some point from the industrial WW. The HMs are generally altered to other forms, which need to be eliminated. Moreover, they are very expensive in terms of energy cost [15].

Research is continuously going onwards and discovering cost-effective and suitable biotechniques. HM removal from WW is regarded as a main field of research concerning environmental and economic considerations. Previous research emphasized biological techniques, such as yeasts, bacteria, and fungi have been studied for HM removal from the WW [16]. Bacteria have a large surface area to volume ratio that presents a huge contact surface, allowing the interaction with HMs in their environment, and have been used successfully as biosorbents [13]. Bacteria have the highest range of potential for the process of bioremediation. Many microbes, such as *Ochrobactrum*, *Acinetobacter*, *Cellulomonas* sp., *Bacillus* sp., *Serratia marcescens*, *Pseudomonas* sp., *Arthrobacter*, and *Desulfovibrio vulgaris* have been recognized to reduce the highly toxic and soluble forms to less toxic and less soluble forms [17]. Many diverse bacteria, such as *B. subtilis*, *endophytes*, and *pseudomonas* have been used in the remediation process [18]. Hayatabad, Peshawar is one of the key industrial area of Khyber Pakhtunkhwa, Pakistan. Heavy metal pollution is one of the key issues in industrial wastewater in this area. The level of heavy metals is increasing because of excessive industrialization. These heavy metals can be degraded or removed from industrial wastes through the direct use of microbes, including bacteria or enzymes.

To date, no comprehensive study is available for the safe and efficient removal of these toxic heavy metals. By keeping these gaps, the present study is designed to categorize the most efficient bacterial species and explore the efficiency of removing HMs (Mn, Cr, Cd, Cu, Co, Ag, Pb, Ni, Ca, and Mg) present in the industrial wastewater in the Hayatabad Industrial Estate, Peshawar, Pakistan.

2. Materials and Methods

2.1. Bacterial Collection and Transportation

The bacterial species, *Bacillus pakistanensis* and *Lysinibacillus composti* were collected from National Agricultural Research Centre (NARC), Islamabad, Pakistan. The bacteria were shifted through a portable delagua kit. The bacteria were transplanted in industrial effluents of the Hayatabad Industrial Estate (HIE) for 14 days at room temperature and a light/dark cycle of 14:10 h [19]. The reason behind the selection of these species is their rapidly growing temperature, high and low pH resistance, and their high tolerance to heavy metals (HMs) toxicity.

2.2. Collection of Industrial Effluents

The water sample was collected from the main source, where all the industrial effluents fall into the main drain in the Hayatabad Industrial Estate, Peshawar. The industrial effluent of the Hayatabad industrial Estate contains organic and inorganic pollutants [3]. Hayatabad Industrial Estate has pharmaceutical, paint, plastic, chipboard, match, steel, rubber, incinerator, and paper industries that generate a huge amount of WW [20]. Before experimentation, the WW sample was analyzed for different parameters, including COD, pH, BOD, TSS, TDS, turbidity, color, fluoride, Ca hardness, Mg hardness, total hardness, electric conductivity, and chloride using standard analytical methods [3]. HMs such as Pb, Ni, Co, Cr, Mn, Cu, Cd, Ag, Ca, and Mg were analyzed using an atomic absorption spectrophotometer (AAS) in a laboratory at the Environmental Protection Agency (EPA) in Peshawar, Khyber Pakhtunkhwa (KP), Pakistan [3].

2.3. Experimental Design

A pot experiment was performed to study the efficiency of bacteria against organic and inorganic pollutants. For this purpose, six pots were used, which were first washed thoroughly with double deionized distilled water, and 10% dilute nitric acid [19]. Three pots served as control pots containing tap water and three as treatment pots containing industrial effluents.

The pots were named C1, C2, and C3, and contained *Bacillus pakistanensis* (5 mL), *Lysinibacillus composti* (5 mL), individually, and a consortium of *Bacillus pakistanensis* (5 mL), and *Lysinibacillus composti* (5 mL) in clean tap water (500 mL each), respectively. The treatment pots were named T1, T2, and T3, and contained *Bacillus pakistanensis* (5 mL), *Lysinibacillus composti* (5 mL), and a consortium of *Bacillus pakistanensis* (5 mL), and *Lysinibacillus composti* (5 mL) in industrial effluents (500 mL each).

2.4. Water Sampling and Analysis

Water samples (500 mL) from each of the six containers (C1–C3 and T1–T3) were obtained for analysis after 14 days of experimentation. All the water samples collected from control and treatment containers were analyzed for pH, EC, temperature, COD, BOD, TSS, TDS, sulfide, color, turbidity, chloride, fluoride, calcium hardness, magnesium hardness, and total hardness using standard analytical methods [3,19–21].

2.5. Heavy Metal Analysis

The water samples were prepared for analysis using the selected metals [22]. Nitric acid (25 mL) and HCl (75 mL) were added to (50 mL) of each sample digestion for 24 h, and afterward, the makeup of the sample was conducted with distilled water to a total of

250 mL [21]. Using ASS, the water samples were analyzed for Pb, Ni, Co, Cr, Mn, Cu, Cd, Ag, Ca, and Mg concentration [20].

2.6. Bioremoval Efficiency (%)

Bioremoval efficiency (%) was calculated using the equation $R = \frac{Ci-Cf}{Ci} \times 100$, where R represents the removal percentage, C_i is the initial concentration of the metal in the water samples, and C_f is the final concentration of the metal in the water samples [22].

2.7. Statistical Analysis

Statistical analysis was performed using software, including SigmaPlot and Microsoft Excel for graphical representation of the data and Statistical Package for Social Science (SPSS) 16.0. The *t*-test was used to determine the significant difference (p) between two and more than two variables of the parameters.

3. Results and Discussion

3.1. Physicochemical Parameters of WW

The physicochemical parameters of the water samples used for the experiment are provided in Table 1. At the initial and final points of the experiment, the water samples were collected from each container (C1, T1, C2, T2, C3, and T3). In the present study, the industrial WW at the Hayatabad Industrial Estate initially had low mean pH values (6.83), which indicates that the acidity and the presence of large amounts of pollutants are in the industrial WW (Table 1). The results show that the influences of bacteria on the pH were insignificant, with the changes ranging from 0.68 to 1.38 units. The low pH in the industrial WW may raise the metal solubility, if released in aquatic ecosystems, and negatively impact aquatic organisms [23]. In previous studies, similar findings (pH of 7.6) were observed by Fito et al. [24], who analyzed the WW in the sugar industry. Khan et al. [25] observed the same results (pH of 6.15) while experimenting with the treatment of industrial WW at the Hayatabad Industrial Estate by algae. Hossain et al. [26] conducted a study on the pollution load of industrial WW discharged from Bangladeshi industries and discovered similar results (pH of 7.28). These pH values can be ascribed to the difference in the water samples collected for analysis.

At the initial stage, the observed EC value for industrial WW was 1.195 mS/cm. The EC values were significantly ($p \leq 0.05$) reduced (11.9–46.5%) in the pot experiment, which could be related to bacterial metal uptake and precipitation (Table 1). This EC value does not agree with the EC value (5.04 mS/cm) found in the industrial WW at the Hayatabad Industrial Estate as observed by Ayaz et al. [3]. A study by Hossain et al. [26] reported on the physicochemical characteristics of WW released from different industries (Bangladesh) and found similar findings (2.64 mS/cm).

The TSS value at the initial stage observed for industrial WW at the Hayatabad Industrial Estate was 232 mg/L. TSS was reduced to a range of 2–90% (Table 1). The high TSS values in WW can have harmful effects on the physical, biological, and chemical characteristics of water [27], while if utilized for irrigation purposes, it can cause soil pore clogging [28]. Aniyikaiye et al. [29] carried out a study on paint industrial WW and found much higher TSS values (2470 mg/L). Similarly, the TDS values at the initial stage for industrial WW samples were observed as 794 mg/L. The high value of TDS in industrial WW can lead to salinity problems when discharged without prior treatment for irrigation [30]. Ayaz et al. [3] experimented on the phytoremediation in the WW and observed much lower TDS values (2.0 mg/L). Similarly, much higher TDS values (7072 mg/L) have been determined in textile industrial WW by Paul et al. [31]. The differences in the findings can be attributed to the different sampling sites.

Table 1. Physiochemical parameters of water samples in both control and experimental groups.

Physiochemical Parameters		C1		T1		C2		T2		C3		T3	
		Mean	**Eff. %**	**Mean**	**Eff. %**	**Mean**	**Eff. %**	**Mean**	**Eff. %**	**Mean**	**Eff. %**	**Mean**	**Eff. (%)**
pH	I	7.3		6.83		7.3		6.83		7.3		6.83	−20.2
	F	7.98		8.02		8.23		8.10		8.27		8.21	
EC (mS/cm)	I	0.44		1.195		0.44		1.195		0.436	46.5	1.195	15.8
	F	0.24	45.45	1.052	11.9	0.37	15.9	1.003	16.0	0.23		1.006	
Temperature (°C)	I	26.6		26.8		26.6		26.8		26.6	45.1	26.8	45.1
	F	14.6	45.1	14.7	45.1	14.4	45.8	14.7	45.1	14.6		14.7	
BOD (mg/L)	I	04		85.5		04		85.5		04	25	85.5	43.8
	F	02	50	21	75.4	01	75	03	96.4	03		48	
COD (mg/L)	I	05		921		05		921		05	20	921	84.7
	F	02	60	179	80.5	01	80	124	86.5	04		140	
TSS (mg/L)	I	02		232		02		232		02	2.0	232	84.4
	F	1.81	9.5	38	83.6	1.22	39	23	90.0	1.96		36	
TDS (mg/L)	I	288		794		288		794		288	17.0	794	18.1
	F	247	14.2	662	16.6	260	9.7	436	45.0	239		650	
Sulfide (mg/L)	I	0.40		5.6		0.4		5.6		0.4	25.0	5.6	83.9
	F	0.32	20.0	1.3	76.7	0.36	10.0	0.7	87.5	0.30		0.9	
Color (TCU)	I	0.34		440		0.34		440		0.34	79.4	440	87.3
	F	0.17	50.0	63.13	85.6	0.26	23.5	63.72	85.5	0.07		55.45	
Turbidity (NTU)	I	1.08		54.65		1.08		54.65		1.080	4.6	54.65	73.9
	F	1.04	3.7	10.35	81.06	1.04	3.7	8.642	84.1	1.03		14.23	
Fluoride (mg/L)	I	0.13		3.6		0.13		3.6		0.13	15.4	3.6	11.1
	F	0.12	7.6	2.7	25	0.10	23.0	3.2	11.1	0.11		3.2	
Chloride (mg/L)	I	54		162		54		162		54	33.3	162	91.3
	F	41	24.0	110	32.0	32	40.7	130	19.7	36		14	
Calcium Hardness (mg/L)	I	280		590		280		590		280	35.7	590	42.3
	F	200	28.5	580	1.6	210	25.0	480	18.6	180		340	
Magnesium Hardness (mg/L)	I	180		395		180		395		180	10.5	395	49.3
	F	170	5.5	210	46.8	157	14.6	90	77.2	161		200	
Total Hardness (mg/L)	I	460		985		460		985		460	25.8	985	45.1
	F	370	19.5	470	52.2	367	20.2	570	42.1	341		540	

C1: Container of control for *Bacillus pakistanensis*; **T1**: Treatment for *Bacillus pakistanensis*; **C2**: Container for control of *Lysinibacillus composti*;**T2**: Container for treatment of *Lysinibacillus composti*; **C3**: Container for control of *Bacillus pakistanensis, Lysinibacillus composti*; **T3**: Container for treatment of *Bacillus pakistanensis, Lysinibacillus composti*;**I**: initial; **F**: final; **% eff.**: % efficiency.

A reduction in the values of BOD and COD was noted in the range of 25–96.4% and 20–86.5%, respectively (Table 1). The BOD at the initial stage, observed for the industrial WW at the Hayatabad Industrial Estate, was 85.5 mg/L, which is comparable to the research study by Ayaz et al. [3]. The similarity may be due to the same sample collection point and study area. However, the findings are much higher and do not agree with the findings (25 mg/L) in the research study performed on the remediation of wastewater by Khan et al. [20]. The COD at the initial stage, reported for the WW, was 921 mg/L. These COD values do not agree with the COD values for the WW at the Hayatabad Industrial Estate obtained by Ayaz et al. [3]. Moreover, these discoveries are considerably similar to the results (1231 mg/L) from the study conducted by Aniyikaiye et al. [29] on effluents in the paint industry.

A decrease in sulfide contents was observed in the range of 10–87.5%. A reduction in the values for color and turbidity were in the ranges of 23.5–87.3% and 7.6–25%, respectively. After experimentation, reductions in the values of fluoride, chloride, calcium hardness, magnesium hardness, and total hardness for the water samples were also observed, as shown in Table 1. The sulfide concentration at the initial stage observed in industrial WW was 5.6 mg/L. This sulfide concentration does not agree with the results (195–1434 mg/L) determined from the physical analysis of industrial WW released from paint industries in Nigeria by Aniyikaiye et al. [29]. Another physicochemical study on the sugar industry WW, conducted by Fito et al. [24], found a much higher concentration of sulfide (30 mg/L). At the initial stage, the turbidity value observed for industrial WW was 54.65 NTU. The present value of turbidity is in line with the results (60 NTU) in industrial WW observed by Momeni et al. [32]. At the initial stage, the fluoride value observed for industrial WW was 3.6 mg/L. A study reported on the quality of groundwater and health risks associated with fluoride contamination in the watershed in India by Adimalla et al. [33] and found similar findings (1.4–5.9 mg/L). At the initial stage, the chloride content observed in industrial WW was 162 mg/L. This chloride concentration did not agree with the findings (733.8 mg/L) determined by the physical analysis of industrial WW released from paint industries in Nigeria by Aniyikaiye et al. [29]. A study reported on the physicochemical characteristics of WW released from ethanol distillery wastewater by Fito et al. [24] and found much higher values (6722 mg/L). These differences in the results can be attributed to the study area differences. At the initial stage, the total hardness value observed in industrial WW was 985 mg/L. An analysis reporting on fishpond water by Stone and Thomforde [34] identified many low findings (103.80 mg/L). These differences in data can be attributed to the different study areas used.

3.2. Heavy Metals

3.2.1. Manganese (Mn)

Mn concentrations checked at the initial stage for both control and treated water were 0.013 and 1.23 mg/L, respectively. At the final stages, the Mn mean concentration ranged from 0.004 to 0.011 mg/L for the control and from 0.008 to 0.049 mg/L for the treated water samples, as shown in Table 2. The highest values were recorded in T1 for the treatment water, while in C2 for the control water samples at the final stage. The study found a 69.2, 96, 15.3, 99.1, 38.4, and 99.3% reduction in the final water samples of C1, T1, C2, T2, C3, and T3, respectively. The highest bioremoval efficiency (R) was shown by T3, whereas the lowest was for C2 (Figure 1). Ali et al. [35], investigated the physicochemical parameters of the water collected from the Kurram River, Pakistan, and found much higher values (4–10 mg/L).

Table 2. Metal concentration (mg/L) in water samples of both control and experimental groups.

HMs		C1	T1	C2	T2	C3	T3
		Mean	**Mean**	**Mean**	**Mean**	**Mean**	**Mean**
Mn	**Initial**	0.013	1.230	0.013	1.230	0.013	1.230
	Final	0.004	0.049	0.011	0.011	0.008	0.008
Cu	**Initial**	0.010	0.810	0.010	0.810	0.010	0.810
	Final	0.003	0.003	0.009	0.013	0.004	0.012
Cr	**Initial**	0.016	2.120	0.016	2.120	0.016	2.120
	Final	0.001	0.102	0.012	1.470	0.001	0.046
Cd	**Initial**	0.012	0.180	0.012	0.180	0.012	0.180
	Final	0.010	0.010	0.005	0.055	0.001	0.081
Co	**Initial**	0.014	0.151	0.014	0.151	0.014	0.151
	Final	0.012	0.102	0.011	0.081	0.009	0.089
Ag	**Initial**	0.054	0.240	0.054	0.240	0.054	0.240
	Final	0.008	0.080	0.019	0.190	0.012	0.100
Pb	**Initial**	0.002	1.120	0.002	1.120	0.002	1.120
	Final	0.001	0.450	0.001	0.420	0.0017	0.104
Ni	**Initial**	0.046	0.113	0.046	0.113	0.046	0.113
	Final	0.013	0.013	0.012	0.012	0.019	0.019
Ca	**Initial**	1.720	14.50	1.720	14.50	1.720	14.50
	Final	0.088	0.880	0.920	1.265	0.110	0.160
Mg	**Initial**	0.040	0.190	0.040	0.190	0.040	0.190
	Final	0.017	0.047	0.007	0.097	0.016	0.016

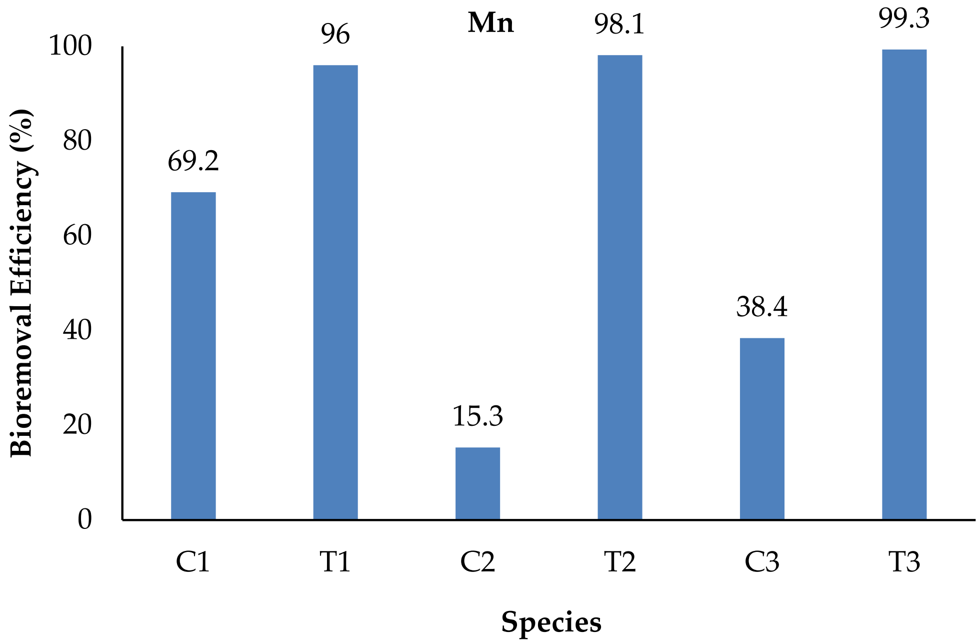

Figure 1. Bioremoval efficiency of *Bacillus pakistanensis* and *Lysinibacillus composti* for Mn in industrial wastewater.

3.2.2. Copper (Cu)

The Cu values observed at the initial stage for both control and treatment water samples were 0.010 and 0.81 mg/L, respectively. At the final point, the mean Cu concentrations ranged from 0.003 to 0.009 mg/L for the control and from 0.003 to 0.013 mg/L for the treated water samples. The highest values were recorded in T2 for treatment water, while in C2 for the control water samples. The study found a 70, 99.6, 10, 98.3, 60, and 98.5% reduction in the final water samples in C1, T1, C2, T2, C3, and T3, respectively. The highest bioremoval efficiency was observed in T1, whereas the lowest was in C2 (Figure 2). In other physicochemical studies on the sugar industry WW, conducted by Fito et al. [24], found similar results (0.7 mg/L).

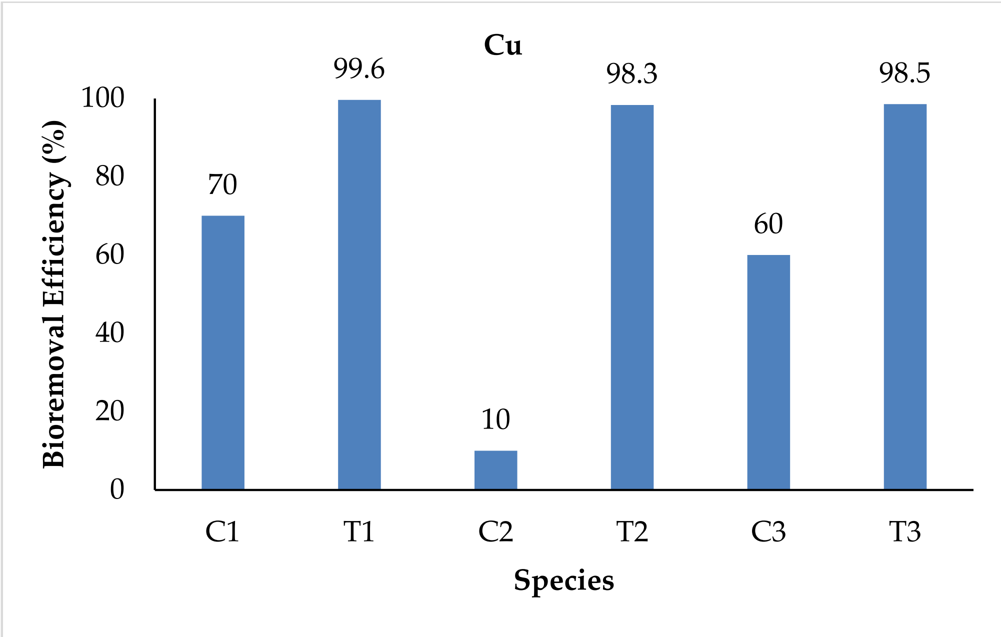

Figure 2. Bioremoval efficiency of *Bacillus pakistanensis* and *Lysinibacillus composti* for Cu in industrial wastewater.

3.2.3. Chromium (Cr)

Cr concentrations, checked at the initial stage for both control and treatment water, were 0.016 and 2.12 mg/L, respectively. At the final stages, the mean concentration ranged from 0.001 to 0.012 mg/L for the control and from 0.046 to 1.47 mg/L for the treated water samples. The highest values were recorded in T2 for treatment water, while in C1 and C3 for the control water samples. The study found 93.7, 95.1, 25, 30.6, 93.7, and 97.8% reductions in the final water samples for C1, T1, C2, T2, C3, and T3, respectively. The highest bioremoval efficiency was observed in T3, whereas the lowest was in C2 (Figure 3). This value for the Cr concentration agrees with the 1.5 mg/L finding by Fito et al. [24] who reported on the physicochemical properties of the sugar industry WW. Ali et al. [35] conducted a study on the physicochemical parameters of water collected from the Kurram River, Pakistan, and found much higher values (8.0 mg/L). These differences in the results can be ascribed to the difference in the collection site.

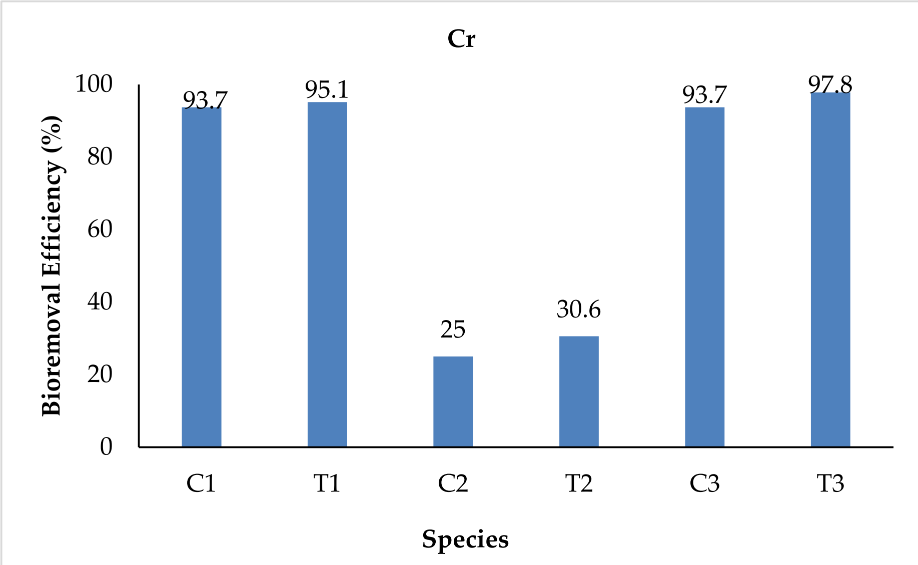

Figure 3. Bioremoval efficiency of *Bacillus pakistanensis* and *Lysinibacillus composti* for Cr in industrial wastewater.

3.2.4. Cadmium (Cd)

The Cd concentrations determined at the initial stage for both the control and treated water samples were 0.012 and 0.18 mg/L, respectively. At the final stages, the mean values ranged from 0.001 to 0.010 mg/L for the control and from 0.010 to 0.081 mg/L for the treated water samples. The highest values were recorded in T3 for the treatment water, while in C1 for the control water samples. The study found reductions of 16.6, 94.4, 58.3, 69.4, 91.6, and 55% in the final water samples of C1, T1, C2, T2, C3, and T3, respectively. The highest bioremoval efficiency was observed in T1, whereas the lowest was in C1 (Figure 4). Ali et al. [35] conducted a study on the physicochemical parameters of water collected from the Kurram River, Pakistan, and found a very similar cadmium concentration (0.14 mg/L). Another study conducted on the industrial WW at the Hayatabad Industrial Estate by Ayaz et al. [3], observed a much higher Cd concentration (4.5–8.0 mg/L).

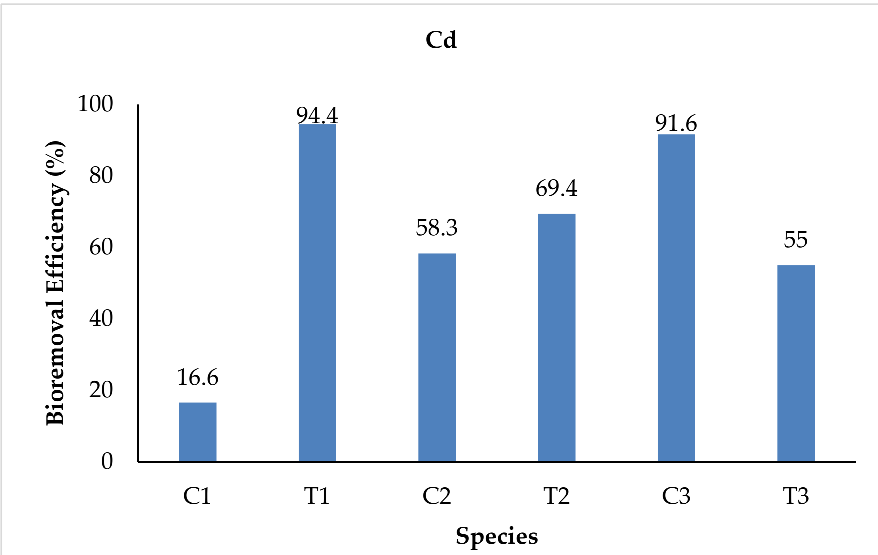

Figure 4. Bioremoval efficiency of *Bacillus pakistanensis* and *Lysinibacillus composti* for Cd in industrial wastewater.

3.2.5. Cobalt

The Co levels checked at the initial stage for both control and treatment water, were 0.014 and 0.151 mg/L, respectively. At the final stages, the mean values ranged from 0.009 to 0.012 mg/L for the control and from 0.081 to 0.102 mg/L for the treated water samples. The highest values were recorded in T1 for the treatment water, while in C1 for the control water samples. The study found 14.2, 32.4, 21.4, 46.3, 35.7, and 41% reductions in the final water samples of C1, T1, C2, T2, C3, and T3, respectively. The highest bioremoval efficiency was observed in T2, whereas the lowest was in C1 (Figure 5). Similarly, Gokalp and Mohammed [36] carried out a research study on the assessment of metal pollution in Heshkaro, Iraq, and determined lower concentrations (0.00002–0.00233 mg/L) of Co. The differences may be ascribed to the difference in the water sample collection site and the initial metal concentration.

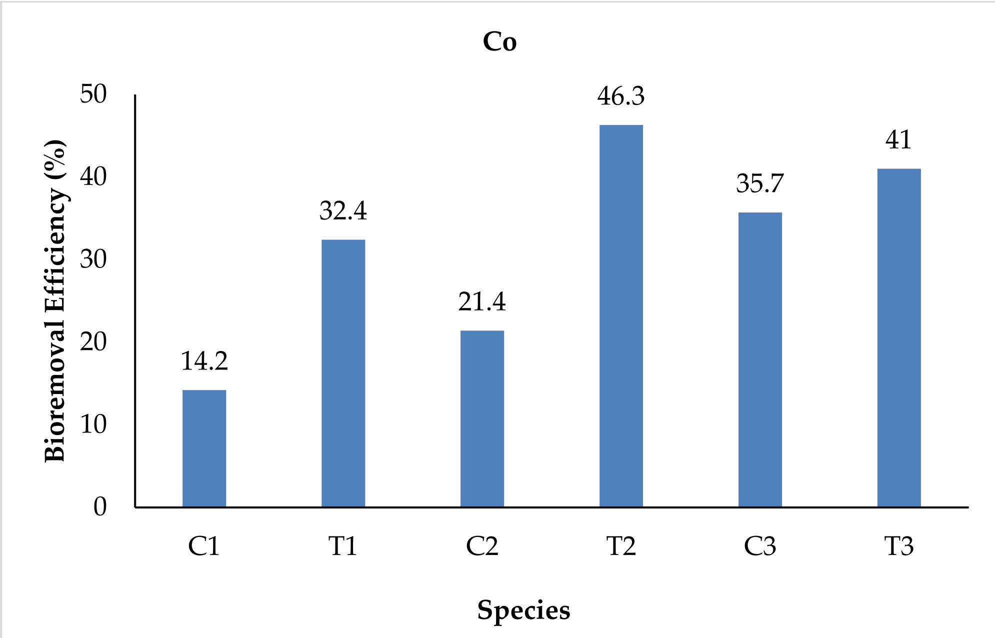

Figure 5. Bioremoval efficiency of *Bacillus pakistanensis* and *Lysinibacillus composti* for Co in industrial wastewater.

3.2.6. Silver (Ag)

The Ag concentrations at the initial stage for both control and treated water were 0.054 and 0.24 mg/L, respectively. At the final stages, the Ag mean values ranged from 0.008 to 0.019 mg/L for the control and from 0.08 to 0.19 mg/L for the treated water samples. The highest values were recorded in T2 for the treatment water, while in C2 for the control water samples. The study found 85.1, 66.2, 64.8, 20.8, 82.3, and 58.3% reductions in the final water samples of C1, T1, C2, T2, C3, and T3, respectively. The highest bioremoval efficiency was observed in C1, whereas the lowest was in T2 (Figure 6). Ali et al. [35] conducted a study on the physicochemical parameters of the water collected from the Kurram River, Pakistan, and found much higher heavy metal concentrations.

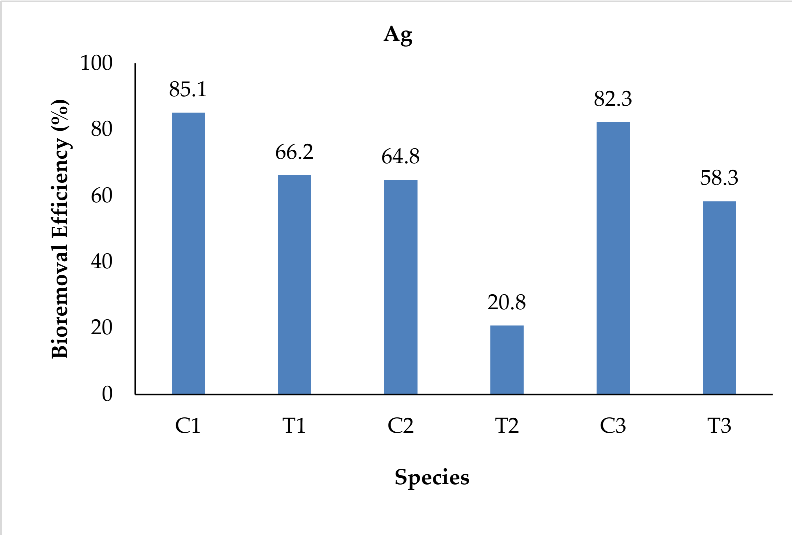

Figure 6. Bioremoval efficiency of *Bacillus pakistanensis* and *Lysinibacillus composti* for Ag in industrial wastewater.

3.2.7. Lead (Pb)

The Pb concentrations checked at the initial stage for both control and treated water were 0.002 and 1.12 mg/L, respectively. At the final stages, the mean values ranged from 0.001 to 0.0017 mg/L for the control and from 0.104 to 0.450 mg/L for the treated water samples. The highest values were recorded in T1 for the treatment water, while in C3 for the control water samples. The study found 50, 59.8, 50, 62.5, 25, and 90.7% reductions in the final water samples of C1, T1, C2, T2, C3, and T3, respectively. The highest bioremoval efficiency was observed in T3, whereas the lowest was in C3 (Figure 7). The initial Pb concentration observed for industrial WW was 1.12 mg/L. In another study, conducted on the industrial WW at the Hayatabad Industrial Estate, by Ayaz et al. [3], a much higher Pb concentration (6.81–8.01 mg/L) was observed. The difference in results may be due to the initial concentration difference. In the initial stage the Ni concentration observed in the industrial WW was 0.026 mg/L. Ali et al. [35] analyzed the physicochemical parameters of the water collected from the Kurram River, Pakistan, and found a much higher Ni concentration (2.0–10.5 mg/L). These results differences can be ascribed to the difference in the study area and initial concentrations of Ni.

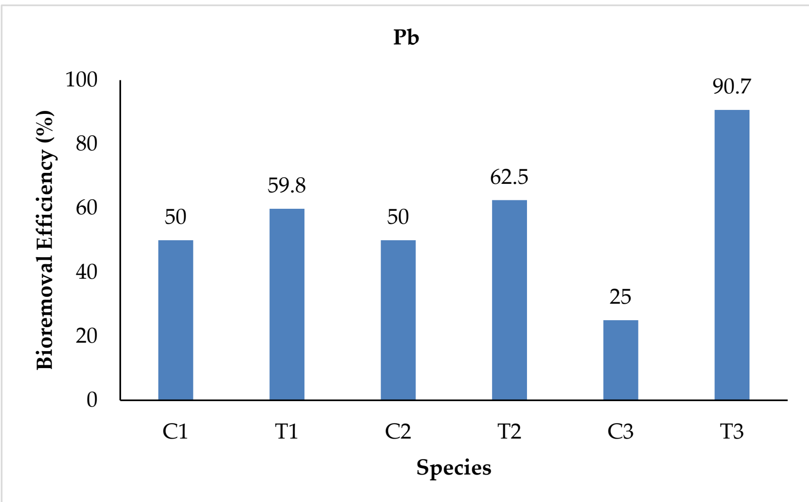

Figure 7. Bioremoval efficiency of *Bacillus pakistanensis* and *Lysinibacillus composti* for Pb in industrial wastewater.

3.2.8. Nickel (Ni)

The Ni concentrations checked at the initial stage for both the control and treated water were 0.046 and 0.113 mg/L, respectively. At the final stages, the mean values ranged from 0.012 to 0.019 mg/L for the control and from 0.012 to 0.019 mg/L for the treated water samples. The highest values were recorded in T3 for the treatment water, while in C3 for the control water samples. The study found 71.7, 88.4, 73.9, 89.3, 58.6, and 83.1% reductions in the final water samples of C1, T1, C2, T2, C3, and T3, respectively. The highest bioremoval efficiency was observed in T2, whereas the lowest was in C3 (Figure 8). A study was conducted by Kamika and Momba [37] on the bioremediation of the selected bacterial species (*Pseudomonas putida*, *Bacillus licheniformis*, and *Peranema*) on HMs in IWW. The removal efficiencies (Co-71%, Ni-51%, Mn-45%, and Cu-49%) observed by Kamika and Momba [37] were much lower and were not in agreement with the present results.

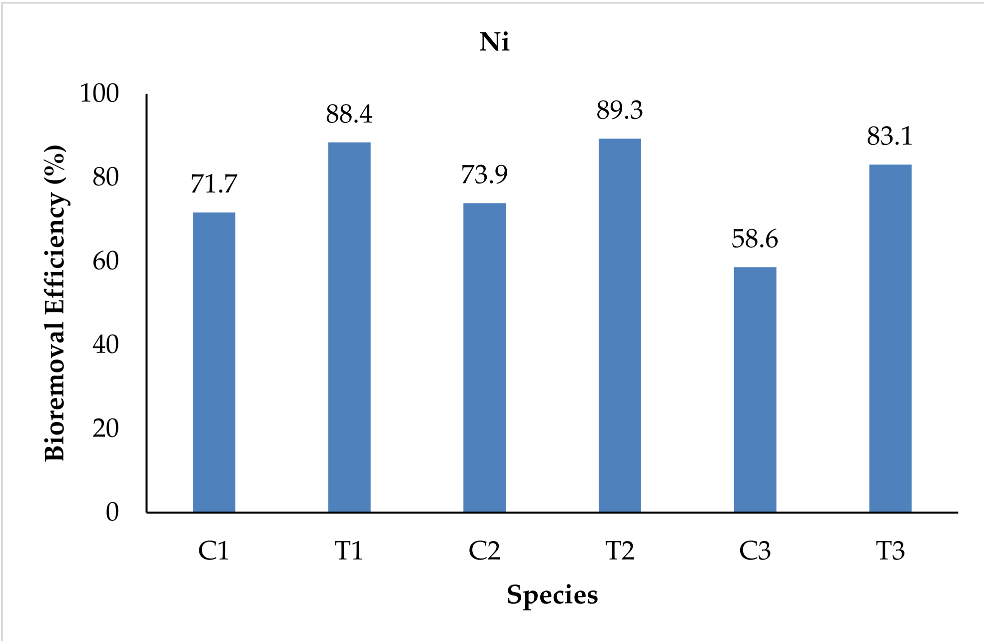

Figure 8. Bioremoval efficiency of *Bacillus pakistanensis* and *Lysinibacillus composti* for Ni in industrial wastewater.

3.2.9. Calcium (Ca)

The Ca levels checked at the initial stage for both the control and treated water were 1.72 and 14.5 mg/L, respectively. At the final stages, the mean values ranged from 0.088 to 0.920 for the control and from 0.880 to 1.265 mg/L for the treated water samples. The highest values were recorded in T2 for the treatment water, while in C2 for the control water samples. The study found 94.8, 93.9, 46.5, 91.2, 93.6, and 98.8% reductions in the final water samples of C1, T1, C2, T2, C3, and T3, respectively. The highest bioremoval efficiency was observed in T3, whereas the lowest was in C2 (Figure 9). The findings are not in agreement with the findings (376.9–468 mg/L) of the study by Fito et al., which reported on the physicochemical properties of the sugar industry WW [24].

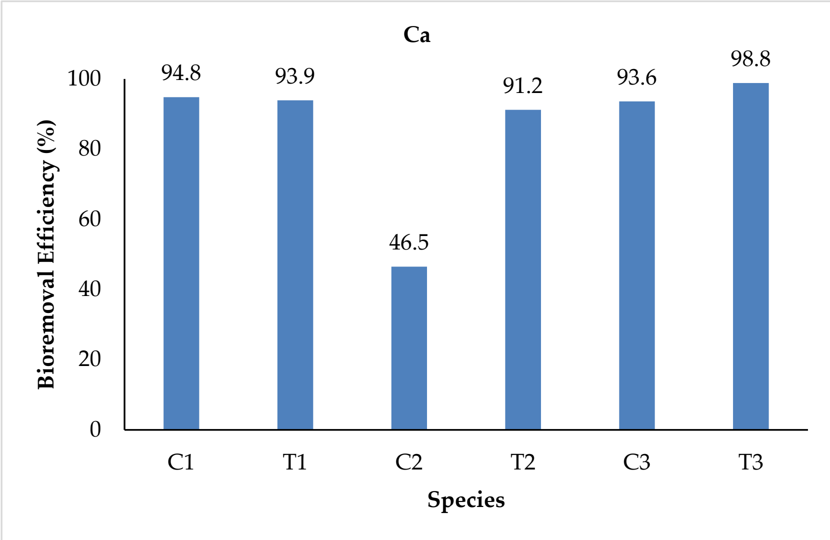

Figure 9. Bioremoval efficiency of *Bacillus pakistanensis* and *Lysinibacillus composti* for Ca in industrial wastewater.

3.2.10. Magnesium (Mg)

The Mg levels checked at the initial stage for both the control and treated water were 0.040 and 0.19 mg/L, respectively. At the final stages, the mean values ranged from 0.007 to 0.017 mg/L for the control and from 0.016 to 0.97 mg/L for the treated water samples. The highest values were recorded in T2 for the treatment water, while in C1 for the control water samples. The study found 57.5, 75.2, 82.5, 48.9, 60, and 91.5% reductions in the final water samples of C1, T1, C2, T2, C3, and T3, respectively. The highest bioremoval efficiency was observed in T3, whereas the lowest was in T2 (Figure 10). However, in another study conducted by Nirgude et al. [38] on the physicochemical evaluation of industrial effluents from Indian industrial areas, much higher values (34 to 3246 mg/L) were observed. The recent finding is not in agreement with the results (274.2–341.0 mg/L) of the study by Fito et al., which reported on the physicochemical properties of the sugar industry WW [24]. The difference may be ascribed to the difference in the initial concentration, water sample collection site, and season of collection.

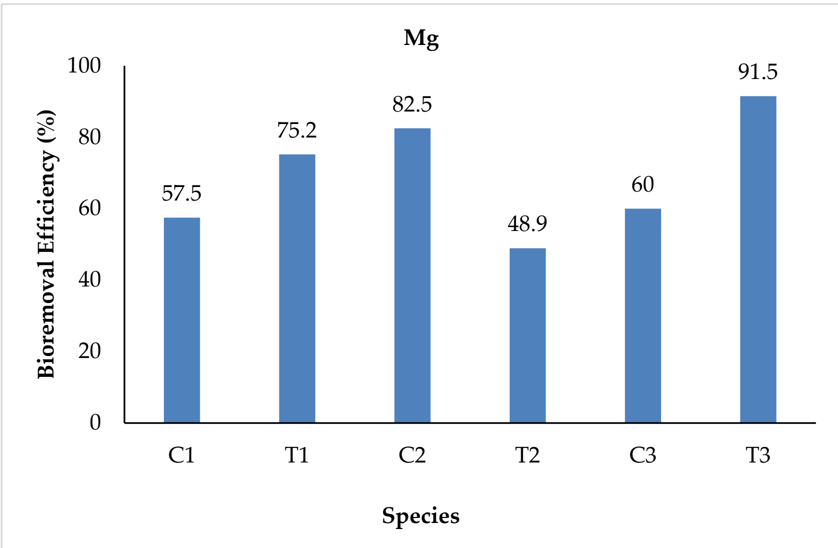

Figure 10. Bioremoval efficiency of *Bacillus pakistanensis* and *Lysinibacillus composti* for Mg in industrial wastewater.

4. Conclusions and Recommendations

The concentrations of the metals found in the Hayatabad Industrial Estate's industrial WW exceed the permissible limits. The bacterial species, individually and in a consortium, have a noteworthy role in the remediation of metal from industrial WW. The *Bacillus pakistanensis* had the best removal efficiency in the C1 control for Ag, and in the T1 treatments for Cu and Cd. Overall, the concentrations of the metals (Ag, Cu, Cd, and Co) in the industrial WW significantly reduced ($p \leq 0.05$) following the treatment of T1 and C1. The *Lysinibacillus composti* had the best removal efficiency in the treatment of T2 for Mn and Ni. Similarly, treating the bacterial consortium T3 showed the best removal efficiency for Cr, Pb, Ca, and Mg. Overall, the concentrations of the metals (Mn, Ni, Cr, Ca, and Mg) in the WW significantly reduced ($p \leq 0.05$) in the T2 and T3. The study showed that the bacterial species *Bacillus pakistanensis* is more competent in most of the metal (Mn, Cu, Cr, Ag, Pb, Ca, and Cd) uptake. Moreover, this species is more efficient in the control pot (C1), which indicates that the species can remediate wastewater, even if the metals are at trace levels, unlike traditional methods. The results also showed that the use of treatment with pot T3 (consortium of *Bacillus pakistanensis* and *Lysinibacillus composti*) was more efficient in metal removal, compared to the other treatments. Therefore, it is concluded that at higher metal concentrations the consortium of both these species is more effective than simply using individual bacterial species (T1 and T3). These selected species can survive under stress triggered by the metal concentrations. Therefore, this character can encourage evidence for these selected bacterial species to be utilized for the bioremediation of polluted water, either individually or in a consortium. Thus, it can be concluded that bioremediation is environmentally friendly, cost-effective, and can be used for industrial WW remediation. This study serves as a baseline for the removal of toxic heavy metals by using promising bacterial species, while these species can be used for the safe removal of heavy metals from other areas of the country. However, further research is needed to identify some other novel bacteria that have similar modes of action against toxic heavy metals.

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