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Removal of Toxic Heavy Metals Using Genetically Engineered Microbes: Molecular Tools, Risk Assessment and Management Strategies

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Abstract

As a result of the increasing rates of heavy metal pollutants in many environmental resources, it was necessary to discover and develop new methods for treating heavy metal pollutants, and one of the most effective methods is the use of genetically modified microorganisms such as bacteria (Shewanella Oneidensis and Cupriavidus Metallidurans) which is very useful in getting rid of heavy metal pollutants. This study aims to determine and evaluate the effectiveness of genetically modified bacteria and organisms such as Shewanella Oneidensis and Cupriavidus Metallidurans. In getting rid of heavy metal pollutants, genetically modified bacteria were used and obtained from MB Genetics, which is one of the leading companies in this field. Strains of genetically modified bacteria were obtained from MB Genetics, a company specialising in synthesising genetically modified microbes. Then, these genetically modified bacteria were used to reduce the presence of harmful heavy metals in the water to acceptable levels that meet the permissible limits and values. These bacteria were used under different conditions and different pH numbers. The results indicated the effectiveness of genetically modified bacteria in eliminating levels of heavy metals in water. Both bacterial strains successfully eliminated more than 91% of lead at a pH of 7. The study highlighted the significant influence of acidity and pH levels in determining the levels of heavy metals present in water and the environment in general. High levels of pollution with heavy and harmful metals in water can be treated by using genetically modified bacteria such as (Shewanella Oneidensis and Cupriavidus Metallidurans); this treatment is highly effective in removing harmful heavy metals from water when it is used correctly and by the principles of scientific research and scientific theories.

Keywords:

Heavy Metals, Genetically Modified Bacteria, Shewanella Oneidensis, Cuprividus Metallidurans.

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Introduction

In light of the development of technology, especially the field of industry, the percentage of pollutants in the environment has increased, whether they are pollutants resulting from harmful thermal emissions or increasing industrial activities, resulting in the presence of many toxic and harmful heavy metals in the environment, which poses a threat to environmental systems and human health **[1].** This required further studies and research to discover methods and techniques that would get rid of these heavy harmful metals and get rid of them in order to achieve environmental sustainability **[2].**

One of the most important technologies and methods that have emerged recently and proven effective in this subject is the removal of harmful heavy metals using genetically modified bacteria. It is considered one of the most important technologies that have contributed to the existence of accurate and good solutions for removing harmful heavy metal pollutants from the environment in general, from water in particular **[3].**

Many genetically modified bacteria such as (Pseudomonas aeruginosa, *Shewanella oneidensis,* and *Cupriavidus metallidurans.* It is the cornerstone of the revolution in treating water and the environment from heavy metal pollutants, as they effectively contribute to getting rid of these harmful heavy metals and reaching permissible levels locally and internationally [4]. A type of bacteria called (Pseudomonas aeruginosa) has been modified. It is a bacterium that can easily adapt to any environmental conditions and the pH or pH of the water, in addition to its superior capabilities in getting rid of heavy metals, as many previous studies have proven **[5].** Genetic modification and genetic modification of P. aeruginosa bacteria allowed it to absorb, store, and dispose of harmful heavy metals, making it an important and effective tool in the bioremediation of the environment **[6].**

Also, one of the types of bacteria that can be genetically modified and used as an effective tool to get rid of heavy metals present in the environment in general and in water are bacteria (*Shewanella oneidensis.* Many studies confirm that these bacteria can get rid of harmful heavy metals, as they reduce and stabilize the metals; this is done by genetically modifying it, or what is called genetic manipulation. *S. oneidensis.* We can contribute to enhancing the ability of these types of bacteria to reduce the levels of harmful metals in the water and reach the permissible limits under different pH numbers and degrees of acidity, in addition to its tremendous ability to adapt to all environmental conditions, including temperatures, humidity, etc. **[7].**

Also, another type of bacteria and organism called (Cupriavidus metallidurans) has been genetically modified to take advantage of its advantages, as it is characterized by its innate resistance to a wide range of metals and its ability to bind to them. Therefore, it has been genetically modified to enhance its ability to bind to metals **[8].** It enhances its ability to convert harmful heavy metal ions into smaller, less harmful ions and forms, making them important and powerful tools for vital environmental treatment and removing harmful heavy metals **[9].** The effectiveness and mechanism of using genetically modified bacteria to get rid of heavy metals depends on the fact that they are modified according to precise genetic mechanisms and procedures that allow them to absorb harmful substances after reducing their shapes and sizes to less dangerous degrees. Where many techniques were used, such as... (CRISPR-Cas9), It is used to modify the genetic structure of bacteria to enhance their ability to get rid of heavy metals, remove them, and adapt to all environments. It is also considered a technical CRISPR-Cas9 Innovative technique for genetically altering and modifying bacteria genes, as this

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technique changes specific genes in microbial genomes **[10].** It has enabled many researchers and scholars to use this technology to modify and increase the number of proteins that can bind to metals and contribute to accelerating the process of absorbing ions. It also contributes to improving the ability of these bacteria to deal with and absorb these harmful ions and enhance their ability to adapt to the environment, as mentioned by many studies and research **[11].**

Biosynthetic biology has greatly contributed to developing strategies and distinct genetic mechanisms specifically designed to enhance the ability of bacteria to absorb and eliminate heavy metals and adapt to different environments. Using genetic modification technology and the use of artificial gene networks technology, scientists were able to enhance the ability of many species of bacteria to tolerate high levels of metals, and thus, understanding this expanded the scope of their use in many applications and many different environments and contributed to increasing their ability to adapt. In these environments, pollutants can be removed very effectively **[12].** Some metabolic pathways have been identified by enhancing the effectiveness of eliminating microbial heavy metals **[13].** The discovery of new paths would enhance the ability to get rid of metals, transform them, and reduce their effects. However, this required many measures, including changing important enzymes related to metabolic processes **[14].**

Despite the effectiveness of using genetically modified living organisms and bacteria to get rid of harmful heavy metals and improve environmental biology, there are many obstacles and challenges facing the issue of using genetically modified bacteria to get rid of heavy metals and reduce their levels. The most important of these challenges and obstacles is the spread of bacteria. In a significant way, reducing environmental diversity and developing the ability and resistance of bacteria to any genetic modifications leads to reducing their ability to carry out the tasks for which they were genetically modified. Therefore, it was necessary to develop strategies and measures to reduce these obstacles and risks and other strategies to evaluate the risks of genetic modification of bacteria [**15**]. One of the most important of these strategies was conducting comprehensive assessments of the risks resulting from environmental examination and the unexpected consequences of releasing genetically modified organisms into the environment and how to control them **[16].**

One of the most important risk assessment methods, as mentioned by many studies and research, is a comprehensive examination of the ability of genetically modified bacteria to survive, spread, and transfer genes in the environment **[17].** Also, one of the most important techniques used recently is machine learning techniques to develop models that can predict the behaviour of transmission and spread of genetically modified bacteria and predict the extent of the damage that can be caused; this leads to the spread of these bacteria. Many researchers have emphasized that it is necessary to consider the biological location of genetically modified bacteria and the possibility of their horizontal transmission and to enhance the changes occurring in genetically modified bacteria **[18].**

This study aims to clarify and evaluate the effectiveness of using genetically modified bacteria, specifically Pseudomonas aeruginosa, *Shewanella oneidensis*, and *Cupriavidus metallidurans.* To eliminate heavy metals and get rid of them to preserve the environment and achieve environmental sustainability, for example, due to its superior ability to absorb these harmful metals and due to its great ability to adapt to different environmental conditions. This study is important because it contributes to developing environmental and biological remediation solutions to eliminate heavy metals in the environment using genetically modified bacteria. The importance of this study is also because it sheds light on the assessment of relevant risks related to the genetic modification of bacteria, and it clarifies the appropriate genetic modification mechanisms and how to Get rid of heavy metals present in water in the environment in general and water.

Method

By obtaining groups and samples of genetically modified bacteria from MB Genetics Company, which, as previously mentioned, is one of the leading companies specialized in the field of manufacturing genetically modified bacteria and organisms such as bacteria and fungi, genetically modified bacteria were obtained (Cupriavidus metallidurans and Shewanella oneidensis), where MB Genetech uses genetic modification technology called "directed mutagenesis" to change and modify the genes of bacteria (Cupriavidus metallidurans and Shewanella oneidensis (through a technology-based approach) CRISPR-Cas9) is the specific name for the bacteria Shewanella oneidensis, which is "Shewanella oneidensis Mr-1". " The designation specified for Cupriavidus metallidurans is "Cupriavidus metallidurans". CH34This is removing heavy metals from water using genetically modified bacteria, as stated in a study.

Through a set of procedures, it is clarified how and how to use genetically modified bacteria to get rid of harmful heavy metals, which are as follows:

- Prepare a sample of wastewater or drinking water and examine it to confirm the proportions of harmful elements in it, according to known tables, limits, and values. Tables (1, 2, 3)
- Preparing and determining the types of genetically modified bacteria suitable for conducting the practical steps based on the analysis results and the concentration of heavy metals present in the samples taken.
- Adjusting the pH of the sample by adding alkaline materials, taking into account the type of specific mineral and genetically modified bacteria.
- Genetically modified bacteria are grown in wastewater. Bacteria multiply and attach to heavy metals in healthy or drinking water. When bacteria multiply and attach to heavy metals, the metals precipitate out of the water.
- This can be accomplished by introducing a chemical agent that causes the heavy metals to precipitate. After the bacteria and heavy metals precipitate, the microorganisms are removed using either filtration or centrifugation. The bacteria can then be disposed of or reused.

A set of criteria must be taken into consideration, which are as follows**:**
- The pH level must be appropriate to the nature of the genetically modified bacteria, as the pH is one of the important factors determining the optimal conditions for the absorption of heavy metals, especially from wastewater, which usually contains heavy metals in large quantities. The pH is either neutral or neutral. Somewhat alkaline.
- Genetically modified bacteria show enhanced abilities to absorb lead in wastewater with a pH range of 5.5 to 7. Adding an alkaline chemical to the effluent can achieve this.
- The process of removing heavy metals from water takes place in two stages, as follows:
- Mineral absorption: Genetically modified bacteria reduce the volume of heavy metals and absorb them
- Metal accumulation: Absorbed metals accumulate in the cytoplasmic membrane of genetically modified bacteria, as Figure (1) shows.

As the figure shows, when the mixture is transferred to **(**HF), due to increasing pressure, the solution passes through the filtration membrane, represented by vertical lines. This process separates the saturated particles (HM) using the nitrocellulose filter. It is a bifilter with a diameter. It is 0.45 micrometres. The continuous stirred tank reactor (CSTR) creates the essential environment for cell proliferation. The filtrate obtained from the filter is purified by

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removing mixed waste. Blue continuous stirred tank reactors (CSTRs) represent cells resuspended in a non-growth medium, while yellow CSTRs show cells resuspended in a growth medium.

Figure (1): shows bioaccumulation bioremediation schemes for continuous biological processes

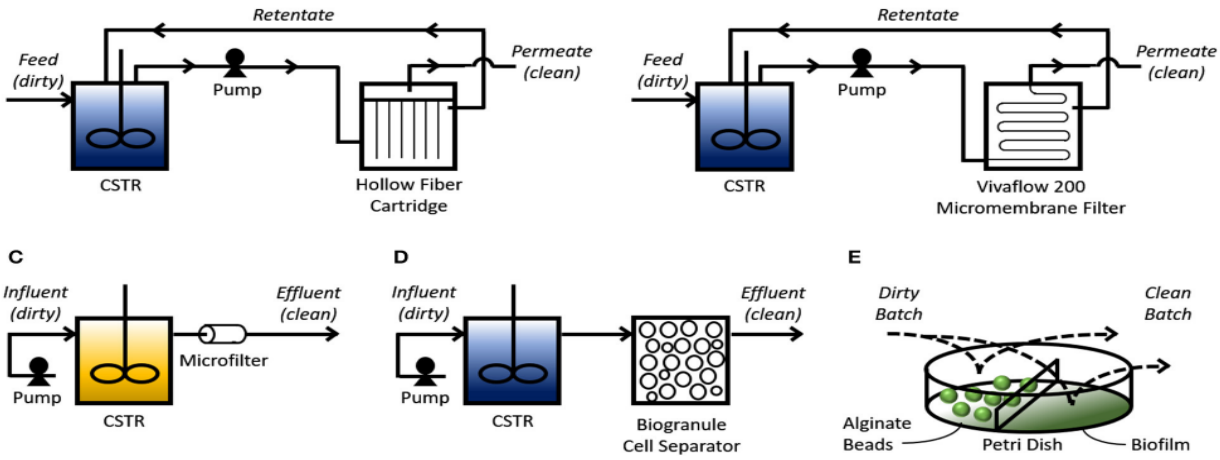

Figure No. (1) shows bioaccumulation biotreatment schemes for continuous biological processes

Table (1): Permissible levels of heavy metals in water

It is known that the removal and disposal of heavy metals is one of the important and effective procedures in the biological treatment of the environment. By understanding the relationship of the optimal pH to the ability of bacteria to absorb heavy metals, it is possible to understand the mechanism of disposal of heavy metals and their effectiveness. Table (4) shows the maximum permissible pH cards. Bacterial absorption of heavy metals.

These pH suggestions emphasize the importance of customizing ambient conditions for bioremediation techniques. It is known that bacteria show different abilities to absorb metals as a result of pH levels; therefore, as a result of this knowledge, vital information is provided through which strategies can be developed that help in accurate and effective treatment and enable scientists and environmental engineers to use this information for further development and improvement processes related to improving and reducing the percentage of Heavy metals and harmful pollutants in water in general and the environment in particular.

Table (2): Actual value for the percentages of heavy metals in a water sample from sewage

Metal	Metal value in wastewater Sample (%)
Lead (Pb)	0.05
Mercury (Hg)	0.01
Cadmium (Cd)	0.02
Zinc (Zn)	0.001
Copper (Cu)	0.2
Iron (Fe)	1

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Also, through the values in table (2), it is the table that shows the percentages present in the water sample obtained from wastewater, and by comparing these percentages, the permissible quantities and the limited quantities are shown in table (1). It is possible to evaluate and determine whether the existing values comply with the standards; thus, the water quality can be determined. As previously mentioned, it must be noted that monitoring the levels of heavy metals in the water is the most important step and stages necessary to prevent environmental pollution and maintain and adhere to the laws related to protecting public health and the environment.

Table (3): Types of genetically modified bacteria that can absorb heavy metals

Bacteria type	Heavy metals
Pseudomonas aeruginosa, Shewanella oneidensis, Cupriavidus metallidurans	Lead (pb)
Pseudomonas aeruginosa, Shewanella oneidensi, Cupriavidus metallidurans	Cadmium (Cd)
Cupriavidus metallidurans, Pseudomonas putida, Shewanella oneidensis	Copper (Cu)
Shewanella oneidensis ‹Pseudomonas putida ‹Ralstonia eutropha	Iron (Fe)
Shewanella oneidensis, Cupriavidus metallidurans, Ralstonia eutropha	Mercury (Hg)
Shewanella oneidensis, Cupriavidus metallidurans, Ralstonia eutropha	Zinc (Zn)

Table 3 shows the types of genetically modified bacteria used to remove toxic heavy metals. It also demonstrates the capabilities of each type of genetically modified bacteria in reducing the percentage of pollution with heavy metals through their absorption. Still, it must be considered that these results may have major repercussions on the natural biological treatment of the environment and that using genetically modified bacteria effectively and safely achieves the goal during risk assessment.

Table (4): determines the appropriate pH for the absorption of each heavy metal

Metal type	pH
lead (Pb)	5.5:6
Mercury (Hg)	5:6
Cadmium (Cd)	6:7
Zinc (Zn)	6:8
Copper (Cu)	4:6
Iron (Fe)	5:6

Efficient metal detoxification in environmental bioremediation relies on a comprehensive understanding of the bacteria's optimal pH range for absorbing heavy metals. The pH ranges advised for the absorption of particular heavy metals by bacteria are presented in Table (4).

These pH suggestions emphasize the significance of customizing ambient conditions for bioremediation techniques. Bacteria demonstrate selective metal uptake capacities in response to different pH levels, offering vital information for developing precise and effective bioremediation strategies. Scientists and environmental engineers can use this information to improve conditions for more effective removal of heavy metals, which helps find sustainable solutions for polluted ecosystems.

Results

After using more than a million bacteria, *Shewanella oneidensis* is the appropriate type for all metals present in the sample: Lead, iron, copper, cadmium, mercury, and zinc, at pH numbers)5,6,7) These were the results.

Table (5): Results at pH=5 with *Shewanella oneidensis* bacteria

pH	Metal	Metal value in wastewater sample	Removal rate (%)
5	lead (Pb)	0.009	82%
	Mercury (Hg)	0.002	80%
	Cadmium (Cd)	0.005	75%
	Zinc (Zn)	0.000192	81%
	Copper (Cu)	0.0596	70%
	Iron (Fe)	0.394	61%
6	Metal	Metal values in a wastewater sample	Removal rate (%)
	lead (pb)	0.004	92%
	Mercury (Hg)	0.0017	83%
	Cadmium (Cd)	0.004	80%
	Zinc (Zn)	1E-04	90%
	Copper (Cu)	0.04	80%
	Iron (Fe)	0.3	70%
7	Metal	Metal values in a wastewater sample	Removal rate (%)
	lead (pb)	0.004	92%
	Mercury (Hg)	0.0017	83%
	Cadmium (Cd)	0.004	80%
	Zinc (Zn)	1E-04	90%
	Copper (Cu)	0.04	80%
	Iron (Fe)	0.3	70%

Cupriavidus *metallidurans* is the suitable species for all the metals found in the sample, including lead, iron, copper, cadmium, mercury, and zinc, at pH levels of 5, 6, and 7. The outcome is presented in Table 6.

Table (6): Results at pH=5 with Cupriavidus *metallidurans*

pH	Metal	Metal values in a wastewater sample	Removal rate (%)
5	lead (pb)	0.0045	91%
	Mercury (Hg)	0.0018	82%
	Cadmium (Cd)	0.0038	81%
	Zinc (Zn)	9.5E-05	91%
	Copper (Cu)	0.0386	81%
	Iron (Fe)	0.292	71%
6	Metal	Metal values in a wastewater sample	Removal rate
	Lead (pb)	0.006	88%
	Mercury (Hg)	0.002	80%
	Cadmium (Cd)	0.0038	81%
	Zinc (Zn)	0.00011	89%
	Copper (Cu)	0.05	75%
7	Metal	Metal values in a wastewater sample	Removal rate
	Lead (pb)	0.006	92%

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	Mercury (Hg)	0.002	82%
	Cadmium (Cd)	0.0038	83%
	Zinc (Zn)	0.00011	89%
	Copper (Cu)	0.05	75%
	Iron (Fe)	0.2	85%

At the same pH

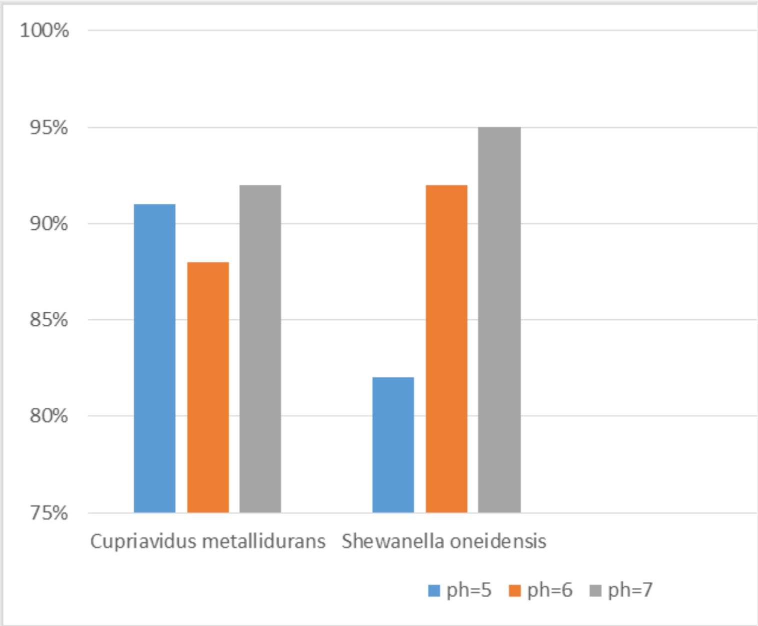

Fig (2) Comparison between the metal removal rate using *Shewanella oneidensis* and bacteria with bacteria (*Cupriavidus metallidurans*) at the same pH.

Discussion

This study aims to study and clarify the role of genetically modified bacteria, including Shewanella oneidensis and Cupriavidus metallidurans, in removing and reducing levels of harmful metals from the environment in general, especially in water. The study contributed to enhancing the clarification of the importance of the extent and effectiveness of using genetically modified bacteria as environmentally friendly and effective tools for eliminating harmful metals. It presented a new approach and new strategies to control the proportions of harmful metals by using bacteria appropriate to the nature and proportions of the metals. The results of the study agreed with many previous studies related to the rates of heavy metal removal at high pH levels, especially since bacteria, such as Pseudomonas aeruginosa, Shewanella oneidensis, and Cupriavidus metallidurans, do not. It is distinguished by its ability to remove heavy metal pollutants with high efficiency and adapt to different environments in different environmental sources **[19].**

The results from evaluating heavy metal removal in wastewater at different pH levels are consistent with previous research highlighting the significant influence of pH on the effectiveness of metal removal procedures.

Many previous studies have shown that the pH level is significantly related to the proportions and forms of heavy metals in water. Therefore, the results showed a relationship between pH levels and metal absorption rates. Previous research shows that the pH level affects the formation of different chemical forms of heavy metals in water. The results demonstrate a relationship between increased pH levels and higher metal removal rates **[15].**

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Genetically modified bacteria strains (Pseudomonas aeruginosa, Shewanella oneidensis, and Cupriavidus metallidurans) are strains that can absorb harmful metals with high efficiency and have the ability to adapt to any environmental conditions to a great extent **[20].**
The association between genetically modified bacteria and their ability to absorb certain molecules and heavy metals highlights the importance of biological treatments in wastewater treatment **[21].**
Higher pH levels contribute to improved heavy metal removal and environmental sustainability. Studies show that adjusting pH can limit the environmental impacts of metal contamination and that using microbes is an effective strategy to remove these metals. The ideal pH range is 3 to 8, which results in lower absorption **[22].** Bacteria also show greater efficiency in acidic environments, which indicates the importance of studying microorganisms adapted to these conditions **[23].**

The findings align with research into using microbes to remove heavy metals. Gaining a comprehensive understanding of the pH dependence of these processes offers useful insights for formulating efficient remediation solutions. Wastewater treatment facilities can improve the effectiveness of heavy metal removal techniques by regulating pH conditions.

Typically, a pH range of 3 to 8 is optimal, as metal ions are present in the hydroxide state in solutions with high pH, hindering the adsorption process **[24].** Furthermore, bacteria exhibit enhanced behaviour in an acidic environment, making it crucial to investigate microorganisms specifically adapted to low pH conditions **[25].** It is crucial to control the duration of cell culture to save costs, as the amount of adsorption does not achieve saturation after reaching equilibrium. According to reports, excessive adsorption material might lead to competition among adsorption groups, while too little adsorption material can result in inadequate adsorption **[26].** The presence of other ions can occupy the adsorption sites, decreasing the efficacy of adsorbing the desired ion.

Ultimately, the findings regarding eliminating heavy metals from wastewater at varying pH levels enhance the current knowledge base, providing valuable practical guidance for enhancing wastewater treatment approaches. The correlation between our findings and prior research highlights the significance of pH as a crucial element in formulating sustainable and efficient methods for addressing heavy metal contamination in water systems **[27].**
The number of genetically modified bacteria is directly proportional to the concentration of metals. The higher the metal concentration, the more necessary it is to increase the number of bacteria to eliminate harmful heavy metals with high efficiency and effectiveness **[28].** Chemical and physical molecular properties also play a fundamental role in the extent to which genetically modified bacteria can absorb and store harmful metals **[29].**
The development of synthetic biological treatment technology by identifying specific genetic pathways in microorganisms and discovering new pathways enhances the ability of these genetically modified organisms to Binding to metals **[30].** Risk assessment procedures must be comprehensively considered to control the potential and emergency environmental impacts of genetic modification of bacteria **[31].** Biocontrol techniques are essential to prevent the release of modified organisms into the environment, whether through developing self-safe organisms or using physical containment measures **[32].**

Conclusion

- There must be comprehensive and multiple strategies and development procedures to reduce and eliminate heavy metals using genetically modified bacteria effectively.

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- Reducing harmful metals depends on many important factors, including choosing the appropriate type of bacteria according to the types and percentages of harmful heavy metals present. According to the pH numbers, each bacterium is more effective at certain pH numbers.

- Hybrid strategies can be developed to take advantage of the advantages of each strategy when conducting biological, physical, and chemical analyses and treatment, as well as when using genetically modified bacteria to reduce the levels of harmful metals in the environment in general and water.

- Although many genetically modified bacteria can adapt to environmental conditions, it must be considered that environmental conditions may affect the performance of some bacteria, and results vary according to different environments.

- Assessing the risks of genetic modification of bacteria and developing strategies that accurately assess and control risks are among the most important factors of security, safety, and safety for human life and the environment**.**

Limitation of the study

Sometimes, some results in studies lack accuracy, so we find many differences in the results, which may be minor or major. Therefore, the results may depend on the complexity of natural environments and the data's accuracy, quality, and diversity. The more data in terms of size and diversity, the more accurate the results will be. It must also be considered that the difference in environmental conditions and sometimes the heterogeneity of samples and strains of genetically modified bacteria may make the evaluation difficult, considering ignoring the interactions between different types of metals. Considering the challenges facing the field of biological containment in terms of adherence to genetic modification legally and taking into account ethical and regulatory factors, genetic modifications are extremely dangerous procedures that may lead to catastrophic results if they are not controlled, and their risks are not well assessed. Therefore, more studies are needed to determine the social, ethical, and regulatory aspects of such procedures and conduct a comprehensive cost analysis and risk assessment to expand the scope of these technologies while clarifying that the study may not have predicted all possible environmental implications.

Conflict of Interest

The authors have no conflict of interest

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