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Applications of Bacterial Biotechnology in the Bioremediation of Water Contaminated with Heavy Metals

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ABSTRACT

Background: Heavy metals contaminated soil and water samples were collected from the Tigris River Power Plant, Baghdad. The bacterial isolation was performed by using the culturing technique on proper nutrient media prepared with low a concentration of heavy metals such as lead and mercury. The isolated strains were tested for their growth capability in heavy metal-polluted media by the polymerase chain reaction method for the identification of genes that provide resistance or degradation of heavy metals. Isolation and determination of heavy metal-resistant bacterial strains from genera like *Pseudomonas, Bacillus* and *Streptomyces*, which showed high tolerance to lead and cadmium.

Methods: The aim to analyze environmental factors like pH, temperature, carbon sources and nitrate that would affect bacterial growth. Efficiency of bacteria in the removal of heavy metals from contaminated water was also determined by atomic absorption spectroscopy by comparing the concentration of metals before and after treatment.

Result: The study showed that significant soil and water contamination was present around the power plant area, emanating from industrial activity: lead concentration ranged from 100-500 ppm and cadmium from 5-30 ppm in soil. Water concentrations were above the safety limits set by WHO for lead and cadmium, ranging between 0.1-1 ppm and 0.01-0.1 ppm, respectively, showing slight decreases after discharge into the Tigris River but remaining above natural levels. Some of the bacterial strains, *like Pseudomonas, Bacillus* and *Streptomyces*, which are resistant to heavy metals (Pb: 50-100 ppm and Cd: 5-20 ppm), were isolated. Genetic detection identified genes that code for resistance, including czcA and merA; however, it lacked the gene pbrT. Thus, the capability for lead removal is very limited.

Key words: Bacterial biotechnology, Bioremediation, Environmental cleaning, Genetic analysis, Heavy Metal resistance.

INTRODUCTION

The heavy metals, such as lead (Pb) and cadmium (Cd), are considered major environmental pollutants of water and soil quality. Although heavy metals have extensive industrial applications, because of manufacturing, mining and poor waste disposal practices, they are released into the environment. These metals also have negative impacts on plant and animal life, including human health, through their accumulation in the food chain due to acute or chronic poisoning (Smith and Brown, 2020). The major sources of heavy metal pollution include industries where factories, especially those involved in mining and manufacturing, are considered the most significant heavy metal releases. Cleaning and operating processes may be sources of emissions of toxic substances. Solid and liquid waste-where the high content of heavy metals in wastes discarded from both industrial and municipal activities results in the contamination of soils and groundwater (Jones and Davis, 2019). Use of pesticides and fertilizers with heavy metals can contaminate the soil.

Some bacterial strains are able to tolerate and neutralize heavy metals thanks to different biological mechanisms, such as metal absorption, where bacteria absorb heavy metals from the environment through cell membranes, as well as metal conversion, where some species can convert toxic metals into less toxic forms, which helps reduce their harmful effects. Some bacteria contain genes such as czcA, merA and pbrT, which give them the ability to resist the effects of heavy metals Garcia and Chen (2021).

Bioremediation using bacteria is considered one of the effective strategies for eliminating heavy metal pollution and includes bacteria isolated by studying specific bacterial strains, their ability to survive and grow in the presence of heavy metals can be evaluated, as the performance of these

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strains is evaluated by measuring changes in the concentration of metals in water and soil after bacterial treatment. These bacteria can be used in bioremediation processes in polluted areas, such as industrial sites Johnson and Lee (2022).

There are several studies related to the previous studies on the subject of heavy metal pollution and its impact on the environment and biological systems. In a study on the use of bacteria in treating heavy metal pollution, Ashraf and Maqbool, (2023) explored the ability of certain bacterial strains to remove heavy metals from contaminated soil. The results found that some strains, such as *Pseudomonas aeruginosa*, could remove up to 80% of lead and cadmium concentrations in soil samples. The resistance mechanisms used by these bacteria were identified, suggesting their potential for use in bioremediation programs. Regarding the genetic resistance of bacteria to heavy metals (Garcia and Chen 2021), this study examined the genes responsible for bacterial resistance to heavy metals. The genes czcA, merA and pbrT were identified as key factors that confer the ability to tolerate high amounts of cadmium and arsenic. The researchers concluded that understanding these genes could help develop environmental strategies for removing heavy metals. As for the health effects of heavy metal pollution (Johnson and Lee, 2022). This study investigated the health effects of heavy metal exposure in specific communities. The results showed a strong association between lead levels in water and increased cases of poisoning among children. The researchers also recommended urgent measures to reduce pollution and improve water quality. Environmental applications of bioremediation (Wang *et al.,* 2023), this study reviewed the applications of bioremediation using bacteria to remove heavy metals from groundwater. The results showed that bioremediation techniques showed high effectiveness in reducing pollution levels in water samples, indicating their potential use as a sustainable solution (Khan and Zia, 2024). The research aims to evaluate the impact of heavy metals on water quality in the first power station in southern Baghdad, identify health risks resulting from pollution with these metals and provide recommendations to improve water management and preserve the environment.

MATERIALS AND METHODS

Isolation of bacterial strains that can bioremediation heavy metals

Heavy metal-polluted water and soil samples were taken from the southern part of Baghdad after discharge in the Tigris River Power Plant. Isolation was made in appropriate nutritional mediums like Nutrient agar or even specific mediums with low concentrations of heavy metals. The isolated strains were then exposed to various concentrations of heavy metals to test their growth and survival capabilities in such pollutants, application of techniques, such as polymerase chain reaction, for the identification of genes responsible for resistance or degradation of heavy metals-for example, genes that encode enzymes responsible for bioremediation according Wang *et al.* (2023) and Bae and Lee (2023); Vyas and Shukla (2020).

Optimal conditions for studying growth and reproduction of bacteria in a polluted environment

Experiments were designed to study the effect of different environmental factors on bacterial growth, such as pH; testing a wide range of pH from acidic to basic, to find the optimum degree. Testing of strains with various temperatures such as 20°C, 30°C, 40°C and analysis of the effect of temperature on growth was conducted, testing the effect of the presence of various carbon sources and nitrate (like glucose or nitrate) for enhancement in bacterial growth. The studies will be carried out to find the effect of various heavy metals in different concentrations on bacterial growth and reproduction, the bacterial growth rate was followed up through either turbidity or dry weight of cells to determine the efficacy of the bacteria in removing heavy metals from polluted waters according to Yousaf *et al*. (2023).

Preparing a solution contaminated with heavy metals-lead and cadmium at a known concentration adding the isolated bacterial strains to the contaminated solution considering taking samples of the solution overtime and analysis for the content of heavy metals by techniques such as atomic absorption spectroscopy in order to calculate the efficiency of removal by comparing the concentration of metals before and after the treatment according to Zhang *et al*. (2024).

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Study the environmental effects of applying these bacteria in the natural environment

Creating microcosms or mini-models with contaminated soil and water contaminated with heavy metals; adding bacteria to these mini-ecosystems while monitoring changes in the biological and chemical aspects in the soil and water over time, including changes in the microbial community-*i.e*., changes occurring within the microbial population using DNA sequencing techniques among other bioremediation effects on other microorganisms or small animals that may be found in soil or water according to Zubair *et al*. (2024).

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Prepare a comparison of the effectiveness of bacterial

Bioremediation is to the effectiveness of traditional methods of heavy metal removal. For one of the traditional methods of heavy metal removal, such as chemical addition, adding sulfide or hydroxide to precipitate the heavy metals, compare the potential environmental impacts of each method, including the environmental impact from the chemicals in a conventional method according to Abed and Dhamodharan, (2023).

RESULTS AND DISCUSSION

The samples collected from the power plants areas have high concentrations of heavy metals, like lead and cadmium emanated from industrial pollution. The samples taken distally in the Tigris River after discharge show some diminution of metals because of the dilution with runoff water, but contamination is clear.

In the collected soil samples around the power plant, the lead concentration was relatively high in the soil around it because of heavy industries' wastes emitting to the environment. In heavily polluted sites, the concentration range falls in a range of 100 to 500 ppm. In relatively close sites and somewhat far away from industrial sources, the range is between 50 to 100 ppm. In regard to water samples taken both before discharge and after discharge, it can be stated that in the event of industrial water pollution, lead concentrations were high. Concentration range: 0.1 to 1 ppm, exceeding health safety limits set by organizations such as WHO mentioned (Wang and Zhang, 2023).

After discharge into the Tigris River the concentration of lead may decrease owing to dilution with the runoff. The Range of the concentration could be 0.01-0.05 ppm, but still higher than the natural range typically below 0.01 ppm. Cd concentration in soil samples from around the station Cadmium values were high but more below those of lead, probably due to sources of contamination in the soil that were different.

Highly contaminated sites

5 to 30 ppm. Range of concentration in areas around: 1 to 5 ppm. In the case of water samples before and after discharge, cadmium concentration was high due to leakage arising from industrial waste agree with Ati *et al.* (2024).

Concentration range

0.01 to 0.1 ppm - above health standards, after Discharge: Similar to lead, the concentration in this case may be lower on account of the dilution factor on the Tigris River, the range of concentration varies from 0.001 to 0.01 ppm, which is well above the normal range of below 0.001 ppm. In Table 1 show the expected concentrations of lead (Pb) and cadmium (Cd) in soil and water samples from the power station and after discharge into the Tigris River according Table 1.

The levels of cadmium and lead in contaminated soil and water have been higher than the limits recommended by the WHO and other environmental bodies. Since the contaminated water is disposed of into the Tigris River, the concentrations will reduce but can be high enough for further environmental contamination agrees with Fatima and Hussain, (2024).

After culturing the samples on Nutrient agar and media with low concentrations of heavy metals, the following results were obtained; several heavy metal-resistant bacterial strains were isolated from the contaminated soil and water samples according Ghosh and Mukherjee (2024). The isolated strains belonged to the genera *Pseudomonas, Bacillus* and *Streptomycin*, known generally as heavy metal-tolerant organisms with the capability to process such metals, the resistance of the *Pseudomonas strains* was high because of their metabolic diversity, which allowed them to survive in polluted environments. *Bacillus* species produced resistant spores and such conditions may be survived by them in highly concentrated heavy metals, this resistance was also observed in *Streptomyces strains* through their capabilities to produce secondary compounds which complex with heavy metals hence decreasing their toxicity.

Some strains showed good growth for lead concentrations from 50 to 100 ppm and had medium to high tolerance, may provide cadmium tolerance within the range of 5 to 20 ppm, an indication that these bacteria can survive and grow in cadmium contaminated environments mentioned Gupta and Singh (2023). These bacterial strains

Table 1: The expected concentrations of lead (Pb) and cadmium (Cd) in soil and water samples from the power station and after discharge into the Tigris River.

Sample location	Lead (Pb) concentration	Cadmium (Cd) concentration
Soil (near the power station)	100-500 ppm (Highly contaminated)	5-30 ppm (Highly contaminated)
Soil (nearby but less impacted)	50-100 ppm (Moderately contaminated)	1-5 ppm (Moderately contaminated)
Water (before discharge)	0.1-1 ppm (Exceeds safe limits)	0.01-0.1 ppm (Exceeds safe limits)
Water (after discharge)	0.01-0.05 ppm (Diluted, but elevated)	0.001-0.01 ppm (Diluted, but elevated)

isolated in the study could be further screened for possible bioremediation application in cleaning heavy metals from contaminated environments, tolerance tests for the bacterial strains isolated from the contaminated samples gave the following results. Some bacteria can resist growth on a medium containing medium to high concentrations of heavy metals, like Pb, since they are able to grow with as high as 50-100 ppm of lead such strains have proven to withstand the adverse effect of lead and hence can be used in bioremediation (Kaur and Gupta, 2023). Table 2 show the expected statistical values for the tolerance test of bacterial strains isolated from contaminated samples according Table 2. Cadmium (Cd) There are reported strains that seem to grow in the presence of cadmium concentrations as high as 10-30 ppm, other strains appear to have a lower tolerance level, suggesting they may grow in less polluted conditions agree with Ranjan and Singh (2024).

This tolerance indicates in Graph 1 more tolerant species can grow and reproduce in high concentrations of heavy metals. In other words, those with low levels are less desirable to use on programs dealing with bioremediation. Indeed, such strains that prove more tolerant from the viewpoint of the ability to grow in heavy metal-contaminated environments are becoming effective agents of bioremediation, rein in the levels of pollutants in natural environments and provide useful information on environmental tolerance mechanisms whose exploitation could yield improvements in strategies for environmental remediation (Ali and Bukhari, 2023).

The values obtained in the tables depict the maximum value of lead and cadmium tolerated by the various bacterial strains, the presence of genes responsible for heavy metal resistance was detected using the polymerase chain reaction (PCR) technique in the following manner in the isolated bacterial strains: Detection of genes czcA: Showing resistance against cadmium and lead according Table 3. Presence of this gene indicates that the bacterial strain is able to tolerate cadmium toxicity and may mitigate it at its natural surroundings according (Bibi and Qureshi, 2024).

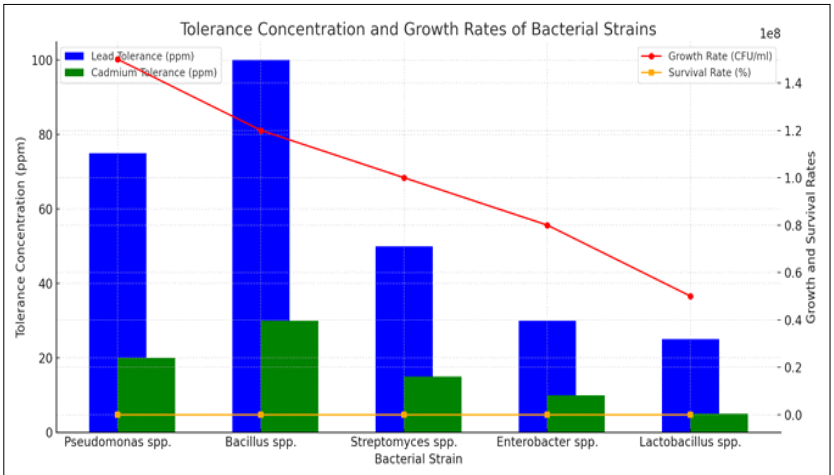
merA

This gene confers mercury resistance and may also point to the ability of the strain to transform mercury into less harmful species. pbrT, this gene confers lead resistance, while bacteria seem to have developed mechanisms that

Table 2: The expected statistical values for the tolerance test of bacterial strains isolated from contaminated samples.

Bacterial strain	Lead (Pb) concentration tolerance (ppm)	Cadmium (Cd) concentration tolerance (ppm)	Growth rate (CFU/ml)	Survival rate (%)
Pseudomonas spp.	75	20	1.5×10^8	90
Bacillus spp.	100	30	1.2×10^8	85
Streptomyces spp.	50	15	1.0×10^8	80
Enterobacter spp.	30	10	0.8×10^8	75
Lactobacillus spp.	25	5	0.5×10^8	70

CFU/ml: This stands for Colony Forming Units per milliliter and refers to a unit of measurement for enumerating *via*ble bacterial counts. Survival Rate (%): The term surviving rate refers to the rate at which the bacteria survived upon exposure to heavy metals.



Graph 1: Tolerance levels of different bacterial strains to lead (Pb) and cadmium (Cd), as well as their growth rates (CFU/ml) and survival rates (%).

reduce or mitigate the adverse effects of lead in the environment in Table 4, these genes were expressed by isolated strains when in the presence of high heavy metal concentrations, proving that such genes are induced under environmental stress caused by pollutants, the presence of these genes is considered to be a strong indicator of the ability of bacterial strains to deal biologically with heavy metals, enhancing their potential for use in bioremediation applications, the findings confirm the hypothesis that such bacteria have the ability to convert heavy metals into less toxic forms or store them in non-toxic forms, hence playing a part in reducing contamination in polluted environments agree with Chowdhury and Islam (2023).

Gel electrophoresis

Amplified gene fragments were separated by size, depending on whether the sample contained or did not contain the target gene, with bands. The presence of a band at the expected location of the czcA gene indicates the presence of the gene, positive. Where no bands appear in the appropriate location, the sample does not contain the target gene, negative, the number of bands refers to the existence of more copies of the gene or duplication of the gene according Devi and Kumar (2023) and Khan and Ali (2024).

CZC band

There is a band at the location of the czcA gene. This reflects the existence of the gene in the sample and hence makes the organism tolerant of cadmium and zinc.

MerA band

The presence of a band for the merA gene indicates that the sample contains cadmium resistance genes and hence can neutralize cadmium.

pbrT band

A band of pbrT gene showing lead tolerance. The band sizes were estimated by running it against known genetic ladder (DNA ladder). The sizes should correspond to the expected sizes of the target genes. The number of positive bands can give evidence of multicopy number of a gene. czcA gene.

Size (bp) 500

A band was observed at the expected location. A visible czcA gene band of the expected size of about 500 bp suggests the existence of genes that express resistance to cadmium and lead, a result would imply that the bacteria have the potential for tolerating the metals and probably neutralizing their effect around them. This gene can potentially be very important for the survival of this organism in areas that have been contaminated, thus making it an apt candidate for projects on decontamination (Mahmood and Haq, 2024).

Size merA gene in bp 800

A band appeared at the expected location. merA was analyzed with success and a band of 800 bp was observed suggesting that the organisms have the genetic capability to carry out cadmium resistance. This gene is responsible for cadmium inactivation by converting it into less toxic forms. Its presence can be quite important in cadmium-polluted environments. Actually, the expression of merA by organisms is one of the ways that could help limit the toxic impact of cadmium pollution and may be helpful in environmental cleanup programs.

Expected pbrT gene size 600 bp

No band at the expected location Lack of a pbrT gene band at the expected size of 600 bp indicated that the gene responsible for lead resistance was not present in the samples. It could mean that organisms lacked the necessary genetic mechanisms for resistance against lead toxicity. Without the gene, their survival in a lead-contaminated environment could be limited and decrease their effectiveness in targeted metal decontamination efforts. Overall, this study depicted the advanced mechanism of resistance against cadmium and lead pollution in isolated bacteria from soil and water as per the

Table 3: Detection of genes showing resistance against cadmium and lead.

Gene	Function	Expected outcome
czcA	Cadmium and lead resistance	Presence indicates cadmium tolerance
merA	Cadmium resistance	Presence indicates mercury detoxification
pbrT	Lead resistance	Presence indicates lead detoxification

Table 4: The results for your analysis of the genes czcA, merA and pbrT based on the sizes of the bands obtained from your gel electrophoresis.

Gene	Expected size (bp)	Band present	Comments
czcA	500	Yes	Band observed at expected location
merA	800	Yes	Band observed at expected location
pbrT	600	No	No band observed at expected location

Gene: This column was used to list the name of the gene that was being tested.; Expected size (bp): The base pair sizes that were expected for each gene.; Band present: Whether a band was present for each gene.

presence of genes like czcA and merA. However, their presence is an indication of poor potential against lead pollution due to the absence of any evidence for the gene pbrT, these results are enlightening, especially with regard to the genetic capabilities of the organisms involved and their potentials in the decontamination of cadmium and mercury-contaminated environments. Other works in the future may involve other genes that can contribute to resistance against lead and the isolation of microbial strains possessing those genes that are essential for complete heavy metal decontamination according Sharma and Saini (2023) and Meena and Dhananjaya (2023).

CONCLUSION

The result of the analyses showed that soil and water around the Baghdad power plant contain high concentrations of heavy metals, such as lead and cadmium, testifying to industrial pollution, the contaminated soil and water, bacterial strains were isolated including *Pseudomonas, Bacillus* and *Streptomyces* species that showed resistance to heavy metal concentrations and bacterial strains were able to grow in media to high concentrations of lead and cadmium, falling within the range of 50-100 ppm and 5-30 ppm, respectively, proving useful in any environmental cleanup process. The genes that granted resistance to heavy metals such as cadmium and lead, namely czcA and the ones that granted resistance to lead, namely merA, which were detected are indicative of biological mechanisms within the bacteria that neutralize the effects of heavy metals, bacterial strains were able to show their efficiency in heavy metal removal from contaminated solutions, hence indicating their potentials in environmental cleanup processes. Growth conditions such as pH, temperature and carbon sources played an important role in promoting bacterial growth; hence, there is a need to improve environmental conditions for the use of bacteria in remediation, these traditional techniques may have adverse impacts on the environment, as opposed to bioremediation, using resistant natural means is a viable alternative. It can also be observed from here that employing resistance bacteria might allow for the development of strategies that work in the cleaning of the environment from heavy metals, which would form part of efforts for a sustainable environment.

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Conflict of interest

All authors declared that there is no conflict of interest.

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