Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 04 | 2023
DOI 10.17605/OSF.IO/CV3MU

BIOREMEDIATION OF HEAVY METALS FROM WASTEWATER THROUGH SOIL BACTERIA

ALIYA KHALID

Department of Microbiology, Abdul Wali Khan University Mardan, KP, Pakistan.

YAHYA KHAN

Department of Agriculture and Veterinary Medicine, University of Padua, Italy.

RANA HADI

Department of Zoology, Jinnah University for Women, Karachi, Sindh, Pakistan.

SAYYADA GHUFRANA NADEEM

Department of Microbiology, Jinnah University for Women, Karachi, Sindh, Pakistan.

SANA AHMAD

Department of Biochemistry, Bahria University Medical and Dental College, Karachi, Sindh, Pakistan.

ATEEQ AHMAD AFRIDI

Department of Microbiology, Abdul Wali Khan University Mardan, KP, Pakistan.

Dr. MAJID HUSSIAN*

Department of Forestry and Wildlife Management, University of Haripur, KP, Pakistan.
***Correspondence Author Email: majid@uoh.edu.pk**

Abstract

Currently, water contamination is one of the leading problems worldwide. Unprocessed industrial waste removal into the environment creates a serious problem for freshwater, marine living organisms, and human beings. Among pollution-causing industries, the textile industry achieves major attention from environmentalists due to the expenditure of a large volume of wastewater and chemicals during various manufacturing processes. Wastewater contains carcinogenic organic and inorganic materials such as heavy metals. Nearly all the physicochemical procedures do not provide essential parameters for removing heavy metals from industrial wastewater. Bioremediation is a successful way the removal of heavy metals from wastewater. Most of the bacteria are resistant to heavy metals and are used as a potential source of bioremediation of heavy metals. In this research, four bacterial species were isolated from soil samples of District Mardan KP, Pakistan. Morphological, physiological, and biochemical characteristics of these isolates were observed. Wastewater samples were collected from Sugar Mill and Marble industries located at Nowshera road District Mardan KP, Pakistan. After that wastewater containing heavy metals was analysed by Atomic Absorption Spectrometry and three metals, Cadmium, Chromium, and Iron was identified. After that four-isolated bacterial species (*Bacillus subtilis, Acinetobacterboumannii, Pseudomonas aeruginosa,* and *Lactobacillus acidophilus*) were used to treat the heavy metals in industrial wastewater. After the successful incubation period of 5 days, among these four isolates, three bacterial isolates successfully eliminated the heavy metals from the industrially contaminated water. The present study shows that heavy metal tolerant bacterial species effectively degrade the heavy metals from industrial wastewater.

Keywords: Bioremediation, Heavy Metals, Wastewater, Soil Bacteria, Pakistan

1. INTRODUCTION

Environmental pollution is one of the most emerging problems globally. Pollution is present in different forms based on its source, however pollution due to heavy metals is gradually increasing and gaining global attention, and there is a large diversity of natural resources for metals in the environment. Different processes are involved in forming metals such as extraction and mixing rock material with atmospheric downfall like a volcanic eruption. Metals pollutants are produced due to human activities, and their level of concentration is not measurable due to the involvement of diverse natural resources such as groundwater, seawater, aquifers, and freshwater) (Silva and Araújo 2003b, Weber 2004, Burak *et al*., 2010, Singh *et al*., 2010, Fakayode., 2005). Heavy metals are toxic and resist biodegradation, which increases the burden of pollutants on the environment (Bahadir *et al*., 2005).

When the density of metal crosses the 5g cubic centimeter range, it is called heavy metal. A wide range of elements follows these criteria. These include chromium, cadmium, iron, copper, arsenic, mercury, and zinc. As they are toxic, they pose a high risk for ecological pollution (Nagajyoti *et al*., 2010; Jaishankar *et al*., 2013), and some other toxic consequences like it badly affecting cell generation and progress (Babel and Kurniawan 2004). The most common toxic heavy metals in contaminated water are nickel, cadmium, copper, chromium, lead, arsenic, and zinc, which are hazardous to both the ecosystem and the biological system (Lambert *et al*., 2000). Heavy metals acquire different mechanisms by which they enter the ecosystem. Still, transmission depends on the source of metal i.e., industrial effluents, natural weathering of the earth’s crust, insect or disease control agents applied to crops, soil erosion, mining, urban runoff, sewage discharge, and many others (Morais *et al*., 2012). The hydrophilic nature of metals makes them more prone to dissolve in water and their absorption in different foods, which are then taken by humans and cause different health problems such as cancer (Babel and Kurniawan 2004).

Among all the heavy metals, lead (Pb), and cadmium (Cd) are highly toxic to the human body because of their non-essential nature to humans and involvement in other tissues damages (nervous, skeletal, circulatory, kidney) and systemic damage (enzymatic, endocrine, and immune systems) (Yang *et al*., 2006, Zhang *et al*., 2012). Moreover, chromium also causes oncogenic effects in the liver, kidney, and gastrointestinal tract (Jarup., 2003; Zukowska and Biziuk 2008; Chervona *et al*., 2012). Arsenic and zinc are also highly toxic heavy metals involved in skin cancer and lesion formation on the skin, while zinc is involved in the mutation of DNA structure (Navoni et al., 2012; Bobillo *et al.,* 2014; Navoni *et al.,* 2014). Mercury is also involved in the clinical disease of different organs, including the heart, liver, brain, and kidneys, thus leading to death rarely (Zietz *et al*., 2003; Muhammad *et al*., 2011). The mechanism of iron pathogenicity depends on the iron-oxidizing and reducing state. The oxidation and reduction processes lead to the formation of free hydrogen ions which then attach to cellular material such as DNA and

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 04 | 2023
DOI 10.17605/OSF.IO/CV3MU

cause damage, mutation, and transformations in the genetic makeup, leading to a cancerous state (Grazuleviciene *et al*., 2009).

The mechanism by which heavy metals affect the living organism includes disrupting the cell membrane, destabilizing the enzymatic activity, and causing mutation in the DNA structure and nature. Altogether these processes are involved in the causation of oncogenic and mutational effects (Diels *et al*., 2002). These problems enhance the abnormal functioning of the living body and contribute to causing cancer (Rajbansi., 2008). The conversion of metals into harmless forms is the most challenging problem because these metals are freely present in the environment (Kadirvelu *et al*., 2001). Treatment of heavy metals poses many other issues like high cost, partial degradation of metals, the requirement for higher efficiency, and release of toxic materials after treatment (Naz, T. *et al*., 2016).

Various physical, chemical, and biological methods are used to eliminate heavy metals from the environment, specifically from wastewater. Commonly used physicochemical processes such as sedimentation, filtration, ion exchange, and floatation are not only economically expensive but are not eco-friendly (Mulligan *et al*., 2001: Kadirvelu *et al*., 2002). Moreover, the side effect of the physicochemical method is partial metal elimination, many chemicals and potential requirements, and removal of poisonous or contaminant, which require proper management for disposal; all the processes are expensive and not standard procedure for heavy metals from the aquatic ecosystem. Due to all these issues, the discovery of advanced and environmentally friendly technologies is the main concern (Volesky and Naja. 2007).

The discovery of metal persistent microorganisms plays a crucial role in cleaning the ecosystem (Filali *et al*., 2006). Due to the high potential of heavy metals, bioremediation bacteria have recently attempted a rapid consideration (Farhadian *et al*., 2008). Bioremediation is considered the safest and most affordable way of cleaning the environment and acts as an alternative chemical and physiochemical method. Due to the toxic nature of heavy metals, persistent microbes are a top priority for consideration (Filali *et al*., 2006). Different mechanisms involved in the bioremediation of heavy metals such as bio-sorbent, electrostatic interaction, ions exchange mechanisms, biosorption (metal sorption to the cell surface by physicochemical mechanisms), biomineralization (heavy metal immobilization through the creation of non-soluble sulphides or polymeric compounds), bioleaching (heavy metal utilization through the elimination of organic acids or methylation reactions), enzyme-catalyzed transformation (redox reactions), and intracellular accumulation but all the mechanisms adopted by the bacteria is dependent on the physiological and environmental condition such as the ionic form of metals, PH, temperature, and toxicity (Lloyd JR and Lovley 2001, Vimala& Das 2009; Naja *et al*., 2009), as can be seen in Fig. 1.

ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 04 | 2023
DOI 10.17605/OSF.IO/CV3MU

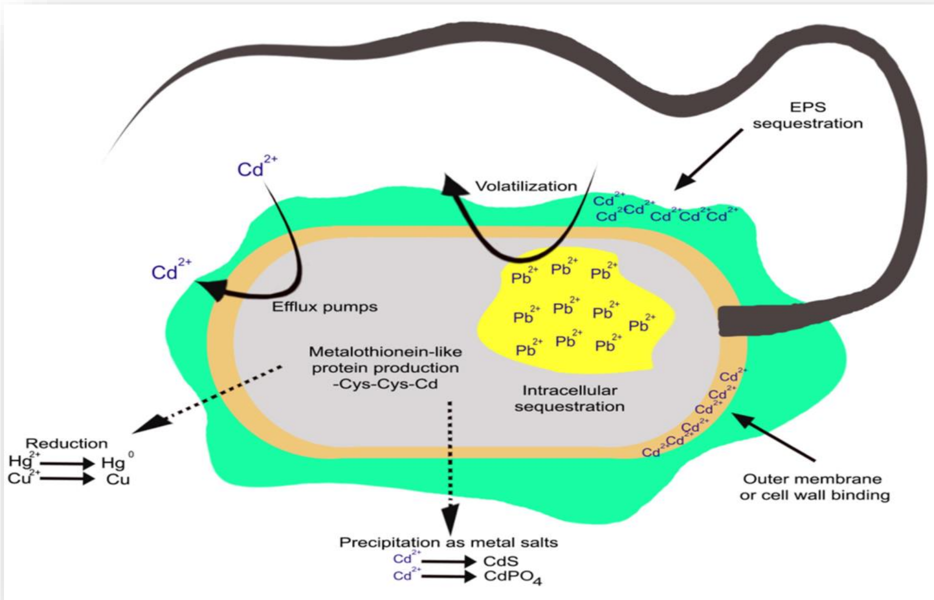

Fig. 1: Bioremediation mechanism of different heavy metals in Bacteria

Microorganisms, particularly bacteria, are essential in heavy metal' biological removal. Bacteria have acquired unique mechanisms by which they protect themselves from the lethal effects of metals like methylation, uptake, adsorption, reduction, and oxidation (Fernandez PM, *et al*., 2012). Mostly individual bacterial growth is used for contaminant removal from the environment, but enriched microbial growth is quite efficient (Adarsh *et al*., 2007). In the case of chromium remediation, the enriched growth of microorganisms is recommended because of their existence and increased consistency in sewage places and where multiple metals are present (Tahri-Joutey N *et al*., 2011). Therefore, it is of utmost importance to learn how microorganisms remove heavy metals from wastewater. This study isolated heavy metals from industrial wastewater in the district of Mardan, Khyber Pakhtunkhwa (KP), Pakistan. For the effective bioremediation of isolated heavy metals in wastewater, metal-resistant bacteria were isolated from soil: Bacillus subtilis, Pseudomonas aeruginosa, Acinetobacter boumannii, and Lactobacillus acidophilus, and these bacterial species were used for the potential bioremediation of heavy metals chromium, cadmium, and iron from wastewater.

2. SAMPLE COLLECTION SITES

2.1.1 Wastewater Sample Collection Site

Water samples were collected from Sugar Mill and Marble Industries, located near the industrialized area of Nowshera road District Mardan Khyber Pakhtunkhwa, Pakistan.

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 04 | 2023
DOI 10.17605/OSF.IO/CV3MU

Four water samples (S1, S2, S3, and S4) were collected from both sites. Water samples were collected in sterile plastic bottles.

2.1.2 Soil Sample Collection Site

Soil samples were also collected randomly from Nowshera Road District Mardan Khyber Pakhtunkhwa, Pakistan. About five samples were collected in sterile plastic bags and were air-dried at room temperature.

2.2 Soil Sample Processing

About 3 grams of the soil was taken in the beaker from each sample to maintain moisture, 25 ml of distilled was also added. The soil was correctly mixed by a rotator shaker at 150r/min for 30 min.

2.3 Physicochemical Characterization of Wastewater Sample

Characterization of the effluent for various constraints such as Biochemical Oxygen Demand (BOD), Total Dissolved Solids (TDS), and Heavy Metals (Cd, Cr & Fe) were analyzed by the methods of the American Public Health Association (APHA) (1998).

2.4 Serial Dilution

Serial dilution was performed by the principle of 10-5 dilution. A sterile test tube (10ml) was taken, and 9 ml of sterile water was added to each tube. After that, 1 mg of soil sample was added for dilution. Then 1ml from the first test tube was taken and added to the second test tube. The same procedure was repeated for all the test tubes. The process of serial dilution is depicted in Figure 2.

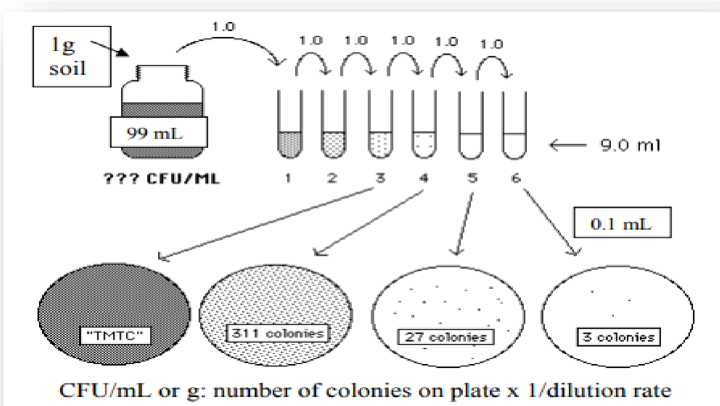

Figure 2: Process of Serial Dilution

2.5 Isolation of Bacteria

The cell counting technique was followed in Nutrient Agar (NA) media plates to determine the population density. Serial dilutions were done at five dilutions of the same soil sample.

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 04 | 2023
DOI 10.17605/OSF.IO/CV3MU

After those solutions from 10-3, 10-4, and 10-5 dilutions were spread on the Nutrient agar medium and were incubated at room temperature. Pure bacterial strains were identified after sequential transfer of individual colonies on NA plates and their accurate incubation temperature and time is 37ºC for 24 hours.

2.6 Identification of Bacteria

Different biochemical tests were performed to identify heavy metals and persistent bacteria. We performed mannitol, sucrose, and lactose tests to analyze their glucose fermentation potential. The isolates were similarly tested for indole and citrate tests. Further biochemical tests included in identification are oxidase, catalase, and urease test. We used selective media such as blood agar and MacConkey agar to isolate selected species of bacteria. Biochemical identification of bacterial species helped identify the genus of metals-resistant bacteria.

2.7 Bacterial Inoculum Preparation

The suspension of 3 days old bacteria cultures was used to degrade heavy metals from the wastewater. It was prepared in saline solution (0.90% sodium chloride). A loop full of each bacterial culture was inoculated into 5 ml of saline and incubated at $37^{o}C$ for 3 hours (shown in Figure 3).

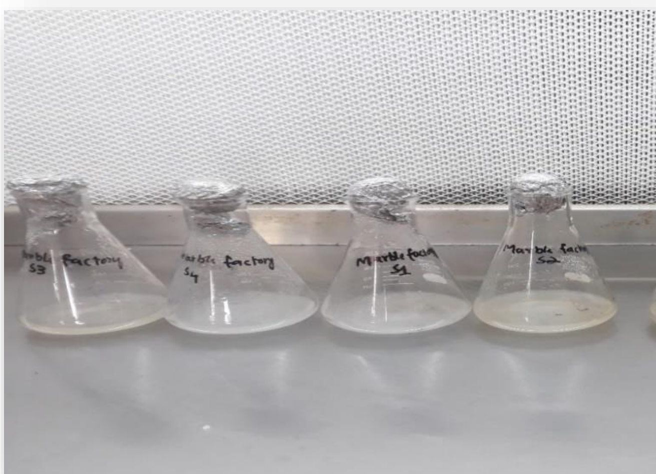

Figure 3: Bacterial Inoculum Preparation

2.8 Treatment of Wastewater with Isolated Heavy Metal Resistant Strains

Bioremediation of heavy metals experiments was carried out in 250 ml of separate flasks containing 100 ml of wastewater collected from study sites S1, S2, S3, and S4. The Wastewater flasks were kept in a mechanical shaker at a speed of 100 rev/min and incubated at 37ºC for 5 days. The pH was adjusted to 7 using NaCl and H_2SO_4. Then, the

ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 04 | 2023
DOI 10.17605/OSF.IO/CV3MU

flasks were autoclaved at 121ºC for 15 minutes. The autoclaved flasks were inoculated with 5ml of bacterial inoculum of each isolate.

2.9 Metals Analysis

50 ml of treated Wastewater was taken in a bottle and was digested using 5 ml of triple acid solution (HNO_3, H_2SO_4, and $HClO_4$ in 9:2:1 proportion, respectively) till the waste became colorless. The digested sample was filtered using a Whatman number filter for three made up to 100 ml and subjected to heavy metal assay using Atomic Absorption Spectroscopy (Mac: SL 176 Double beam Spectrophotometer) as per the standard method recommended by APHA, 1998. Three replications were maintained for each treatment (shown in Figure. 4).

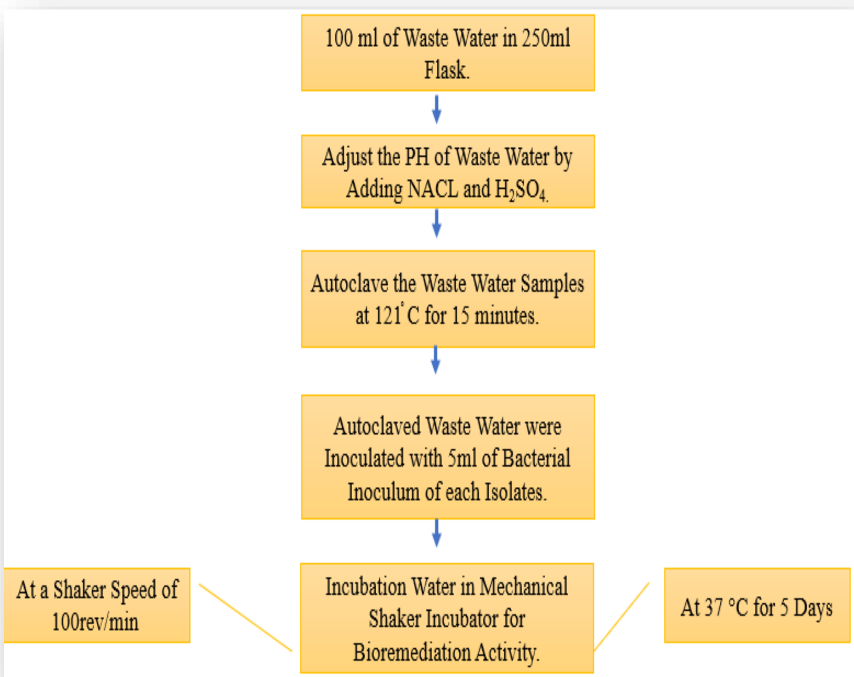

Figure 4: Procedure for Wastewater Treatment

ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 04 | 2023
DOI 10.17605/OSF.IO/CV3MU

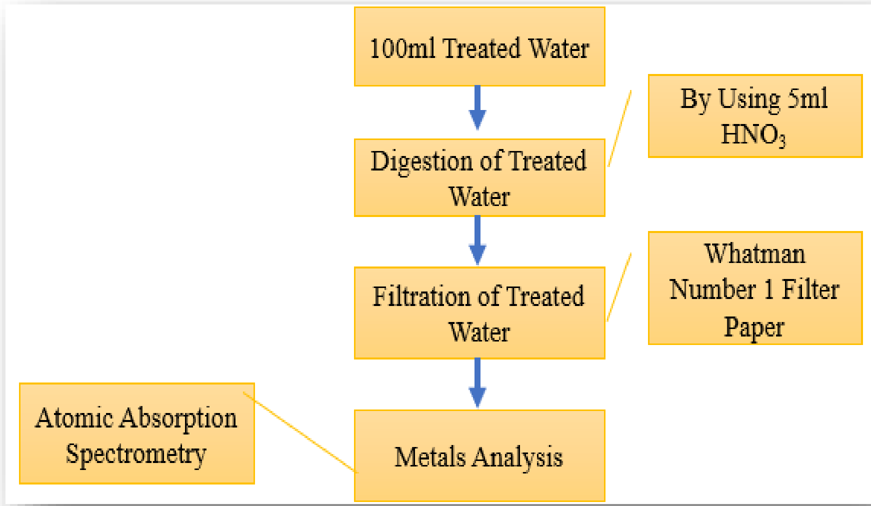

Figure 5: Metals Analysis Procedure

3. RESULTS

3.1 Physio-Chemical Characterization of Industrial Wastewater

The wastewater was collected randomly from different locations of District Mardan with specific high concentrations of pollutants compared with standards of NEQS (2010). The different samples were in dark brown and white milky but mostly depended on the pollutants. Almost all the samples had a pungent odour (Table 1). In the pH values of the effluents, there was a lot of variation which differ from 4.5 to 10, indicating the acidic and basic nature of the effluents. The BOD values were also very high in all the textile dye effluent samples. The heavy metals such as chromium, cadmium, and iron content in the samples' wastewater were in large concentrations. Hence all the wastewater samples collected from the study area specify large quantity of pollutants.

Table 1: Physio-chemical Analysis of the Wastewater Samples

Parameters	Sugar Mills Samples		Marble industries Samples		NEQS Limits
	S1	S2	S3	S4	
Colour	Dark Brown	Light Brown	Chalk like	Chalk like	Colour Less
Odour	Burnt Sugar	Burnt Sugar	Pungent	Pungent	Odour Less
PH	4.5	5.7	10	9.8	6-9
BOD	553.00	1842.00	480.00	270.00	80-250
Chromium	1.44 mg/l	1.68 mg/l	2.39 mg/l	1.22 mg/l	1.0 mg/l
Cadmium	0.22 mg/l	0.40 mg/l	0.30 mg/l	0.26 mg/l	0.1 mg/l
Iron	9.54 mg/l	10.01 mg/l	5.30 mg/l	5.89 mg/l	8 mg/l

**NEQS National Environmental Quality Standard (2010).*

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 04 | 2023
DOI 10.17605/OSF.IO/CV3MU

3.2 Isolation and Characterization of Bacterial Strains from Soil Samples

2. These isolated microbial strains were streaked on Petri plates and isolated bacterial colonies were used for the bioremediation of heavy metals from wastewater. After the sub culturing of sub culturing isolates on solid media (Nutrient agar, blood, and MacConkey agar) four bacterial strains of microbes were collected from S1, S2, S3, and S4 locations, the bacterial strains which were isolated included bacillus subtilis, lactobacillus acidophilus, and Pseudomonas aeruginosa. For further identification different biochemical tests were performed. The biochemical characterization of bacterial strains is given in Table 2.

Table 2: Biochemical Characterization of Isolated Bacterial Stains from Soil

Tests	Bacillus Subtilis	Pseudomonas aeruginosa	Lactobacillus acidophilus	Acinetobacterbo umannii
Gram staining	Gram Positive	Gram Negative	Gram-positive	Gram Negative
Colony Colour	Slightly Yellowish	Yellowish/ GREEN	Cream Colour	Cream Colour
Shape	Rod shape	Rod shape	Rod Shape	Cocco-bacillus
Motility	Motile	Motile	Motile	Non-Motile
Catalase	+	+	+	+
Oxidase	-	+	-	-
Nutrient Agar	+	+	+	+
Sucrose	+	-	-	-
Mannitol	+	+	+	-
Lactose	+	-	+	-
Indole	-	-	-	-
Citrate	+	+	-	+
Urease	-	-	-	-

3.3 Bioremediation of Heavy Metals from Wastewater

3.3.1 Bioremediation of Cadmium

In all the samples, the Cadmium concentration was higher than NEQS, (2000) standards. But after adding a starter culture to wastewater samples with identified bacterial species, there was a notable decrease in Cadmium concentration in all the samples. Similarly, *Bacillus subtilis* showed high heavy metal remediation potential (96.6%) compared to other bacterial strains. *Acinetobacter boumanni* showed remediation potential (83.33%), while *Pseudomonas aeruginosa* showed a maximum of 99.54% of Cadmium remediation potential from the wastewater (Table 3).

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 04 | 2023
DOI 10.17605/OSF.IO/CV3MU

Table 3: Bioremediation of Cadmium from Wastewater Using Bacterial Strains

Bacterial Isolates	Sample 1 Sugar Mills			Sample 2 Sugar Mills			Sample 3 Marble industry			Sample 4 Marble industry		
	Ic	Fc	%	Ic	Fc	%	Ic	Fc	%	Ic	Fc	%
Bacillus Subtilis	0.22 mg/l	0.04	**81.81**	0.40 mg/l	0.03	**92.5**	0.30 mg/l	0.01	**96.6**	0.26 mg/l	0.06	**76.92**
Pseudomonas aeruginosa	0.22 mg/l	0.001	**99.54**	0.40 mg/l	0.01	**97.5**	0.30 mg/l	0.02	**93.33**	0.26 mg/l	0.10	**61.53**
Acinetobacter boumanni	0.22 mg/l	0.1	**54.54**	0.40 mg/l	0.09	**77.5**	0.30 mg/l	0.05	**83.33**	0.26 mg/l	0.13	**50**
Lactobacillus acidophilus	0.22 mg/l	0.17	**22.72**	0.40 mg/l	0.35	**12.5**	0.30 mg/l	0.22	**26.66**	0.26 mg/l	0.19	**26.9**

Ic = Initial concentration, Fc=Final concentration,

3.3.2 Bioremediation of Chromium

In all the wastewater samples S1, S2, S3, and S4 collected from different study areas, Chromium was found to have the highest concentrations. After the inoculation of water samples using all isolated bacterial strains, there was a significant decrease in chromium concentrations from all the samples. Similarly, *Bacillus subtilis* showed maximum heavy metal remediation (86.90%) compared to other bacterial strains. *Pseudomonas aeruginosa* showed a maximum of 91.21%, and *Acinetobacter boumanni* showed 79.49% of Chromium remediation potential from the wastewater (Table. 4)

Table 4: Bioremediation of Chromium from Wastewater Using Bacterial Strains

Bacterial Isolates	Sample 1 Sugar Mills			Sample 2 Sugar Mills			Sample 3 Marble industry			Sample 4 Marble industry		
	Ic	Fc	%	Ic	Fc	%	Ic	Fc	%	Ic	Fc	%
Bacillus Subtilis	1.44 mg/l	0.30	**79.16**	1.68 mg/l	0.22	**86.90**	2.39 mg/l	0.27	**88.70**	1.22 mg/l	0.17	**86.06**
Pseudomonas aeruginosa	1.44 mg/l	0.43	**70.13**	1.68 mg/l	0.19	**88.69**	2.39 mg/l	0.21	**91.21**	1.22 mg/l	0.22	**81.96**
Acinetobacterboumanni	1.44 mg/l	0.67	**52.47**	1.68 mg/l	0.52	**69.04**	2.39 mg/l	0.49	**79.49**	1.22 mg/l	0.61	**50.83**
Lactobacillus Acidophilus	1.44 mg/l	1.30	**9.72**	1.68 mg/l	1.43	**15.33**	2.39 mg/l	2.19	**20**	1.22 mg/l	1.09	**10.65**

Ic=Initial concentration, Fc=Final concentration

3.3.3 Bioremediation of Iron

All the bacterial isolates were added to wastewater for five days. In all samples, there was a significant decrease in the heavy metal concentration. *Bacillus subtilis* removed 82.85% of iron (Fe) from the treated samples. *Acinetobacter boumanni* effectively removed (65.70 %) Fe from all samples. *Pseudomonas aeruginosa* showed bioremediation (79.05%) activity (Table. 5).

Table 5: Bioremediation of Iron from Wastewater Using Bacterial Strains

Bacterial Isolates	Sample 1 Sugar Mills			Sample 2 Sugar Mills			Sample 3 Marble Industry			Sample 4 Marble Industry		
	Ic	Fc	%	Ic	Fc	%	Ic	Fc	%	Ic	Fc	%
Bacillus Subtilis	9.54 mg/l	2.01	**78.93**	10.01 mg/l	2.90	**71.002**	5.30 mg/l	1.21	**77.16**	5.89 mg/l	1.01	**82.85**
Pseudomonas aeruginosa	9.54 mg/l	3.53	**62.99**	10.01 mg/l	2.81	**71.92**	5.30 mg/l	1.11	**79.05**	5.89 mg/l	1.89	**67.91**
Acinetobacter boumanni	9.54 mg/l	4.11	**56.91**	10.01 mg/l	4.55	**54.54**	5.30 mg/l	2.31	**56.41**	5.89 mg/l	2.02	**65.70**
Lactobacillus acidophilus	9.54 mg/l	9.33	**2.20**	10.01 mg/l	9.29	**7.19**	5.30 mg/l	5.05	**4.71**	5.89 mg/l	5.49	**6**

Ic=Initial concentration, Fc=Final concentration

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 04 | 2023
DOI 10.17605/OSF.IO/CV3MU

3.5 Overall Bioremediation Activity

A comparison of the complete bioremediation activity of all the bacterial strains against all three heavy metals is shown in Figure 13.

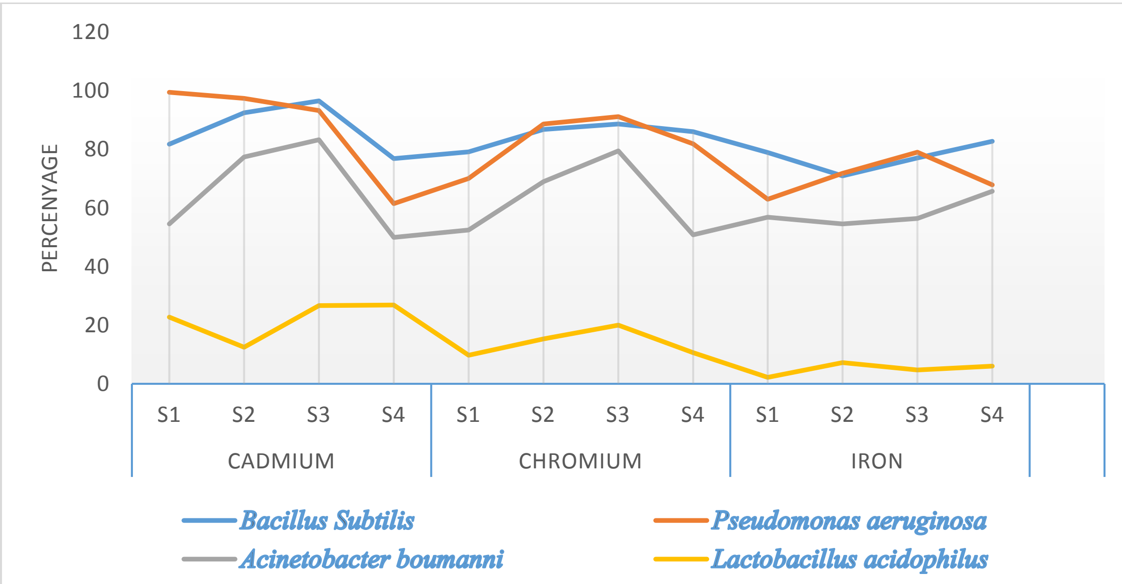

Figure 6: Bacterial Strains Comparison against All Wastewater Samples

4. DISCUSSION

The problem of water contamination is gradually increasing nowadays and is considered a main environmental and public health problem in many low-economic countries. Disposing of these wastes without any processing makes it necessary to innovate rapidly and advance techniques that are easy to use, affordable and accessible. Previous literature has focused on different techniques used to treat waste effluents in water. In this research study, we mainly focused on the wastewater treatment of district Mardan and selected two different industrial areas in Mardan and collected samples randomly. We specifically used the bioremediation technique to degrade the heavy metals from wastewater. The samples collected from the industrial area showed a high level of contamination. This research was compared with some previous studies based on pollutant content (physio-chemical characterization) (Rajaganesh *et al*., 2014). A study by Manisha *et al*., 2011, suggested that numerous bacteria are involved in the bioremediation of metals from the aquatic ecosystem (Manisha *et al*., 2011). In this research study, we isolated metal's persistent bacteria such as *Bacillus subtilis, Pseudomonas aeruginosa, Lactobacillus acidophilus,* and *Acinetobacter boumanni* from soil samples.

The biochemical analysis of mentioned isolated bacteria is shown in Table 2. Industrial wastewater contains heavy metals like cadmium, chromium, and iron. The amount of cadmium in wastewater ranges from 0.22 mg/l to 0.40 mg/l, the amount of chromium

Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/
Journal of Xi'an Shiyou University, Natural Sciences Edition
ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 04 | 2023
DOI 10.17605/OSF.IO/CV3MU

ranges from 1.22 to 2.39, and the amount of iron ranges from 5.89 to 10.01. The primary goal of this research work was to eliminate the heavy metals or to reduce their concentration from industrial wastewater samples collected from District Mardan KP, Pakistan. The net decrease in cadmium concentration after bacterial dilution is about 0.22 mg/l to 0.04, 0.40 to 0.03 mg/l, 0.30 to 0.01 mg/l, and 0.26 to 0.06 mg/l (Table 3). These results are following a study conducted by Ashok *et al.* (2010), which verified that bacterial species are involved in the elimination of toxic metals from water. Hanjun *et al*., 2010 also confirmed the bioremediation or elimination of heavy metals from the aquatic ecosystem using plants loving *bacillus* bacteria species.

In wastewater, the chromium concentration is 1.22 mg/l to 2.39mg/l in four given samples. After the mixing of the starter culture of isolated bacteria in wastewater, and their incubation, a gradual decrease in chromium concentration was identified, these ranging from 1.44 to 0.30, 1.68 to 0.22, 2.39 to 0.27, 1.22 to 0.17 mg/l (Table 4). Current study resembles that of Ganguli and Tripathi (2002) in which the *pseudomonas aeruginosa* entirely remediated the chromium from water. Wangand Xiao, 1995 investigates that *bacillus* species release a specialized enzyme that is involved in chromium elimination. The isolated strains of bacteria, i.e., Bacillus subtilis, Pseudomonas aeruginosa, Lactobacillus acidophilus, and Acinetobacter boumanni, are added to wastewater it was observed a continuous reduction of iron that ranged from 9.54 to 2.01, 10.01 to 2.90, 5.30 to 1.21, 5.89 to 1.01 mg/l. This result is in accordance with Guo *et al*. (2010) identified an 81% reduction of iron by using iron-eliminating bacteria. In the current study, bioremediation activity among four bacterial species was compared with each other in which *pseudomonas aeruginosa* and *bacillus subtilis* showed the highest bioremediation activity against cadmium (99.54% and 96.6%) respectively, *Acinetobacter boumannii* shows intermediate bioremediation activity (83.33%), but *lactobacillus acidophilus* showed a negligible bioremediation activity against all type of metals.

Microorganisms play an essential part in the bioremediation of heavy metals and wastewater contaminants by adapting different mechanisms by which they can attract metal ions towards their cell surface. These mechanisms include extracellular precipitation, Vander Waals forces, redox interactions, covalent bonding, and electrostatic interactions (Blencowe and Morby, 2003). The negatively charged carboxyl and phosphoryl groups play the most crucial role in the metal ions interaction (Wase and Foster, 1997). The results obtained in this work concluded that heavy metal tolerant bacteria could survive in soils with a high amount of Cd, Cr, and Fe, showing a high potential for bioremediation that should be explored further. It was also observed that some of the bacteria did not resist some of the heavy metals or some of the concentrations used which might be due to the diverse nature of microbes resistant to heavy metals. Isolation of the most abundant strains strongly proposed that further efforts must be made to understand this metal-contaminated environment fully.

ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 04 | 2023
DOI 10.17605/OSF.IO/CV3MU

5. CONCLUSION

Bacteria were obtained from the -contaminated soil and heavy metals were isolated from the wastewater of the industrial area of Nowshera Road District Mardan KP, Pakistan. A large concentration of heavy metals was identified from wastewater, and four bacterial strains were isolated from the soil. The present study shows that heavy metal tolerant bacterial species effectively degrade the heavy metals from industrial wastewater. In this study, three out of four bacterial strains, namely *Bacillus subtilis, Pseudomonas aeruginosa,* and *Acinetobacterboumannii* reduced a maximum of 99.54% of cadmium, 91.21% of chromium, and 82.85% of iron from the wastewater samples. It may be concluded that microorganisms can show tolerance against heavy metals and are armed with various resistance and catabolic potentials. This catabolic potential of microorganisms is enormous and is advantageous to mankind for a cleaner and healthier environment through bioremediation. Further investigations are also needed to thoroughly evaluate the potential of heavy-metal resistant microorganisms or heavy-metal resistance. The search must be extended to genetic determinants conferring resistance to several heavy metals to accomplish these objectives more clearly. In the genome-based study, RNA analysis would be helpful to quantify the expression of heavy metal resistance genes in the environment. After analyzing the results, a new screen should be carried out using different culture-dependent strategies to assess the culturable bacterial isolates that must exist.

Conflict of Interests

The authors declared no conflict of interests in the publication of this article.

References

1. Adarsh VK, Madhusmita Mishra, Sanhita Chowdhury, 2M. Sudarshan, A.R. Thakur and 1S. Ray Chaudhuri. (2007). “Studies on Metal Microbe Interaction of Three Bacterial Isolates from East Calcutta Wetland”. 7(2) 80-88.
2. Ashok, K., Balwant, S.B and Vishnu, D.J. (2010). “Biosorption of heavy metals by four acclimated microbial species, Bacillus spp., Pseudomonas spp., Staphylococcus spp. And Asperdillusniger”. J. Bio Envi Sci. 4(12): 97108.
3. Babel, S., Kurniawan, T.A., (2004). “Cr (VI) removal from synthetic wastewater using coconut shell charcoal and commercial activated carbon modified with oxidizing agents and/or chitosan. Chemosphere” 54 (7), 951–967.
4. Bahadir U, Ahmed N, Beswick AJ, et al. (2005). “Screening of copper tolerant bacterial species and their potential to remove copper from the environment”.
5. Blencowe, D.K and Morby, A.P. (2003). “Zn (II) metabolism in prokaryotes”. FEMS Microbiol Rev. 27, 291-311.
6. Bobillo C, Navoni JA, Olmos V, Merini LJ, Villaamil LE, Corach D (2014). “Ethnic characterization of a population of children exposed to high doses of arsenic via drinking water and a possible correlation with metabolic processes”. Int J MolEpidemiol Genet 1:1–1
7. Burak DL et al. (2010). “Geochemistry and spatial distribution of heavy metals in Oxisols in a mineralized region of the Brazilian Central Plateau”. Geoderma 160:131–142

ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 04 | 2023
DOI 10.17605/OSF.IO/CV3MU

8. Chervona Y, Arita A, Costa M. (2012). “Carcinogenic metals and the epigenome: understanding the effect of nickel, arsenic, and chromium. Metallomics”. 4(7):619–627
9. Diels L, van der Lelie N and Bastiaens L. (2002). “New development in the treatment of heavy metal contaminated soils. Reviews in Environmental Science and Biotechnology”. 1: 75–82.
10. Fakayode SO. (2005). “Impact assessment of industrial effluent on water quality of the receiving Alaro River in Ibadan, Nigeria”. Ajeam-Ragee 10: 113.
11. Farhadian, M., Vachelard, C., Duchez, D., Larroche, C. (2008). “In situ bioremediation of monoaromatic pollutants in groundwater: a review”. Bioresour. Technol. 99, 5296–5308.
12. Fernandez PM, Martorell MM, Farina JI, and Figueroa LIC., (2012). “Removal Efficiency of Cr6+ by Indigenous Pichia sp. Isolated from Textile Factory Effluent”. The Scientific World Journal., Article ID 708213, 6 pages
13. Filali BK, Taoufik J, Zeroual Y, et al. (2006). “Wastewater bacteria resistant to heavy metals and antibiotics”. Current Microbiology 41: 151–156.
14. Ganguli A, Tripathi A K. (2002). “Bioremediation of toxic chromium from electroplating effluents by chromate-reducing Pseudomonas aeruginosa A2Chr in two bioreactors”. Applied Microbiology and Biotechnology”. 58: 416–420
15. Godlewska-Zylkiewicz, B. (2006). “Microorganisms in inorganic chemical analysis”. Anal. Bioanal. Chem., 384(1): 114–23
16. Hanjun, G., Shenglian, L., Liang, C., Xiao, X., Qiang, X., Wanzhi, W., Guangming, Z., Chengbin, L., Yong, W., Jueliang, C and Yejuan, H. (2010). “Bioremediation of heavy metals by growing hyperaccumulator endophytic bacterium Bacillus sp. L14”. Bioresource Technology. 101, 85998605.
17. Jaishankar M, Mathew BB, Shah MS, Gowda KRS. (2014). “Biosorption of Few Heavy Metal Ions Using Agricultural Wastes”. Journal of Environment Pollution and Human Health. 2(1): 1–6.
18. Jarup L. (2003). “Hazards of heavy metal contamination. Br Med Bull”. 68(1):167–182
19. Kadirvelu K, Thamaraiselvi K and Namasivayam C. (2001). “Adsorption of nickel from aqueous solution onto activated carbon prepared from coir pith. Separation and Purification Technology “. 24: 477–505.
20. Kadirvelu K., Senthilkumar P., Thamaraiselvi K., Subburam V, (2002). “Activated carbon prepared from biomass as adsorbent: elimination of Ni (II) from aqueous solution”. Bioresource Technology 2002; 81 87–90
21. Lambert M, Leven BA, Green RM. (2000). “New methods of cleaning up heavy metal in soils and water”. Environmental science and technology briefs for citizens. Kansas State University, Manhattan, KS.
22. Lloyd JR and Lovley DR, (2001). “Microbial detoxification of metals and radionuclides”. Current Opinion in Biotechnology; 12 248–253.
23. Manisha, N., Dinesh, S and Arun, K. (2011). “Removal of heavy metal from industrial effluent using bacteria”. International journal of environmental science. 2.
24. Morais S, Costa FG, Pereira ML. (2012). “Heavy metals and human health, in Environmental health – emerging issues and practice (Oosthuizen J ed)”. pp. 227–246, InTech.
25. Muhammad S, Shah MT, Khan S. (2011). “Health risk assessment of heavy metals and their source apportionment in drinking water of Kohistan region, northern Pakistan”. Microchem J 98:334–343
26. Mulligan C. N., Yong R., Gibbs B. F. (2001). “Remediation technologies for metal contaminated soils and groundwater: an evaluation”. Engineering Geology 60 (1–4) 193– 207.

ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 66 Issue 04 | 2023
DOI 10.17605/OSF.IO/CV3MU

27. Nagajyoti PC, Lee KD, Sreekanth TVM, (2010). "Heavy metals, occurrence and toxicity for plants: a review". Environ Chem Lett 8(3): 199–216.
28. Naja, G., Murphy, V. &Volesky, B. (2009). "Biosorption, metals. In: Encyclopedia of Industrial Biotechnology": Bioprocess, Bioseparation, and Cell Technology". (M. C. Flickinger, ed.). 29 pp.
29. Navoni JA, De Pietri D, Garcia S, Villaamil LE, (2012). "Health risk for the vulnerable population exposed to arsenic in the province of Buenos Aires, Argentina". Pan Am J Public Health 31:1
30. Navoni JA, De Pietri D, Olmos V, Gimenez C, Bovi Mitre G, De TittoE, Edda CVL. (2014). "Human health risk assessment with spatial analysis: a study of a population chronically exposed to arsenic through drinking water from Argentina". Sci Total Environ 499:166–174
31. Naz, T. et al. (2016). "Biosorption of heavy metals by Pseudomonas species isolated from sugar industry". Toxicology and Industrial Health, 32(9), pp. 1619–1627.
32. Rajaganesh, K., Sumedha, N.C and Ameer Basha, S. (2014). "Characterization of textile dye effluent from komarapalayam, Namakkal district, Tamil Nadu, India. Indian Streams". Research Journal. 4(1).
33. Rajbansi A, (2008). "Study on heavy metal resistant bacteria on Gusehwori sewage treatment plant". Our Nature 6: 52–57.
34. Silva RCA, Araújo TM, (2003b). "Groundwater quality in urban areas of Feira de Santana, State of Bahia". Ciência e SaúdeColetiva 9(4): 1019–1028.
35. Singh A., et al. (2010). "Health risk assessment of heavy metals via dietary intake of foodstuffs from the wastewater irrigated site of a dry tropical area of India". Food Chem Toxicol 48:611–619.
36. Tahri-Joutey N, Bahafid W, Sayel H, El Abed S and El Ghachtouli N, (2011). "Remediation of hexavalent chromium by consortia of indigenous bacteria from tannery waste-contaminated biotopes in Fez, Morocco". International Journal of Environmental (686) 901-912.
37. Vimala, R. & Das, N, (2009). "Biosorption of cadmium (II) and lead (II) from aqueous solutions using mushrooms: A comparative study". Journal of Hazardous Materials 168, 376–382
38. Volesky B. and Naja, G,. (2007). "Biosorption technology: Starting up an enterprise". Int. J. Technol Transf. Commer., 6: 196–211.
39. Wase, J., and Foster, C. (1997). "Bio sorbent for metal Ions". Taylor and Francis, Ltd, London.
40. Yang QW, Lan CY, Wang HB, Zhuang P, Shu WS. (2006). "Cadmium in soil–rice system and health risk associated with the use of untreated mining wastewater for irrigation in Lechang, China". Agric Water Manag 84:147–152
41. Zhang Y, Li Y, Li J, Sheng G, Zhang Y, Zheng X. (2012). "Enhanced Cr (VI) removal by using the mixture of pillared bentonite and zerovalent iron". ChemEng J 185–186:243–249
42. Zietz BP, Vergara JD, Dunkelberg H. (2003). "Copper concentrations in tap water and possible effects on infant's health—results of a study in lower Saxony, Germany". Environ Res 92(2):129– 138
43. Zukowska J, Biziuk M. (2008). "Methodological evaluation of a method for dietary heavy metal intake". J Food Sci 73:21–29.