The ability of locally is elated bacteria species to remove some heavy metals form aqueous media

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ABSTRACT

The study included the efficiency of two types of bacteria *Psendomonas aeruginosa* and *Ecsherichia coli* For the purpose of using it in the biological removal of the elements cadmium, copper and iron from the water media, and for two sites in the city of Baghdad, which is the city of Dora, near the Dora refinery, and the city of Al-Waziriyah, near the general origin of the electrical industries. The two isolates proved their ability to remove heavy metals, as the removal percentage reached copper , iron and cadmium 95.4 , 99.5 , 90.1% by isolation *P.aeruginosa* Respectively, while the metal removal percentage was above 90.9
, 95 , 80.5 % coli, and the two isolates demonstrated their ability to remove heavy metals at low concentrations of 5 mg / L and their ability to remove them at a concentration of 75 mg / L. The percentage of heavy metal removal using the live and dead mass of bacteria was *Psendomonas aeruginosa* 96.8 ,97.9 , 95.8% and 95.9 , 99.9 , 92.5% for the elements Cu , Fe and Cd, respectively, while the percentage was reached using the live and dead biomass of the isolate *E.coli* 99.8
, 98.4 , 91.8 % and 95.8 , 97.9 , 92.2 % for the elements Cu , Fe , Cd respectively where isolation has been demonstrated *P.aeruginosa* High efficiency in the biological treatment of water contaminated with heavy elements in the Dora region, near the Dora refinery, as well as in the Al-Waziriyah region, near the General Establishment for Electrical Industries. The efficiency of the live and dead biomass of bacteria has also been proven *P.aeurginosa* In the biological treatment of contaminated water and for both site.

Keywords: Heavy metals , aqueous media , bacteria

Introduction

Ability to The water environment, especially surface and fresh water, is one of the major environmental problems at the present time, as it is polluted with many pollutants, such as water pollution with industrial and agricultural waste, including various heavy elements. Pollution with heavy elements is considered one of the most important types of enviro

Given the difficulty of dealing with heavy elements in terms of breaking down and environmental removal because of their ability to bind to their atoms and their great tendency to unite with other chemical compounds or their penetrate vital pollutants into food chains and various environmental media and their biological and chemical accumulation and stay in the environment for a long time, this made researchers think seriously about finding techniques There are different ways to deal with this problem, but most of these physical and chemical techniques need specialized management and cost huge sums (Ali and cupta,2007) Therefore, studies and research directed to alternative techniques that depend on the natural resources of the environment, and among these bioremediation techniques, the bioremediation method is one of the most important and best modern methods, as microorganisms are used to remove or break down pollutants. Microorganisms, bacteria or fungi, may be tools for this treatment, so scientific ideas were generated to use These different organisms are matched to test their ability to bioremediate and withdraw environmental toxins (Mustapha and Halimo on,2015). The subject of collecting heavy metals in the bodies of microorganisms has received great attention from many researchers, because some of these microorganisms have the ability to remove

heavy elements and break them down from pollutants and industrial waste. that you can remove (Rani and Dhania,2014) Applied studies in this field also nmental pollution as a result of emissions from heating and vehicle and factory exhaust And many other human activities (Malizia *etal* . 2012) Heavy elements go through many paths after their use, part of them evaporates or evaporates as a result of the sun's rays and moves to the layers of the atmosphere, which causes air pollution, and this in turn leads to the descent of these pollutants with rain to the soil. directly different water) long and shu.,2010) .

indicated the great difference in the material costs required by chemical and physical treatments and their specialization in dealing with one aspect of the problem compared to biological methods that can treat more than one pollutant at the same time, whether by using sequential treatments or joint farms, and this depends on the nature of the distribution of neighborhoods In a water body in the surface layer, water column, or benthic zone) Priyadarshani *etal*,2011).

Aim of the study

1. Isolation of types of bacteria from some polluted water sites
2. Testing the efficiency of isolated bacterial species in removing heavy metals

1- Literature Review

1-1 Environmental pollution

Environmental pollution increased with the development of man, but the world did not realize its danger until a century ago, and since that time calls began to increase to address these risks. It can be described as the addition of man, with his various activities, to the environment, materials and compounds that would have a negative impact on the ecosystem . Pollution is also known as the change in the physical and chemical properties of air, water and soil, and it results in damage to human life in the field of industrial and agricultural activity, causing damage and damage to environmental resources. In addition to that, there are countless benefits for humans) Gadzala- Kopciuch etal.,2004)

2.2 Water Pollution

Water pollution is defined as a change in the quality of natural water due to the addition of harmful substances in the water in increasing concentrations or the introduction of effects on it such as an increase in its temperature or even a decrease in some of its basic pollutants as a result of human intervention until this water becomes unfit for biological uses or even industrial uses (Hoffman *etal.*,2006).

2-3 Heavy metals

They are chemical elements with a

specific density of at least five times more than water, and their number in the periodic table reaches 38 elements, and they differ from environmental pollutants by their natural presence in the components of the earth's crust such as soil, air and water, and they have negative and positive effects on the environment (Tucker *etal*.,2003)

And that small amounts of these elements are found naturally in the environment and have an impact on public health, but large quantities of any of them will cause acute or chronic toxic effects and may have dangerous toxic effects at the cellular and genetic levels and may cause mutations in animals, humans and plants) Wuana and okieimen;;2011)

The presence of these elements in the environment and the exposure of organisms to different concentrations, according to most studies, leads to damage to the functioning of the human central nervous system and the destruction of some components of the blood, lung, liver, kidney and other vital organs. The physiological, muscle and nervous system causes Alzheimer's

disease and may cause sensitivity in people who are in direct contact with some of these elements and may cause cancer (vara and Helena.,1999)

Pollution with heavy elements is one of the most difficult types of pollution, as these minerals remain in different environments without disintegrating. These aquatic organisms are also affected by these minerals because they are the base of the food chain in environmental systems. Also, the elements that are removed by bacteria, algae, fungi and some aquatic plants return a large part of them For water after the death or disintegration of organisms

2.3.1 Cadmium element

It is found in nature and is one of the elements of the earth's crust. Its texture is smooth and its color tends to be silvery white. It is not often found in its pure form. Rather, it is found united with other elements, forming multi- element rocks in its forms such as sulfur oxides, sulfates and carbonates, and in other metal ores such as zinc, lead and copper. Cadmium is considered a very strong mineral. Toxic to all living organisms. It is used in the manufacture of batteries, plastics, and paint granules. It can be present in the soil due to the use of pesticides, fungicides, and phosphate fertilizersm) Mustapha and Halimoon,2015)

2.3.2 Copper element

Some natural soils contain the element copper, but it is found in very low concentrations that may not exceed one part per million. On the other hand, it is considered a fast-reacting element in the presence of atmospheric moisture, which results in toxic copper oxide with a green color. The conditions of solubility and movement of copper are in oxidizing and acidic conditions. Its concentration in the basic and reducing conditions is little, and its presence in the reducing environment leads to the formation of a copper sulfide compound, which is insoluble and immobile and forms complexes with organic materials and is more powerful to form complexes with these materials compared with zinc, cadmium and lead (Wang *etal*.,2009 (

2.3.3 Iron element

It is a metallic chemical element, and it is the most widely used and abundant metal after oxygen, silicon and aluminum. Iron is in the pure state and isolated from air in standard conditions in the form of a silver-gray metal, and its surface is soft and smooth. On the other hand, contact with the oxygen of the air and the presence of moisture leads to the reaction of iron and the formation

of a reddish-brown layer of hydrated iron oxides, which is called (rust). Iron has two common oxidation states, iron (II) and (III) iron. Iron shares general properties with the properties of other transition metals. Iron forms a large number of chemical compounds in various oxidation states (Dedhisk, 1999) (Demazeau and Pouchard, 1982)

2.4 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is an aerobic, motile, gram-Negative rod able to grow and survive in almost any environment lives primary in water, soil and vegetation. However, despite abundant apportunities for spread, *P.aeruginosa* rarely causes community-acquired infections in incompetent patients.

P.aeruginosa produces several extracellular products that after colonization can cause extensive tissue damage, blood stream invasion, and dissemination in viro studies have shown that mutants defective in the production of exotoxin A, exoenzyme S, elastase or alkaline protease are essential for maximum virulence of P.aeruginosa (oliveira *etal* ,2006)

2.5 Escherichia coli

Escherichia coli was first described by Escherich in 1885 after isolation form infant stool suffering from enteritis, and it was soon shown that this organism could also be isolated from the stool of healthy infants and adults (Edward and Ewing 1986). The organism typically colonizes the infant gastrointestinal tract (GIT) within hours of life, and, thereafter., E. coli and the host derive mutual benefit. This organism is distributed in the environment as well as in the bowel of human and animals, it is also present in water supplies as an indicator of recent fecal contamination and potential presence of enteric pathogens (Mackie and Maccartartney 1996).

2- Materials and methods

The samples were isolated from the waters of the Tigris River, where bacteria were isolated *pseudomouas aeruginosa* From the Dora area near the Dora refinery, while bacteria were isolated *Escherichia coli* From the Al- Waziriya area near the general facility for electrical industries, and it was diagnosed based on the agricultural, morphological, and biochemical characteristics. The isolates were kept at refrigerator temperature and in the solid nutrient medium until use.

2-1 Study of the two bacterial isolates in the removal of heavy metals

Flasks were incubated containing 100 ml of the nutrient medium (N.B) and added to it 100 mg/L of heavy metals separately and inoculated with (5) ml of the active bacterial culture *P.aeruginosa* In flasks containing Fe, Cu, and Cd metals. The same applies to the isolate *E.coli*, at a temperature of 30 °C and a pH of (7), and was placed in a shaking incubator with a cycle of 150 cycles / min for a period of 24 hours (Hietala *etal*,2009).

(C) (B) (A)

A- The two bacterial isolates in medium containing iron

B- The two bacterial isolates in medium containing cadmium

C- The two bacterial isolates in medium containing copper

2-2 A study of the efficiency of the two bacterial isolates in removing low concentrations of heavy metals.

Flasks containing 100 ml of sterile liquid nutrient media (N, B) were incubated and heavy metals were added to them Fe , Cu , Cd Each separately and at a concentration of 10 mg/L and supplemented with (5) ml of active bacterial culture *P.aeruginosa* The same applies to the isolate *E. coli*, at a temperature of 30 degrees Celsius, and placed in a rocking incubator for 24 hours, with a number of cycles of 150 revolutions / minute, according to the method (APHA,1998) .

2-3 Studying the ability of the biomass of the two bacterial isolates to remove heavy metals.

This experiment was conducted according to the method presented(Quin telas *etal* , 2008) Where both bacteria *E.coli* and *P.aeruginosa* On the liquid nutrient medium after being inoculated with (5) ml of bacterial inoculum prepared for both isolates and for each (50) ml of the medium, then a rocking incubator was placed at a rotational speed of 150 cycles/min and at a temperature of 37 °C for a period of 24 hours, then centrifugation was performed on it at a speed of 3000 revolutions. The clear part was discarded and the biomass was collected under complex conditions. We add 1 gm of the live mass of *P.aruginosa* to 50 solutions of heavy metals lead, iron and cadmium at a concentration of 50 mg/l, while the same amount of the live mass of *E.coli* bacteria was added to beakers containing the same elements. above and in a shaking incubator at a temperature of 37 ° C for a period of 24 hours and at a number of cycles of 150 cycles / minute for a period of 15 minutes and the clear fraction was taken and the remaining concentrations of heavy metals were estimated using the atomic absorption spectrometer and according to the method used by researche (Gazos *etal.*,2001)

2-4 Studying the ability of dead biomass of the two isolated bacteria to remove heavy metals.

The dead biomass was obtained according to the method reported by the resear (Sineoor *etal* 2010) Then, they were killed by autoclave at a temperature of 121 °C for 15 minutes. (5) g of dead biomass of *P.aeruginosa* was added to every 5 ml of heavy metal solutions lead, iron and cadmium at a concentration of 50 mg / L. The same amount was added to the *E.coli* bacteria, and the beakers were incubated in a shaking incubator at a temperature 37 ° C for 24 hours and a number of cycles of 150 cycles / minute (Gourdon *etal* 1990) After the incubation process, the samples were subjected to centrifugation at a speed of 3000 cycles per minute for 15 minutes and the clear part was taken and the remaining concentration of heavy metals was taken using the spectrometer and according to the method of the researcher (Gazso *etal* 2001)

3- Result and Discussion

3-1 The efficiency of the two bacterial isolates *Pseudomonas aeruginosa* and *Ecsherichia coli* in the removal of heavy metals

Isolation efficiency of Pseudomonas aeruginosa in removing copper , iron and cadmium metals was 95.4, 99.5 and 90.1%, respectively, while the removal percentage for the above elements from the isolate *Ecsherichia coli* reached 90.9, 95 and 80.5%, as shown in Figures (4-1). And (4-2) many studies indicated that the ability of many Gram-negative and Gram-positive bacterial species to remove heavy metals and laboratory waste, so the lipopoly saccharide layer plays the main role in the process of binding metals in the membrane of Gram-negative bacteria, while Gram-positive bacteria play The layer of peptidoglycan with teichuronic teichoic acids plays an essential role in the process of metal binding (Deepti *etal*.,2012), and (Agostinho *etal*,.2012) indicated the ability of the cell wall of Bacillus subtilis to adsorb zinc ions from industrial water pedestrians. General Electric Industries. As confirmed (Daboor *etal*. 2014). The high ability of the same bacteria above to remove lead and cadmium metals from their standard solutions, as indicated (Margaret *etal*; 2016).

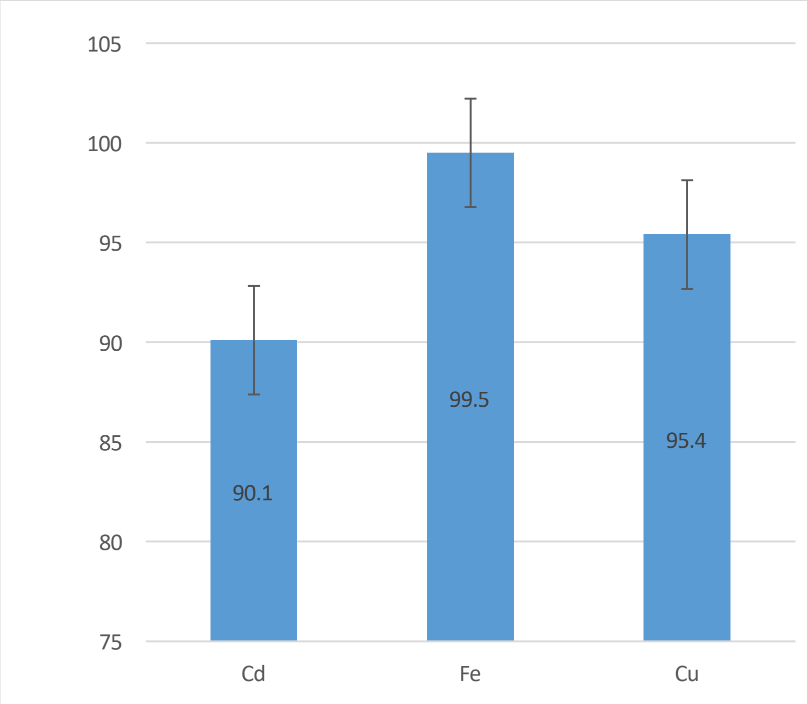

Figure (4-1) the Efficiency of isolate P.aeruginosa in removing heavy metals Cd, Fe, Cu

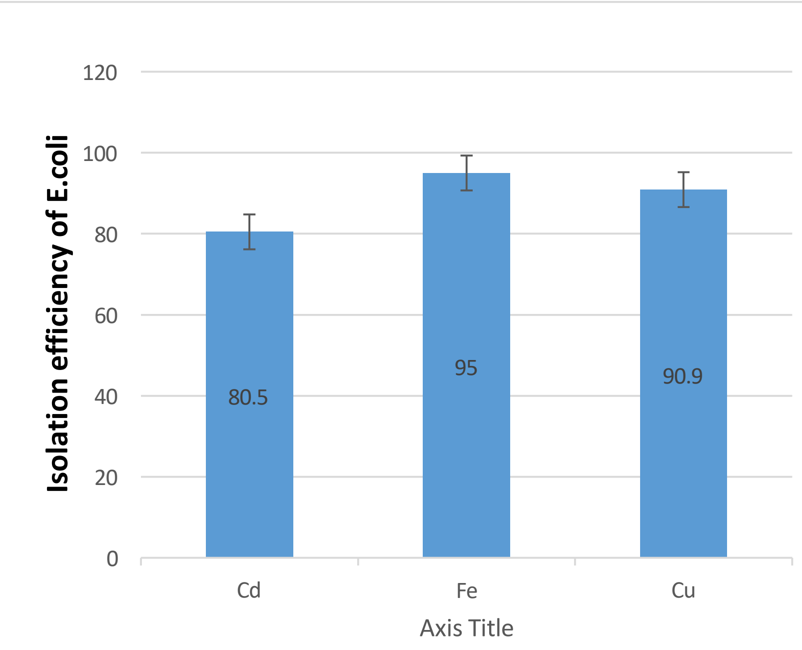

Figure (4-2) Efficiency of isolate E.coli in removing heavy metals Cd, Fe, Cu

The low concentrations of heavy metals are of great importance, due to the inability of traditional methods, whether physical or chemical, to remove small concentrations of metals, and this is what characterized the two isolates *P.aeuginosa and E.coli* and their ability to remove metals such as these small concentrations of (5 mg / liters) and with high removal rates, as in Table No. (1), (2) and this is consistent with (Pandiyan *etal.,* 2011). At low concentrations, all minerals present in the solution will find an opportunity for overlapping and binding, and this leads to an increase in the removal efficiency, and the opposite occurs in the use of high concentrations, where there is a decrease in the yield Adsorption due to saturation of adsorption sites as in Figure (2)

Heavy metals	Cd	Fe	Cu
Removal efficiency at a concentration of 5 mg/L P.aeurginosa isolate	94.7	99.8	96.9
Removal efficiency at a concentration of 5 mg/L E.coli isolate	92.9	95.5	90.1

Table (1): The efficiency of the two isolates in removing heavy metals at low concentrations

Heavy metal	Cd	Fe	Cu
Removal efficiency % at a concentration of 75 mg/L isolate P.aeruginosa	89.8	98.2	95.8
Removal efficiency % at a concentration of 75 mg/L isolate E.coli	90.9	96.8	89.8

Table (2): The efficiency of the two isolates in removing heavy metals at high concentrations

4.2 Removal of heavy metals using live and dead biomass of two isolates *P.aeruginosa and E.coli*

This experiment was conducted with a weight of 5gm of live and dead biomass for the two isolates, as can be seen in Figure (2). There are differences, albeit not significant, between the removal rates using the live and dead biomass of the above isolates, with preference given to the dead biomass if the removal rate of the live biomass is 96.8 and 98.9 and 95.8% for the metals Cu, Fe, Cd, while the percentage of removal of dead biomass was 95.90, 99.90, and 92.5% for the minerals Cu, Fe, and Cd, respectively, using the isolate *P.aeurginosa,* while the percentage of removal of live biomass was 94.8, 98.4, and 91 8.% for the minerals Cu, Fe, and Cd, and the percentage of dead biomass was 94.3, 97.9, and 92.2%, respectively, using the isolate *E.coli*, as in Figures (4-4)

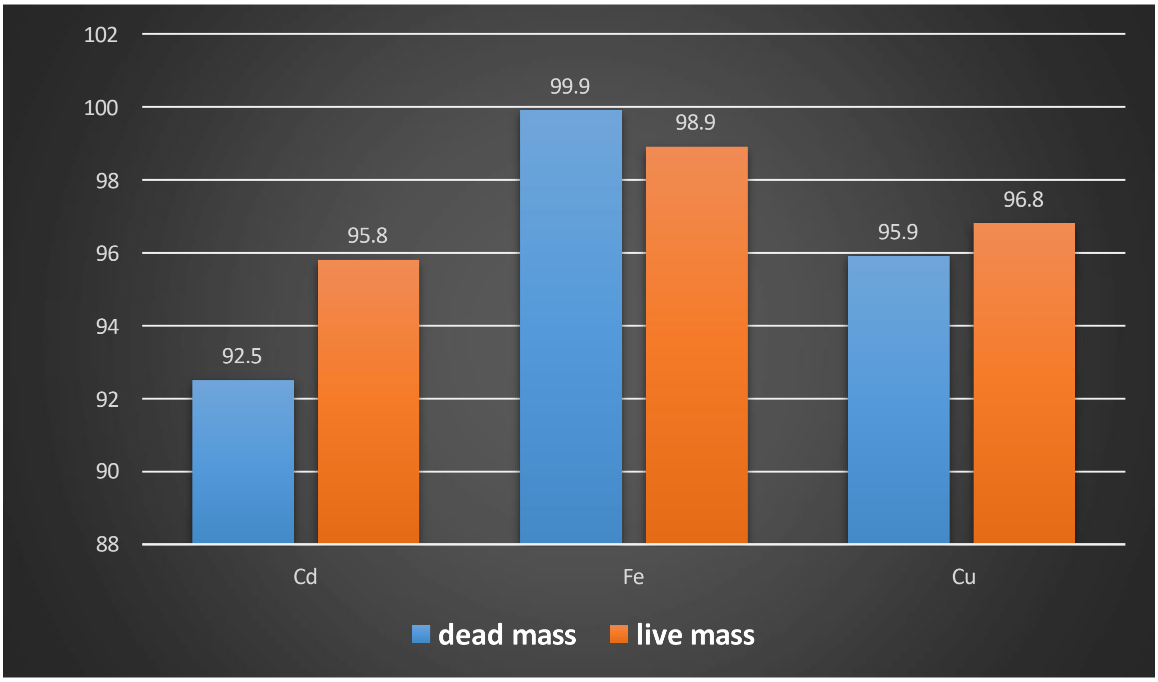

Figure (3) Removal efficiency of heavy metals using the live and dead mass of the isolate *P.aeruginosa*

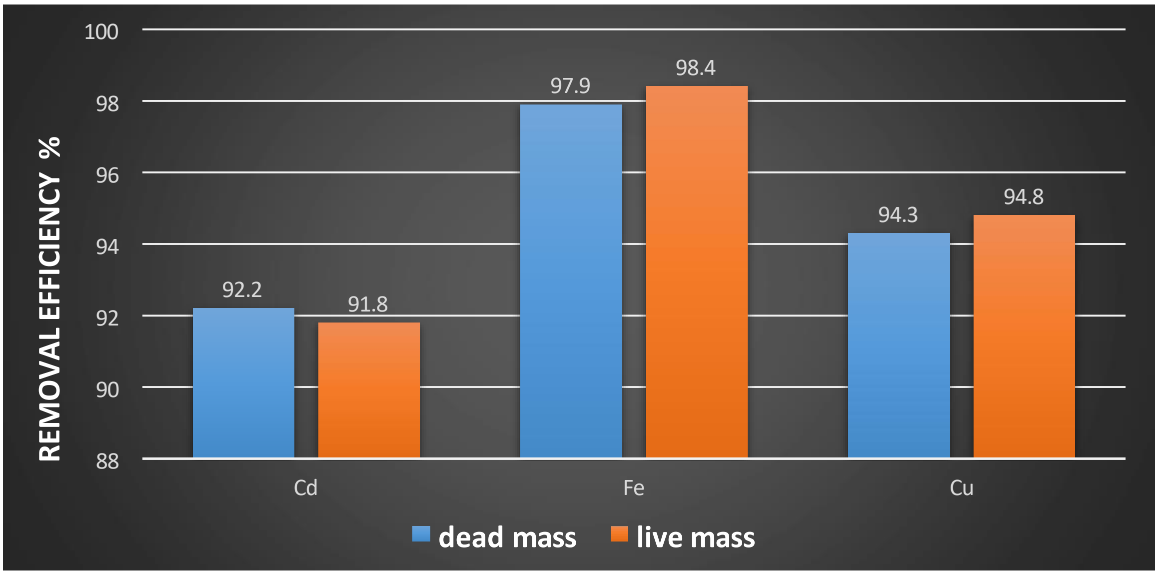

Figure (4) Removal efficiency of heavy metals using the live and dead masses of *E.coli* .

The isolate *P.aeurginosa* proved highly efficient in the biological treatment of polluted water for both sites near the Dora refinery in the Dora region, as well as the polluted water near the world facility for electrical industries in the Waziriyah region.

Conclusions

1. It was found that the isolate *E.coli* showed high efficiency in removing heavy metals at both high and low concentrations.
2. The isolate *P.aeruginosa* showed its effect on removing copper and iron more than its effect on cadmium.
3. Adsorption on live cells is reversed unless their cellular system is destroyed, while dead cells can adsorb heavy metals selectively or non-selectively.
4. The results showed that the ability of bacteria to treat low concentrations of

heavy metals is more complex than treating high concentrations of the same metals.

5. Metal-resistant bacteria play a major and effective role in treating different types of pollutants.

Recommendations:

1. Spreading environmental awareness among citizens and motivating the importance of using vital methods to remove pollutants, including heavy metals
2. Emphasizing that the Iraqi environment is very suitable for applying this type of biological treatment
3. Adding other types of microorganisms such as fungi and bacteria in bio removal studies.
4. The need to use a biological treatment method in light of the problems of polluted water.
5. Urging the use of biological treatment instead of using chemical methods.

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