Bioremoval and Resistance of Some Heavy Metals by Bacterial Isolates from the Sediments of the Diyala River

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ABSTRACT

Background: Heavy metals are pollutants that do not decompose, but enter the food chain, and thus form toxic compounds that have a harmful effect on biological functions. There are an unlimited number of organisms in various environments, including bacteria that are able to degrade and reduce the high levels of many pollutants, most of which have not been as important to researchers as they are interested in pathological microorganisms. **Materials and Methods:** Twelve sediment samples were collected from the Diyala River within the boundaries of the study area, which included four main sites. A number of distinct bacterial isolates were isolated and diagnosed, one of which showed a high ability to grow in culture environments with high concentrations of heavy metals. **Results:** It was possible to characterize fifteen phenotypically different bacterial isolates capable of resisting heavy metals at a concentration of (50 mg/L). Five were chosen. Isolates, including the best isolates capable of growth and resistance to cobalt at concentrations between (400-750 mg/L), and chromium between (400-750 mg/L).1600-2600 mg/L, nickel between (1200-1600 mg/L) and, lead between (1200-2200 mg/L). One of these isolates, (iso 4) showed a high ability to remove heavy metals (chromium, cobalt, nickel, and lead) after the test, with concentrations of (25, 50, and 100 mg/L), and the removal rate for 5 days of chromium was (36, 55, 68, 73 and 77%) respectively, and the removal rate of cobalt was (22, 34, 44, 54 and 59%) and the removal rate of nickel was (23, 40, 56, 68 and 80%) and the removal rate for lead is (58, 68, 74, 80 and 86%). The same isolate also showed high efficiency in removing lead due to the ability to resist high concentrations with a 100% percentage of lead removal at a concentration of 25 mg/L from the first day until the fifth day. The biochemical diagnosis of the selected bacterial isolates was adopted and the diagnosis was made using the VITEK-2 system for isolate No.4, as the results showed that it belongs to the genus *Klebsiella pneumonia*. **Conclusions:** These environmental isolates can be applied in many bioremediation techniques to remove toxic compounds cheaply and safely.

Keywords: Bioremoval, Microorganisms Resistance, Heavy Metals, *Klebsiella pneumonia*.
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1-INTRODUCTION

Heavy metal pollution in all environmental systems is a global problem, especially, in river systems because they are highly hazardous pollutants, with high toxicity, and non-degradable properties (1,2) as well as heavy metals are able to enter the food chain through bioaccumulation.(3)

Bioremediation technologies can be used to remove heavy metals from polluted environments, which are described as environmentally friendly, cheaper, and cost-effective for removing heavy metals, when compared to conventional chemical and physical techniques, which are often more expensive and inefficient, especially for lower element concentrations.(4)

To achieve high efficiency of bioremediation, some factors need to be optimal, such as pH, nutrient content, temperature, and life stage in the microorganism cycle. In addition, the composition of toxic metals and compounds, the bioavailability of pollutants, the biodegradation of pollutants, geological properties, etc. all affected the rate of bioremediation efficiency.(5)

The important mechanisms that depend on it in the bioremediation of heavy metals include biosorption, bioaccumulation, biocatalysis, biomineralization, biotransformation, and adsorption. The effectiveness of these mechanisms depends on many factors including the type and nature of the organism used, the environmental factors present, the availability of nutrients, and the concentration of pollutants present in that environment (6). On the other hand, some Microorganisms possess amazing metabolic pathways that use various toxic compounds as a source of energy and growth, through respiration and fermentation. These microorganisms evolved their characteristic pollutant-specific degrading enzymes and diverse mechanisms to maintain homeostasis and resistance to heavy metals, in order to adapt to toxic metals in the ecosystem (7). Contaminated environments contain many bacterial genera, which have many enzymatic capacities that may not be available in other isolates of contaminated environments (8). Microbial survival in contaminated environments depends on intrinsic and structural biochemical properties, physiological adaptations, and genetics.(9)

The aim of this study is to determine the bacterial species present in the sample collection sites and their ability to grow in an environment rich in high concentrations of heavy metals, as well as to select the most efficient isolates capable of removing and treating contamination resulting from heavy metals. In addition to diagnosing, the selected bacterial isolates using biochemical methods and VITEK 2 technology.

2- MATERIAL AND METHODS

2.1. Sample collection

Sediment samples were collected from the Diyala River within the boundaries of the study area which included four main sites. sample number and code and date), then it was placed in a portable cooler box containing ice boxes in order to maintain a temperature of 4° C until it was stored until it was transported to the laboratory in the ice box and analyzed within two weeks as mentioned in (10).

2.2. Isolation of Bacteria

Sediment samples were isolated from the pooled total sample by the serial dilution method. Then, the bacteria were isolated using the (pure plate method) according to the method presented in (10). Then, the bacterial isolates were purified by plotting on nutrient agar plates. This method was repeated to obtain pure isolated colonies (11). The bacteria were diagnosed by observing the phenotypic and microscopic characteristics and physiological characteristics tests using biochemical tests. Identification of isolates by Gram stain, catalase test, oxidase and other tests. The diagnosis of bacterial isolates was confirmed by examining the isolates with the VITEK device (12).

2.3. Preparation of heavy metal solutions

The Stock solution of heavy metals was prepared to be added to the bacteria growth media and sterilized by cold sterilization using a filter sterilization syringe measuring (0.22 µm) according to the method mentioned. 100 ml was prepared at a concentration of 3000 mg / L each of potassium chromate, cobalt nitrate, lead nitrate, and nickel nitrate by dissolving (0.3, 0.47725, 0.3, 0.47749 g) respectively, and after completely dissolving the solid, the solution was diluted to the final volume with distilled water. (10,13).

2.3.1. Determination of the minimum inhibitory concentration for the selected bacterial isolates

The isolated bacterial strains were examined for resistance to heavy metals. By determining the minimum inhibitory concentration (MIC). The bacteria were inoculated with different concentrations of the heavy metals under study (125 to 2800) mg/L. as an amount (0.1 ml) of the bacterial suspension was spread in dishes containing (20 ml) of nutrient agar, containing different concentrations of heavy metals under study, and then growth on plates after 24 hours of incubation at 37 °C (14, 15).

2.3.2. Experimenting with bioremediation of heavy metals

For determining the ability of bacteria to remove heavy metals, sterile test tubes containing 5 ml of the liquid nutrient medium containing one of the heavy metals under study (chromium, cobalt, nickel, and lead) were prepared separately, in addition to the bacterial inoculum. The heavy metal concentrations were (25, 50 and 100 mg / L) and a pH of (7) and incubated at (33° C) in a shaking incubator at a speed of (120 cycles / min), where the proportion of biological treatment of heavy metals was examined after (1-5 days), control tubes were made Positive and negative to evaluate the removal rate.

The tubes containing concentrations (25, 50, and 100 mg/L) are taken out after the first day to the fifth day of incubation and then centrifuged at (6000) revolutions per minute for (15 minutes), the liquid is filtered and the absorbance rate is measured with an absorbance measuring device. (spectrophotometer) and at a wavelength of 357 nanometers for chromium, 510 nanometers for cobalt, 530 nanometers for nickel, and 380 nanometers for lead. The percentage of bioremoval of heavy metals is calculated by measuring the initial and final absorbance of the sample using the equation (8, 16):

Bioremoval % = initial absorbance - final absorbance / initial absorbance x 100

2.4. Bacteriological diagnosis using the VITEK-2 device

The VITEK-2 device was used to diagnose the selected bacterial isolates, which are characterized by accurate and rapid diagnosis, and to learn more about the biochemical characteristics of these environmental isolates used in the biological removal process. A bacterial, suspension was prepared from the selected bacterial isolate, which showed high efficiency in resistance to heavy metals, which were used in bio removal experiments for the purpose of diagnosing them using the VITEK 2 system, which included 47 biochemical and enzymatic tests approved for bacteriological diagnosis, which were grown on MacConkey agar medium and nutrient agar medium. It was incubated at 37°C for 24 hours. After preparing the samples, the carrying tube of the diagnostic card was immersed in the test tube and fixed on a special holder, then it was entered through the filling door to be transferred to the diagnostic card and then transferred to the loading door to be incubated at 37 °C and then the results were recorded after 4-6 hours. After that, all cards were automatically disposed of in a waste container prepared for the purpose of later destruction, and then the report on the diagnostic card was printed, according to the company's instructions.

3-RESULTS

3.1. Isolation of bacteria from sediment samples

Bacteria were isolated from sediment samples taken from the selected sites of the Diyala River through the method of serial dilution, then these isolated bacterial strains were cultured in nutrient agar media, and then the bacterial isolates were treated with a different set of biochemical procedures to characterize and diagnose them. 15 bacterial isolates capable of resisting heavy metals were collected at a concentration of (50 mg / L), and then they were sifted into 5 isolates by increasing the concentration to (100 mg / L) and then treating these bacterial isolates with different concentrations of heavy metals under study in order to select the isolates The most efficient and best in resisting high concentrations of heavy metals, in order to test them in the biological treatment experiment for heavy metals, and Figure(1) shows the selected bacterial isolates.

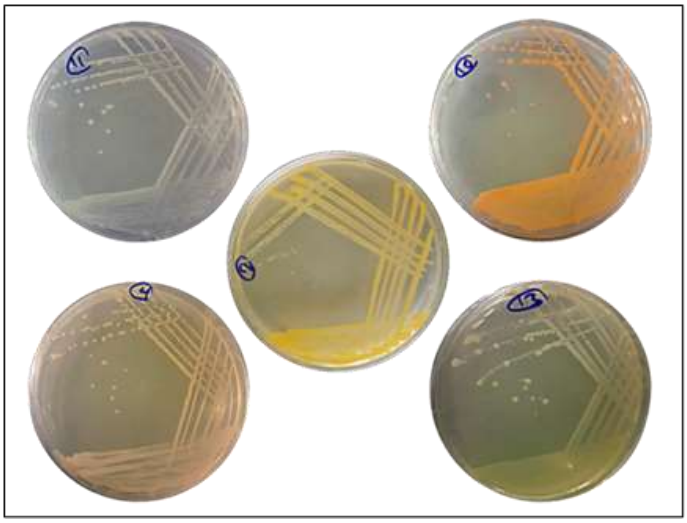

Figure (1): shows the growth of selected bacterial isolates on Nutrient Agar, isolated from sediment samples from the Diyala River

3.2. Biochemical Tests for Selected Bacterial Isolates

Several tests were adopted for biochemical diagnosis of the selected bacterial isolates, which are shown in Table (1), and some results of MR.VP., indole, growth on Simmons Citrate Agar, and tribal sugar agar TSI which are shown in Figure(2).

Table (1): shows the most important biochemical tests for the selected bacterial isolates

Sample ID	Gram stain	Shape	Oxidase	Catalase	MacConkey	Indole	TSI medium				VP	MR	Citrate
							Slant	Butt	H_2S	Gas			
Iso2	+	Cocci	-	+	-	-	A	A	-	-	-	+	-
Iso4	-	Rod	-	+	+	-	A	A	-	-	+	-	+
Iso10	+	Cocci	-	+	-	+	A	K	-	-	-	-	-
Iso11	-	Rod	-	+	+	+	A	A	-	+	-	+	-
Iso13	**+**	Rod	+	+	-	**+**	A	K	-	-	-	+	-
A= Acid , K=Alkaline , += Positive Result , - = Negative Results													

Figure (2): Some results of biochemical tests for the selected bacterial isolates

3.3. Resistance of bacterial isolates to heavy metals

The minimum inhibitory concentration (MIC) was determined for five bacterial isolates from sediment samples. , 1400, 1500, 1600, 1700, 1800, 2000, 2200, 2400, 2600, and 2800 mg / L) for the heavy metals under study (cobalt, chromium, nickel, and lead) and obtained the following results shown in Table (2).

Table (2): The minimum inhibitory concentration for the growth of bacterial isolates on heavy metals

No.	Isolates symbol	MIC (mg/L)			
		Co	Cr	Ni	Pb
1	ISO 2	500	1600	1400	1600
2	ISO 4	500	2200	1200	2200
3	ISO 10	500	2600	1200	1400
4	ISO 11	750	2000	1600	1200
5	ISO 13	500	2600	1400	1400

Several bacterial isolates showed resistance to different concentrations of the heavy metals under study, where the lowest inhibitory concentration of the selected isolates was shown in Table (2). of the most efficient isolates in resistance to cobalt up to a concentration of 750 mg / L, and most of the isolates showed resistance to chromium between a concentration (1600 - 2600 mg / L) and isolates (10, 13) were among the most efficient isolates in resistance to chromium up to a concentration of 2600 mg / L, and showed Most of the isolates are resistant to nickel between a concentration of (1200-1600 mg/l), and isolates (11) were among the most efficient isolates in resisting nickel up to a concentration of 1600 mg/l, and most of the isolates showed resistance to lead between a concentration of (1200-2200 mg/l) and were Isolate (4) is one of the most efficient isolates in resisting bullets up to a concentration of 2200 mg / L.

3.4. Removal of Heavy Metals

The ability of one bacterial isolate (Iso no.4) to remove different concentrations of heavy metals (chromium, cobalt, nickel, and lead) was tested. Figure (3) shows the stock standard solutions of heavy metals at a concentration of 3000 mg / L, which were adopted to prepare the different concentrations under study. The concentrations were tested (25, 50, 100 mg / L) for the biological treatment experiment, as the temperature of the vibrating insulator was determined at (33 ° C) at a speed of (120 cycles/min) for 5 days, and the pH was 7.

Figure (3): Standard solutions of heavy metals used in the biological removal process

The results are shown in Table (3) and the removal rate of chromium was (36, 55, 68, 73 and 77%) respectively for 5 days, and the removal rate of cobalt was (22, 34, 44, 54, and 59%) and the removal rate for nickel is (23, 40, 56, 68 and 80%) and the removal rate for lead is (58, 68, 74, 80 and 86%). Removal rates % by bacterial isolate no.4 of lead, nickel, cobalt, and chromium for five days are shown in Figure (5). Figure (4) and Figure(5) show that a variation of the ability of isolate no.4 to remove the heavy metals under study (chromium, cobalt, nickel, and lead), in different concentrations (25, 50, 100 mg / L) for 5 days. The isolate No. 4, showed high efficiency in removing lead due to the ability of this isolation to resist high concentrations of lead, as shown in Table (2), where the percentage of lead removal was at a concentration of 25 mg / L (100%) from the first day until the fifth day of incubating.

Table (3): The average percentages of removal of heavy metal by bacterial isolates

ISO.4		Chromium									
C mg/L	A_0	Day1		Day2		Day3		Day4		Day5	
		A_1	R%	A_1	R%	A_1	R%	A_1	R%	A_1	R%
25	0.826	0.380	54	0.223	73	0.169	80	0.118	86	0.089	89
50	1.410	0.960	32	0.735	48	0.458	68	0.387	73	0.294	79
100	1.990	1.572	21	1.127	43	0.870	56	0.792	60	0.734	63
Removal %		36		55		68		73		77	
ISO.4		Cobalt									
C mg/L	A_0	Day1		Day2		Day3		Day4		Day5	
		A_1	R%	A_1	R%	A_1	R%	A_1	R%	A_1	R%
25	0.031	0.023	26	0.018	42	0.015	52	0.011	65	0.009	71
50	0.079	0.062	22	0.053	33	0.041	48	0.034	57	0.028	65
100	0.163	0.135	17	0.121	26	0.109	33	0.098	40	0.094	42
Removal %		22		34		44		54		59	
ISO.4		Nickel									
C mg/L	A_0	Day1		Day2		Day3		Day4		Day5	
		A_1	R%	A_1	R%	A_1	R%	A_1	R%	A_1	R%
25	0.017	0.013	24	0.009	47	0.006	65	0.003	82	0.001	94
50	0.037	0.025	32	0.018	51	0.015	59	0.011	70	0.007	81
100	0.074	0.063	15	0.058	22	0.042	43	0.035	53	0.026	65
Removal %		23		40		56		68		80	
ISO.4		Lead									
C mg/L	A_0	Day1		Day2		Day3		Day4		Day5	
		A_1	R%	A_1	R%	A_1	R%	A_1	R%	A_1	R%
25	0.015	0.000	100	0.000	100	0.000	100	0.000	100	0.000	100
50	0.029	0.017	41	0.012	59	0.009	69	0.005	83	0.003	90
100	0.058	0.038	34	0.032	45	0.028	52	0.025	57	0.018	69
Removal %		58		68		74		80		86	

C initial concentration, A_0 initial absorbance, A_1 final absorbance, $R_{\%}$ removal percentage

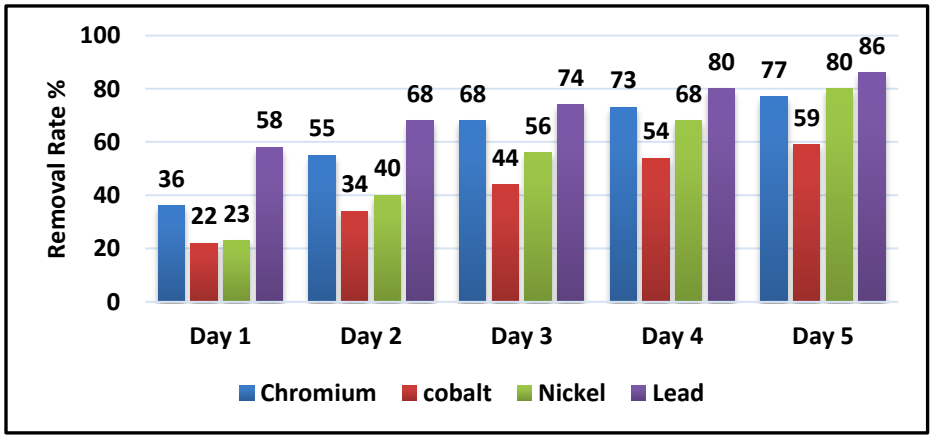

Figure (4): Removal rates % by bacterial isolate no.4 of lead, nickel, cobalt and chromium during fifth dayes

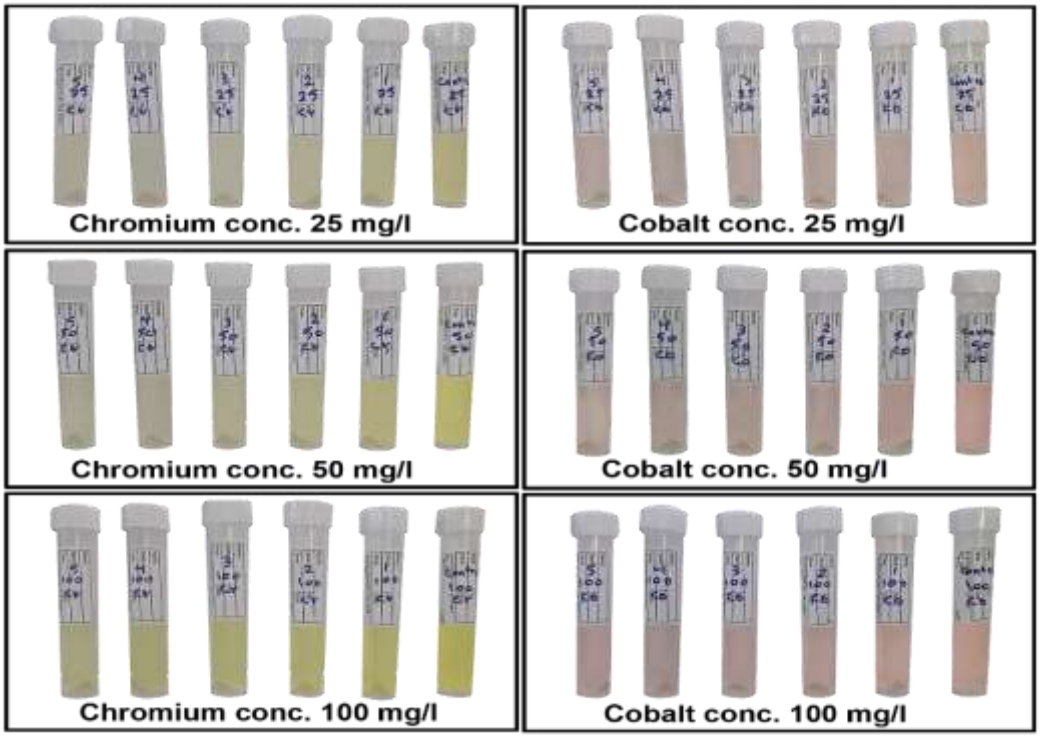

Figure (5): The ability of the bacterial isolates (no.4) to remove chromium and cobalt at a concentration of 25, 50,100 mg / L

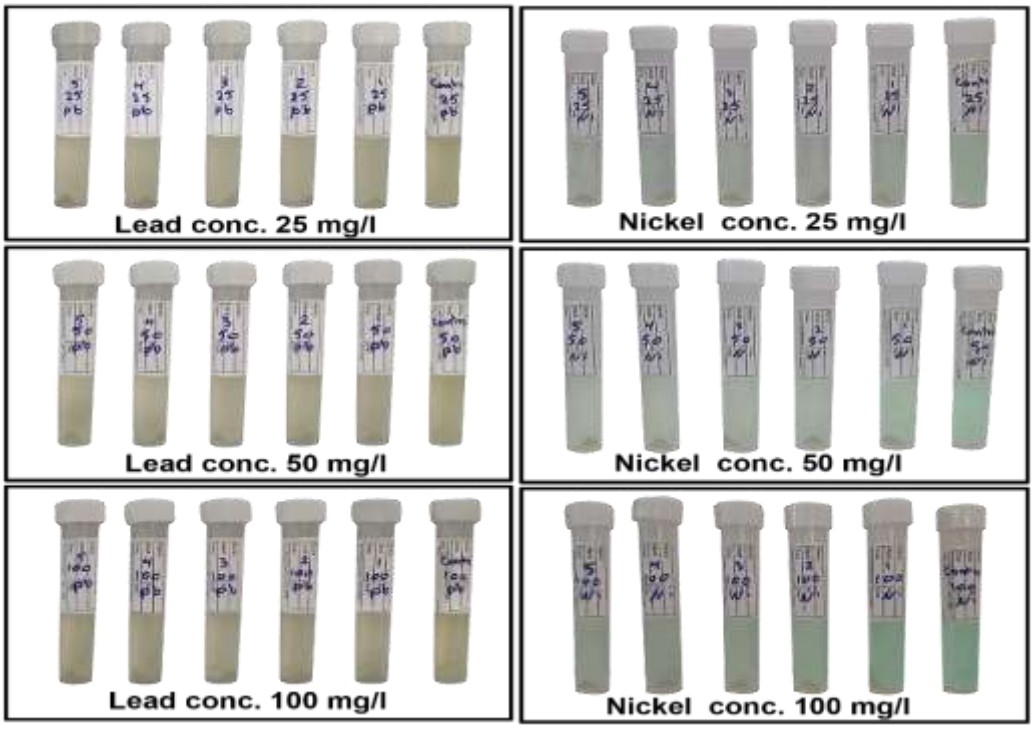

Figure (6): The ability of the bacterial isolate (no.4)to remove nickel and lead at a concentration of 25, 50,100 mg/L

3.5. Identification of bacterial isolates using the Vitek device

To confirm the initial diagnosis of the selected bacterial isolate and to know the races accurately, the diagnosis was resorted to using the Vitek device, which includes 47 vital tests for the selected bacterial isolates that are negative for Gram stain, as shown in Table (4).

The results of the diagnosis showed that there was diversity and heterogeneity in the results of the biochemical tests, as the results shown in Table (4) indicate that isolate 4 gave 23 positive tests out of 47 tests. Through the reports of the results of the examinations of the selected Gram-negative bacterial isolates, it was found that isolate 4 belongs to the genus Klebsiella pneumoniae, with a percentage of 99%.

Table (4): The most important biochemical and enzymatic tests for Gram-negative bacterial isolates

Iso 4 (Gr-)					
No	**VITEK TEST**		**No**	**VITEK TEST**	
1	**APPA**	**-**	**25**	**IARL**	**-**
2	**H2S**	**-**	**26**	**dGLU**	**+**
3	**BGLU**	**+**	**27**	**dMNE**	**+**
4	**ProA**	**-**	**28**	**TyrA**	**-**
5	**SAC**	**+**	**29**	**CIT**	**+**
6	**ILATk**	**+**	**30**	**NAGA**	**-**
7	**GLyA**	**-**	**31**	**IHISa**	**-**
8	**Ol29R**	**+**	**32**	**ELLM**	**-**
9	**ADO**	**+**	**33**	**dCEL**	**+**
10	**BNAG**	**-**	**34**	**GGT**	**+**
11	**dMAL**	**+**	**35**	**BXYL**	**+**
12	**LIP**	**-**	**36**	**URE**	**+**
13	**dTAG**	**-**	**37**	**MNT**	**+**
14	**AGLU**	**-**	**38**	**AGAL**	**+**
15	**ODC**	**-**	**39**	**CMT**	**-**
16	**GGAA**	**-**	**40**	**ILATa**	**-**
17	**PyrA**	**+**	**41**	**BGAL**	**+**
18	**AGLTp**	**-**	**42**	**OFF**	**+**
19	**dMAN**	**+**	**43**	**BAlap**	**-**
20	**PLE**	**+**	**44**	**dSOR**	**+**
21	**dTRE**	**+**	**45**	**5KG**	**-**
22	**SUCT**	**-**	**46**	**PHOS**	**+**
23	**LDC**	**+**	**47**	**BGUR**	**-**
24	**IMLTa**	**-**			

4-DISCUSSION

The resistance of some bacterial strains to heavy metals indicates their ability to adapt to a highly polluted environment where these bacterial strains can tolerate higher concentrations of specific heavy metals present in their surroundings and can reduce these metals locally (17). Bacterial isolates showed multiple tolerance to the various heavy metals under study, as this multiple tolerance to heavy metals indicates that the metals are not found alone in the environment. For example, cadmium is often associated with zinc, while cobalt is often associated with chromium (18).

The reason for the different levels of resistance of bacteria to heavy metals is due to the different concentrations of heavy metals in the environment, as environments contaminated with heavy metals give an opportunity for bacteria to adapt to the environment by developing different mechanisms for resistance and the difference in absorption mechanisms or the transformation of the element enzymatically by oxidation-reduction reactions to less toxic substances. Resistance of bacteria to heavy metals and antibiotics appears in polluted rivers

exposed to sewage and industrial and agricultural streams, as resistance factors to heavy metals and antibiotics occur jointly in systems or habitats contaminated with heavy metals exposed to sources of anthropogenic contamination (19).

Bacteria have several mechanisms to remove heavy metals. The cellular structure of the microorganism can trap heavy metal ions and then absorb them on binding sites in the cell wall (20). This process is called bioabsorption or passive absorption, and it is independent of the metabolic cycle. The amount of metal adsorbed depends on the kinetic equilibrium and mineral composition at the cellular surface. The mechanism involves several processes, including electrostatic interaction, ion exchange, precipitation, and a redox process (21).

The organism in which the heavy metals will accumulate must tolerate one or more of the metals at higher concentrations and must show improved transformative capabilities, changing toxic chemicals into harmless forms that allow the organism to reduce the toxic effect of the metal (22).

The mechanisms of uptake of heavy metals by various biosorbents depend on the cellular surface of microbes, as well as the exchange of element ions and complex formations with element ions on reactive chemical sites on the cell surface. All microorganisms have a negative charge on the surface of their cells due to the presence of anionic structures, which enable them to bind to metal cations. The negatively charged groups involved in the absorption of metals are the alcohol, amine, carboxylate, ester, hydroxyl, sulfhydryl, phosphoryl, sulfonate, and thiol groups (23).

Analysis of cell wall components, which are different for different microorganisms, helps in evaluating the uptake of metals by different microorganisms. The peptidoglycan layer of Gram-positive bacteria, which contains alanine, glutamic acid, meso-diaminobioleic acid, a glycerol polymer, and teichoic acid, and the layer of Gram-negative bacteria, which contains enzymes, glycoproteins, lipopolysaccharides, lipoproteins, and phospholipids, are the sites Active metals involved in the processes of connecting metals (24).

Metals and metalloids bind to these bonds on the surfaces of cells, which displace the essential metals from their normal binding sites. Once the metal and metalloids are bound, microbial cells can convert them from one oxidation state to another.

Biosorption, bioaccumulation, biotransformation, and biomineralization are the techniques microorganisms use to survive in a mineral-contaminated environment. These strategies have been exploited for treatment procedures (25). Living organisms or dead biological material can remove heavy metals.

The microbial cell develops resistance to heavy metals through the secretion of metal-chelating substances. Another mechanism of resistance involves the binding of a metal ion to intracellular molecules, such as metallothioneins, which leads to changes in the distribution of the metal ion (6).

Microorganisms interact with element ions through cell wall-bound metals, intracellular accumulation, extracellular polymeric interactions with transformation, extracellular packaging or immobilization of element ions, and element volatilization (25).

Various factors influence the microbial handling of items. They include mineral bioavailability to the microbe, contaminant concentration, electron acceptors, moisture content, nutrients, osmotic pressure, oxygenation, pH, redox potential, soil structure, temperature, and water activity. The bioavailability of each mineral in the sediment is affected by factors such as buffering capacity, cation exchange capacity, clay mineral content, metal oxides, and organic matter (26). In general, heavy element processing is done by removing the metal ion from the substrate to reduce the risk posed by exposure to such heavy metals.

Atikpo and Ihimekpen (27), confirmed in their study on Klebsiella pneumoniae obtained from soil contaminated with lead in a battery recycling plant in South Africa, where they showed the effectiveness of the bacteria in removing approximately 50% of lead from the solution within the first 3 hours at 80 and 500 ppm of lead These results showed that biosorption is responsible for the initial stage of lead removal, which acts as a means of concentrating lead on the surface of bacteria before biological precipitation occurs**.**

5-CONCLUSION

Up to 15 distinct bacterial isolates from the sediments of the Diyala River were isolated and they had the ability to resist the concentrations of heavy metals under study. For 5 days with different concentrations (25, 50, and 100 mg/l) of the metals chromium, cobalt, nickel, and lead, one bacterial isolate (no4) was tested and the results showed its ability to remove or reduce heavy metals in different percentages. The four metals were chosen in the biological removal process because these metals have an impact on human health due to their toxicity and their impact on vital systems if they are found in high concentrations in the environment. The biochemical characterization using the VITEK-2 system showed that the most efficient selective isolate belonged to the genus Klebsiella pneumonia.

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CONFLICT OF INTERESTS

The researchers confirm that there are no conflicts of interest for publishing this research paper.

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الإزالة الحيوية ومقاومة بعض العناصر الثقيلة بواسطة عزلات بكتيرية من رواسب نهر ديالى

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الخلاصة

خلفية البحث: المعادن الثقيلة هي ملوثات لا تتحلل، بل تدخل في السلسلة الغذائية، وقد تشكل مركبات سامة لها تأثير ضار على الوظائف البيولوجية. هناك عدد غير محدود من الكائنات الحية في البيئات المختلفة، بما في ذلك البكتيريا، القادرة على تحلل وتقليل المستويات العالية للعديد من الملوثات، والتي لم يكن معظمها مهمًا للباحثين بقدر اهتمامهم بالكائنات الحية الدقيقة المرضية. **المواد وطرق العمل:** تم جمع اثنتي عشرة عينة رسوبية من نهر ديالى ضمن حدود منطقة الدراسة والتي شملت أربعة مواقع رئيسية. تم عزل وتشخيص عدد من العزلات البكتيرية المميزة التي اظهرت احداها قدرتها العالية على النمو في بيئات الاستزراع ذات التراكيزالعالية من المعادن الثقيلة.

النتائج : امكن توصيف خمس عشرة عزلة بكتيرية مختلفة مظهريا قادرة على مقاومة المعادن الثقيلة بتركيز (50 ملغم/لتر)، تم اختيار خمس عزلات منها كأفضل عزلات قادرة على النمو ومقاومة الكوبالت بتراكيز بين (400-750 ملغم/لتر)، والكروم بين (400-750 ملغم/لتر). 1600 - 2600 ملغم / لتر والنيكل بين (1200 - 1600 ملغم / لتر) والرصاص بين (1200 - 2200 ملغم / لتر). أظهرت إحدى هذه العزلات بعد الاختبار (Iso 4) ، قدرة عالية على إزالة المعادن الثقيلة (الكروم، الكوبالت، النيكل، الرصاص) وبتراكيز (25، 50، 100 ملغم/لتر). حسبت نسبة الازالة على مدى 5 أيام، فكانت نسبة الإزالة لعنصرالكروم (36، 55، 68، 73، 77%) على التوالي، و نسبة إزالة الكوبالت (22، 34، 44، 54، 59%) و نسبة إزالة النيكل (23، 40، 56 ، 68 ، 80%) ونسبة إزالة الرصاص هي (58 ، 68 ، 74 ، 80 ، 86%). كما أظهرت العزلة نفسها كفاءة عالية في إزالة الرصاص نظراً لقدرتها على مقاومة التراكيز العالية بنسبة 100%عند تركيز25 ملغم/لترمن اليوم الأول حتى اليوم الخامس. تم اعتماد التشخيص البيوكيميائي للعزلات البكتيرية المختارة وتم التشخيص باستخدام نظام VITEK-2 للعزلة رقم 4 حيث أظهرت النتائج أنها تنتمي إلى جنس *Klebsiella pneumonia* .**الاستنتاجات:** يمكن استخدام هذه العزلات البيئية في العديد من تطبيقات وتقنيات المعالجة الحيوية لإزالة المركبات السامة بتكلفة رخيصة وآمنة.

الكلمات المفتاحية: الإزالة الحيوية، مقاومة الكائنات الحية الدقيقة، المعادن الثقيلة، الالتهاب الرئوي بكتيريا الكليبسيلا.