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Bacteria isolated fungal strains from Mohan Nagar Ghaziabad industrial effluents' uptake of lead and cadmium and their possible application in wastewater

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Abstract

These days, one of the main contaminants is heavy metals. Although heavy metals are a necessary part of the atmosphere, their concentration has gone to a safer level as a result of disruptions to their natural geochemical cycles. Both natural and man-made processes introduce these metals into the ecosystem. It is possible for heavy metals that possess the characteristic of not being biodegradable or destroyable to go from a highly poisonous state to a less harmful state. It is now crucial to remove toxic metals from the environment in order to improve its health. These metals are removed using a variety of physiochemical techniques, but they are exceedingly expensive and inefficient. These techniques can be greatly replaced by bioremediation, which is less expensive and safe for the environment. This technique extracts the harmful pollution from the contaminated areas by means of bacteria, plants, or other living things. For this reason, mostly bacteria that consume these hazardous metals are chosen. In the current study, we isolated four strains that are resistant to heavy metals from Mohan Nagar Ghaziabad industrial effluent. Ten isolates were first examined at 50 ppm concentrations on plates containing various heavy metal salts, including as Cu^{+2}, Cr^{+6}, Cd^{+2}, and Ni^{+2}. Because of their excellent tolerance to heavy metals, isolates were selected. The findings demonstrate that all four isolates are resistant to the heavy metals found in industrial wastewater; as a result, these isolates are more useful in bioremediation.

Keywords: Ecosystem, bioremediation, industrial waste water, biodegradable

1. Introduction

The industrial and urbanization processes that have been accelerated and escalated over the past century have deteriorated our aquatic environment and contaminated it with many harmful chemicals. One of the most prevalent environmental issues is wastewater contaminated with heavy metals (HM). When HM are absorbed through the food chain, they have a propensity to accumulate inside the human body. Because organic pollutants are acutely poisonous and carcinogenic, water pollution by them is a severe problem. The global expansion of numerous industries, including mining, fertilizer, pesticide, and electric appliance manufacture, has led to a rise in the amount of organic and inorganic pollutants being dumped into the environment. This rise in disposal also pertains to the release of heavy metals (HMs) into the environment, whether directly or indirectly. Of all the contaminants found in water, heavy metals (HMs) have drawn the most attention because of their toxicity and extended half-life, which prevents them from breaking down naturally in the environment.

Numerous industrial processes, such as those related to mining, electroplating, fertilizer industries, tanneries, paper manufacturing, batteries, etc., produce a surge in the amount of effluents with high concentrations of heavy metals (HMs). The primary sources of heavy metals (HMs) include wastewater discharged from various businesses, including metal plating, alloy manufacture, Cd-Ni battery manufacturing, and hospitals. Because heavy metals (HMs) are hazardous, it is imperative to both eliminate and regulate their negative effects when dealing with ground water contamination. Moreover, the usage of fertilizers, pesticides, car exhaust, leftovers from smelting companies, metalliferous mines, and municipal compost or sludge all contribute to the pollution of a sizable geographical area with these metals. As a result, through the "Plants - Animal - Human" pathway, humans are thought to be the group most exposed to these hazardous metals, either directly or indirectly, through the consumption of food and water polluted with metal. Lead and cadmium are two of the heavy metals (HMs) that are not physiologically significant since they are not involved in biological functions yet are poisonous in trace amounts when exposed. Because HMs are poisonous by nature and cannot be biodegraded, it is difficult to remove them from biological tissues that have been contaminated. This raises serious concerns about the health of all people. According to reports, HMs like lead, cadmium, nickel, zinc, and copper are introduced into the environment through the media of air, food, soil, and water.

Because of their high toxicity, even at low concentrations, pollutants containing heavy metals (HMs) are particularly significant among the numerous contaminants that damage water resources. Since there is no accepted definition for heavy metals (HMs), they can be characterized according to their density. In terms of ecology, non-biodegradable metals that cause pollution in the environment and associated metalloids may be classified as HMs. HMs are metallic elements with high density and somewhat greater atomic weight and atomic number. More than 20 atoms make up HMs (Jiang *et al*., 2017) [1]. They are classified as having a "atomic density higher than 4 g/cm (5 times higher than water)," which puts them in the category of elements with metallic characteristics and metals. The environment and humans are harmed by the poisonous form of metals and their increased concentration. In ground water, industrial effluent, and marine water, the most common heavy metals (HMs) and metalloids are "cadmium (Cd), arsenic (As), chromium (Cr), mercury (Hg), lead (Pb), and nickel (Ni)" among others. HMs are found in the soil and are a natural part of the earth's crust. All life on Earth requires trace amounts of heavy metals. On the other hand, at high concentrations, they are regarded as deadly and toxic to humans, animals, and plants. Humans who are exposed to HMs suffer neurological and physiological harm. Toxic metals (Pb, Hg, Ni, Cr, Zn, Cu, As, Cd, Sn, and Co), precious metals (Ag, Pt, Ru, Au, and Pd), and radionuclides (Th, U, Am, and Ra) are the three categories of heavy metals (Thatheyus and Ramya, 2016) [2]. According to Ramya and Thatheyus (2018) [3], "among these HMs, lead, chromium, mercury, uranium, selenium, zinc, arsenic, cadmium, silver, gold, nickel, and copper may reach up to the toxic level." Three direct pathways allow these metals to reach the environment:

1. The buildup of airborne particles;
2. The discharge of sewage effluents that are "enriched in metal and metalloid."
3. The industries that process products through metal mining and other processes.

The amount of wastewater containing hazardous metals (like lead) is released into water supplies at a higher rate when the number of companies increases. According to the ATSDR's Priority List of Hazardous Substances, lead (Pb) is the second most dangerous metal and a "probable human carcinogen." Anthropogenic and natural sources are major factors in environmental exposure. This element is frequently employed in a variety of industrial processes, including the manufacturing of leaded gasoline, hunting items, electroplating, and lead smelting. The primary causes of lead contamination include mining and smelting, leaded gasoline, welding metal with lead paint, the paper and pulp industries, batteries, and explosives. Lead paint in residential buildings is the main cause of lead pollution. Despite the USEPA's 1978 ban on the use of lead-based paints throughout the nation, lead-based paint is still present in over 21 million households today. Pb is responsible for serious ecological and human health issues, according to a European Commission assessment from 2002. According to Povedano - Priego *et al*. (2017) [5], anthropogenic activities have caused substantial annual deposition rates of metallic Pb in the environment in several western countries, including the USA, Canada, England, India, and others. Because of its extreme toxicity and strong environmental persistence, lead is one of the most widely distributed inorganic contaminants. Due to its detrimental effects on human health, plant growth, productivity, and wide circulation in ecosystems, lead (Pb) contamination in soil, water, and agricultural areas has become a global scientific concern. According to Gowd *et al*. (2010) [6], Pb concentrations in Indian soils range from 10.1 to 67.8 mg/kg, with an average of 38.3 mg/kg close to industrial areas. These concentrations could have a negative impact on crop output and human health.

2. Significance of the study

Byproducts from industrial processes are frequently produced that are hazardous to the majority of earth's life forms, hard to break down, and detrimental to the environment. Heavy metals are harmful substances that are typically found in industrial effluents and need to be managed in an environmentally responsible way. The traditional methods of treating metal-contaminated water-precipitation, ion exchange, electrochemical, and reverse osmosis-are expensive and have a high potential for producing secondary contaminants. We highlight the benefit of bacterial bioremediation in this review study as a solution for metal toxicity. An affordable and environmentally beneficial solution for treating industrial effluent contaminated with metals is bacterial bioremediation. One of the key microbial systems that can be used for bioremediation is thought to be bacteria. By using redox reactions and enzyme-transformation techniques, bacteria use the mechanisms of biosorption and bioaccumulation-ATP-dependent substrate-specific sequestration-to remove pollutants. Potential chemisorption sites for binding and moving metal ions to the bacterial cytoplasm are provided by the bacterial cell wall. By implementing techniques, creating a conducive environment, and employing stress-adapted strains with high metabolic activity, metal selectivity, a membrane transport system, effective biocatalysis, and the capacity to produce chaperones, the potential of bacterial bioremediation can be increased. It is anticipated that the efficacy of bacterial bioremediation will increase significantly with the use of genetic modification techniques such as plasmid exchange, mutation, and transposons. This research could provide fresh insights into the intricate mechanisms and tactics that bacteria have developed for the bioremediation of hazardous heavy metal ions and pave the way for more effective management of metal toxicity.

3. Review of Literature

In order to remove heavy metals (HMs) Cr (VI) and Zn (II) from contaminated water, do Socorro Vale (2021) investigated the "biosorption potential of fungal species A. Niger (dead biosmass) and adsorption equilibrium and kinetic studies determined the adsorption potential of the fungal species." Both the pseudo-first- and pseudo-second-order models were followed by the biosorption process. Moreover, Freundlich and Langmuir models fit the equilibrium. both the Freundlich and Langmuir models were followed for adsorbate concentrations less than 50 mg/L; however, the Freundlich model was the only one that fit the data. Cr (VI) and Zn (II) had respective adsorption potentials of 4.997 mg/L and 3.833 mg/L.

Mondal *et al*. (2021) [7] found that "the response surface methodology (RSM) based on four factorial Box–Behnken experimental designs was used to complete statistical optimization and validate the effect of special parameters like temperature, pH, adsorbent dose, contact time, initial concentration, shaking speed and also the effect of collective interaction of all the parameters throughout the adsorption process" for the "elimination of chromium ions." The values of 1.0 g/L, 33.33 mg/L, 48.45 min, and 4.6 for the adsorbent dose, beginning Cr (VI) concentration, contact time, and pH were discovered to be the standards. The findings verified that at initial pH 2.0, A. niger has a maximum adsorption potential of 11.792 mg/g. Data in equilibrium suit the Temkin and Freundlich isotherms quite well. Cr (VI) biosorption proceeded according to pseudo-second order rate kinetics. An estimate of 103 kJ/mol was made for the activation energy of the adsorption. Studies on thermodynamics verify that the reactions were both possible and naturally occurring. In order to assess the "biosorption ability of A. flavus and A. niger dead biomass, at 20, 30 and 40 ºC temperature, pH 5, 6 and 7 and initial metal concentration of 300, 600 and 900 ppm," Azhar and Iram (2020) [8] carried out batch investigations. At 40 degrees Celsius, it was discovered that A. flavus had adsorbed 257 mg/g of Cr (III) with 85.6% biosorption. The Langmuir adsorption isotherm and pseudo-second-order kinetic model with regression coefficient values > 0.982 were used in the equilibrium and kinetics investigations. The biosorption of Cr (III) on A. flavus was proven by thermodynamic parameters to be a viable, spontaneous, and endothermic process. The industrial revolution is currently altering the natural flow of resources and adding new chemicals to the environment. The amounts of dangerous compounds in surface water, soil, and sediments have risen above average as a result of industrial activity. Hazardous material buildup in food chains can seriously disrupt bioprocesses, weakening the ecosystem (Mistry *et al*., 2010) [9]. Numerous industries release hazardous heavy metal-containing wastewater into the environment (Chandra *et al*., 2004) [10].

4. Objectives of the study

1. Soil sample and industrial effluent physical-chemical characterization.
2. Isolation, screening, and characterization of fungal strains resistant to Pb^{2+} and Cd^{2+}

5. Materials and Methods

The current study examines the use of Aspergillus Niger and Penicillium chrysogenum dead fungal biomass as an affordable biosorbent. Response Surface Methodology has been used to optimize conditions for the removal of Pb^{2+} and Cd^{2+} from aqueous solution.

Samples of soil and effluent were taken from Mohan Nagar Ghaziabad, India. The soil samples were taken at random locations near the ETP, wrapped in plastic bags, and kept cold (4 °C). Every safety measure was done both before and after the collecting. Samples of the electroplating industry's effluent were taken from the ETP's outflow, which is situated on the company's property. The gathered samples were taken to a lab where they underwent many physico-chemical analyses. Soil samples were air dried, ground into a powder, and sieved through a 2 mm sieve before to examination. The Atomic Absorption Spectrophotometer (AAS) was utilized to ascertain the HM concentration in wastewater samples.

In order to investigate the potential industrial applications of Aspergillus Niger and Penicillium chrysogenum, batch mode experiments were conducted under optimum circumstances derived from the removal of Pb^{2+} and Cd^{2+} ions from electroplating effluent using synthetic solutions. The potential of both fungal strains to biosorb Pb^{2+} and Cd^{2+} ions was examined by electroplating raw industrial effluent samples and dilutions of the effluent supplemented with the minimal nutrients required for the growth of fungal strains. The effluent was diluted three times: 25%, 50%, and 75%. Both fungal biosorbents were set up in simultaneous batches. In every instance, the flasks were incubated for three hours at a constant temperature of 30 ºC and 120 rpm on an orbital shaker following the addition of the necessary biomass. Samples were taken from each experiment flask at predetermined intervals following incubation. After the flask's contents were filtered, samples of hydrochloric and nitric acids were used to put the filtrate to acid digestion. After cooling, it was diluted with distilled water to a final volume of 50 mL. Using an atomic absorption spectrophotometer, an aliquot of this solution was utilized to measure the Pb2+ and Cd2+ ions. Using SPSS version 24, the data were correlated, given to one way analysis of variance (ANOVA), and treated to a post-hoc Tukey test.

6. Results and data interpretation

In this study, native fungal strains resistant to lead and cadmium are isolated and identified in order to determine their potential to extract harmful Pb2+ and Cd2+ ions from aqueous solutions. Aspergillus Niger and Penicillium chrysogenum, two fungal strains resistant to lead and cadmium, were identified from the electroplating industry's effluent. The mechanism of the sorption process, as well as all the circumstances or elements impacting it, were examined in order to obtain insight into biosorption technology. Response surface methods as well as traditional one factor at a time were used to optimize the following parameters: pH, temperature, initial concentration of Pb2+ and Cd2+ ions, biomass dosage, and contact time. Thermodynamics, isothermal dynamics, and kinetics were examined. Finally, to evaluate the practical applicability of the biosorption process by both fungal strains, the effectiveness of the isolated strains was also investigated in industrial effluent.

Table 1: Physico-chemical characteristics of the effluent obtained from electroplating industry

S. No.	Parameter	Concentration (mg/L)**	Permissible limits (EPA, 1986)*
1.	pH	2.2 ± 0.02	5.5-9.0
2.	EC (mS/m)	24,750 ± 0.01	250
3.	Color	light green	
4.	Potassium	206.4 ± 0.01	12
5.	Sodium	88,326 ± 0.02	200
6.	Alkalinity	3,430 ± 0.01	200
7.	Chloride	36,388 ± 0.03	250
8.	Sulphate	992.6 ± 0.1	400
9.	Phosphate	4.92 ± 0.01	5
10.	Total hardness	2,997 ± 0.02	200-500
11.	TDS	2,228 ± 0.02	2000
12.	TSS	2,597 ± 0.01	600
13.	BOD	205 ± 0.1	30
14.	COD	1,359 ± 0.4	250
15.	DO	18 ± 0.05	14
16.	Oil and grease	20 ± 0.04	10
17.	Chromium	2.7 ± 0.1	2.0
18.	Zinc	4.6 ± 0.01	5.0
19.	Iron	3.31 ± 0.01	
20.	Nickel	0.8 ± 0.01	3.0
21.	Lead	13.8 ± 0.01	0.1
22.	Cadmium	4.7 ± 0.1	2.0

Table 1 discusses the physico-chemical characteristics of industrial effluent electroplating. Because color has an impact on water clarity, it is a crucial element in the evaluation of water quality. The effluent sample from the electroplating industry was discovered to be dark green in color during the current research. Kumar *et al*. (2011) [11] stated that the electroplating effluent was green in hue as well. The pH scale uses the concentration of hydrogen ions in water to determine how acidic or alkaline the solution is. The value is given by pH = -log [H+], which is the negative logarithm of the hydrogen ion concentration. The pH scale ranges from 0 to 7 for acidic solutions and from 7 to 14 for alkaline solutions, with 7 being the neutral pH. Acids, alkalies, heavy metals, and other hazardous materials are commonly found in industrial effluents.

Table 2: Physico-chemical characteristics of soil samples obtained from electroplating industry

S. No.	Parameter	Concentration (mg/L)*
1.	pH	8.1±0.1
2.	EC (mS/m)	2760±0.01
3.	Color	Light blackish brown
4.	Potassium	10.9±0.1
5.	Sodium	138±0.01
6.	Alkalinity	68 ± 0.02
7.	Chloride	14.41± 0.01
8.	Sulphate	63 ± 0.01
9.	Phosphate	43 ± 0.02
10.	Moisture content (%)	55 ± 0.01
11.	Organic carbon (%)	35 ± 0.02
12.	Calcium	150 ± 0.01
13.	Magnesium	50 ± 0.02
14.	Chromium	5.3 ± 0.01
15.	Zinc	4.8 ± 0.1
16.	Nickel	4.5 ± 0.01
17.	Lead	9.2 ± 0.02
18.	Cadmium	8.7 ± 0.1

In order to isolate fungal strains, the collected electroplating effluent sample was processed using the spread plate and serial dilution methods. Following three days of incubation, colonies of fungal growth were found. These colonies were streaked on various potato dextrose agar plates in isolation.

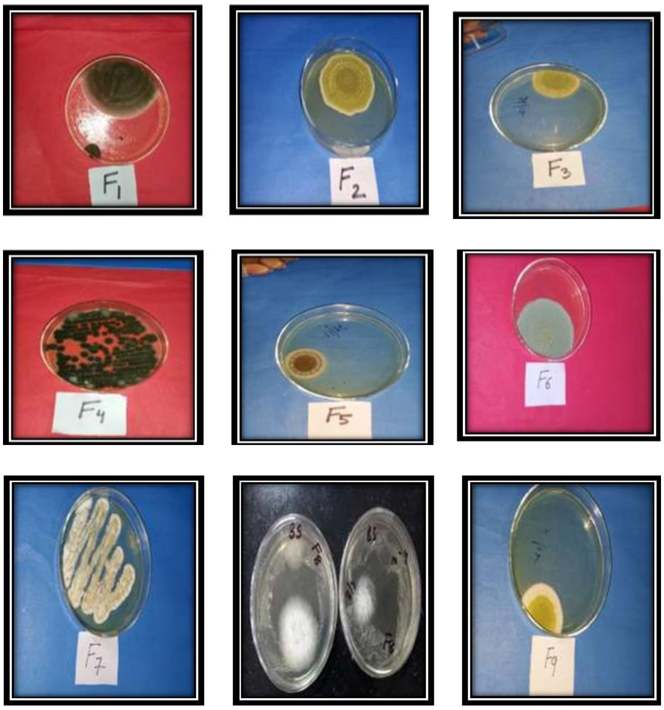

Fig 1: Purified fungal isolates on potato dextrose agar medium

Table 3: Analysis of variance for pb^{2+} ions removal using *Penicillium chrysogenum*

	Sum of Squares	df	Mean Square	F	p-value
Between Groups	8557.238	3	2852.413	28.082	< 0.0001
Within Groups	8126.000	80	101.575		
Total	16683.238	83			

A significance level of α=0.05 was used for the removal of Pb^{2+} ions from electroplating effluent using Penicillium chrysogenum. According to Pearson correlation, there is a substantial positive and strong linear association (r=0.60, $p<0.0001$) between the amount of lead (mg/L) in effluent and the passage of time. Lead concentration in effluent was shown to grow with time, and this increase was rapid due to a tight association. A one-way ANOVA was used to determine whether the average lead content in the effluent varied for different effluent dilutions. The results showed a significant difference in the lead concentration (mg/L) in the effluent, with a $F(3, 80) = 28.08$, $p<0.0001$.

Table 4: Analysis of variance for cd^{2+} ions removal using *Penicillium chrysogenum*

	Sum of Squares	df	Mean Square	F	p-value
Between Groups	9696.667	3	3232.222	27.432	< 0.0001
Within Groups	9426.000	80	117.825		
Total	19122.667	83			

A significance level of α=0.05 was used for the removal of Cd^{2+} ions from electroplating effluent using Penicillium chrysogenum. Pearson correlation analysis revealed a substantial positive and strong linear association (r=0.60, *p*<0.0001) between time and effluent's cadmium concentration (mg/L). Cadmium concentration in effluent increased with time, and because of the close link, this increase happened quickly. A one-way ANOVA was used to determine whether the average cadmium concentration in the effluent varied for different effluent dilutions. The results showed a significant difference in the cadmium content (mg/L) in the effluent, with F (3, 80) =27.43, $p<0.0001$.

7. Conclusion

Because heavy metal contamination is dangerous even at low concentrations, it has become a global issue. Heavy metal poisoning of soil can suppress or even kill certain members of the microbial population. It is frequently irreversible. Most people believe that the development of a tolerant microbial community results from exposure to heavy metals in industrial wastewater. Pb^{2+} and Cd^{2+} ion releases caused by humans are a major global concern. The development of biological treatment methods for lead and cadmium-contaminated wastewater from the mining, tannery, manufacturing, and electroplating industries is urgently needed. One of the more promising techniques for removing Pb^{2+} and Cd^{2+} ions is biosorption. The current work demonstrated the viability of this procedure utilizing isolated fungal strains from settings exposed to lead and cadmium. Fungal strains that are resistant to lead and cadmium were successfully isolated from local surroundings of the electroplating industry for this study. This study sheds light on the ideal physical conditions needed for Aspergillus Niger and Penicillium chrysogenum, two fungal biosorbents, to effectively biosorb Pb^{2+} and Cd^{2+} ions from an aqueous solution. Because there is minimal regulatory control over industrial pollutants, industrial workplaces offer a unique enrichment environment for the natural selection of strong fungal strains that are resistant to metals.

The research encompasses the refinement of parameters necessary for the sorption procedure, as well as the examination and verification of equilibrium data concerning kinetics, isothermal, and thermodynamic investigations. Aspergillus Niger and Penicillium chrysogenum are used in the improved Pb^{2+} and Cd^{2+} ions biosorption process, which has been validated by the RSM study results.

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