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The effect of some bacterial isolates in bioremediation of Cadmium

Asmaa Abd Ali ALrifai
Department of Water Resources, Technical Institute of Al-Mussaib, Al-Furat Al-Awsat Technical University, Babylon, Iraq
Corresponding author email: asmaaabd232@gmail.com

Najeh Hashem Kadhum
Department of Biology, College of Science, University of Kerbala, Iraq

Hassan Jameel Al-Fatlawy
Department of Biology, College of Science, University of Kerbala, Iraq

***Abstract*---**This study was conducted with the aim of applying Bioremediation technology in order to identify the efficiency of some bacterial isolates for improving the quality of wastewater discharging from a tank of Al-Hindiyah district sewage water treatment station in Karbala Governorate. The study included use of bacteria to remove heavy metals (Cd), as 120 bacterial isolates were isolated from wastewater before entering and after leaving the station under study, and it appeared that only 6 isolates of them were tolerant to high concentrations of heavy metals under study, up to 2,400 mg /L. These isolates were diagnosed with the Api20E technique and Vitek2 examination. Four isolates (A49, A35, A15, A1) belongs to the genus *Serratia marcesens* and the isolate A10 belongs to the genus *Enterobacter aerogenes*, while the isolate A32 refers to the genus *Klebsiella pneumonia*. The ability of these isolates was tested in a laboratory experiment in removing heavy metals Severally using the biomass of isolates, A35 isolation was characterized by achieving the highest removal rate for Cd, reaching 71.399% after 72 hours. Also, all isolates very high efficiency in removing (Cd) heavy metal from wastewater, with rates reaching 99.415% after 72 hours.

***Keywords*---**heavy metal, bioremediation, wastewater, bacterial isolates, Cd.

Introduction

Biotreatment refers to the use of biological systems to remove and reduce pollutants from wastewater, which are safe and low-risk methods (Ebrahimbabaie *et al.,* 2020). It can also be defined as one of the safest methods in which microorganisms are used to convert hazardous chemical compounds into safe materials and products (Sivasubramanian *et al.,* 2012). Bacteria have the ability to release large quantities of decomposing enzymes, which in turn break down hydrocarbon and organic materials and convert them into less complex materials and adsorption of a lot of heavy metals on cell wall surfaces in addition to their rapid growth and spread throughout the aquatic environment. *Micrococcus* strains are highly efficient at breaking down organic matter by their degradation enzymes (Ali *et al.,* 2015).

Mauro *et al.* (2003) in their study of the possibility of Chromatium and Bacillus bacteria on analyzing sulfates as well as nitrates and nitrites from wastewater, and their concentrations decreased significantly. Whereas, Arts *et al.* (2007) demonstrated the ability of bacteria to reduce phosphate, nitrate and nitrite concentrations of heavy water and store them in the form of food pellets inside their cells. Bacteria have the ability to secrete a large group of decomposing enzymes that break down organic and hydrocarbon materials and convert them into simpler materials and absorb a lot of heavy metals on their cell wall, as well as their spread throughout the water body and their rapid growth. *Bacillus, Pseudomonas* and some *Micrococcus* strains are highly efficient. To break down dissolved organic materials by their enzymes (Mary *et al.,* 2006).

Brandl and Faramarzi (2006) also indicated that demineralization by a living mass depends on growth and is affected by environmental factors. Conditions are different and affect according to their ability to remove heavy metals. In southern India, Raja *et al.* (2009) that the bacteria isolated from sewage, Proteus vulgaris, showed high resistance to nickel and did not sleep in the presence of chromium and lily, while Pseudomonas aeruginosa and Actinobacter radioresistens showed high resistance to lead. In Kenya, Atieno *et al.* (2013) that the bacteria isolated from wastewater and sheep and goat waste were resistant to lead, nickel, copper, mercury, cobalt and zinc the cause of resistance to the bacteria's possession was attributed to Metallothionein proteins.

Numerous studies have revealed the prevalence of the use of *Bacillus* and *Pseudomonas* in bio-adsorption of heavy gloss due to their good adsorption properties and various means of dealing with high concentrations of heavy metals, usually specialized with one or more types of minerals (Hookoom and Puchooa, 2013). Amrik Bhattacharya *et al.* (2018) indicate to the efficacy of *Serratia marcescens* and *Enterobacter cloacae* in bioremediation of cadmium, with removal rates reaching 96 and 98%, respectively, after 96 hours. Irawati *et al.* (2019) showed that *Klebsiella pneumoniae*, *Acinetobacter calcoaliticus* and *Escherichia coli* isolates isolated from Cikapundung River in Indonesia were resistant to copper metal. This work aims to investigate the efficacy of bacterial isolates isolated from public wastewater in the biological removal of cadmium and the extent of their resistance to high concentrations of it.

Materials and Methods

Water Sample collection

Samples of wastewater were collected from the sewage treatment station located in Karbala Governorate, Al Hindiyah District on the road leading to Babylon Governorate- Iraq. The station is supplied with water from the city of Al-Hindiya through the conveyor line, meaning that the sewage water in this station contains household waste, restaurant waste, and industrial waste.Samples were taken at a depth of 20 cm APHA(1999) using clean and sterile 500 ml glass bottles (bottles were closed after being sterilized with an Autoclave at a temperature of 121 C° and a pressure of 15 pounds for 15 minutes) Samples were taken before entering the water to the treatment station before treatment, from the sedimentation basin, and after entering the sedimentation basin for each sample with three replications. After collecting the samples, the bottles were closed again and under sterile conditions inside the bottle, after that they were kept in a floppy box containing shredded ice until they were transferred to the laboratory. The samples were examined and analyzed for all measurements in the graduate studies laboratory at the Faculty of Science, University of Karbala.

Isolation and diagnosis of bacterial isolates

A portion of the sample was taken and mixed well, then it was transferred by a sterile (Loop) conveyor and cultivated on media of nutrient agar, maconkey agar and sterile blood agar. The dishes were incubated at 37 C° for 24 hours in the incubator. The scientific method used worldwide for diagnosing bacteria and diagnosing the developing colonies according to what was mentioned in (Holt *et al.,* 1994). The diagnosis was confirmed using the API 20 system, and the VITEC 2 system was also used to confirm the diagnosis.

Screening of bacterial isolates

Preparation of Stock Solution for heavy metal salts the storage solution was prepared at a concentration of 1000 mg / liter by dissolving specific weights of cadmium chloride salts and sterilizing by cold method using a ready-made microbial sterilization unit Syringe Filter (0.45µm), the solutions were kept in sterile flasks (Sneath *et al.*, 1986).

Preparation of the solid nutrient medium containing minerals sterilized heavy metal salts solutions were added to the sterile solid nutrient medium of 60 C° and metal concentrations (25, 50, 100, 150, 200, 250, ... 600 mg / liter) were used separately and poured the medium into plastic Petri dishes and left To harden, the plates were planted after hardening by the streaking method of the bacterial isolates that were obtained from the above isolation process. The plates were incubated at a temperature of 37 C° for 48 hours and growth was observed and the results were recorded (Ahmed *et al.,* 2005), after which the bacterial isolates tolerant of high concentrations of the cadmium metal were selected and the rest were excluded Isolates.

Selected bacterial isolates carry high concentrations of heavy metals the prepared cadmium salts solutions were added as in the previous paragraph to the solid nutrient medium at a temperature of 60 ° C at different concentrations including (800, 1000, 1200, 1400, 1600, 1800, 2000, 2200, 2400 and 2600) mg / liter for testing isolates tolerant to high concentrations (Raja *et al.*,2009).

Evaluation of the efficiency of the selected isolates (biomass) in removing heavy metals the method described by Quintelas *et al.* (2008) was adopted in estimating the biomass ability of bacteria to remove heavy metals. The selected isolates were grown in a liquid nutrient broth (prepared according to the instructions of the supplier company (Oxiod) and sterilized with autoclave at 121 C° for 15 minutes at a pressure of 15 lb after being inoculated with 5% of the prepared vaccine (5 ml) activated vaccine is taken from the activated culture and added to 95 ml of cultivated medium (Nutrient broth) and placed in a vibrating incubator at a rotation speed (150 RPM) at a temperature of 37 C° for 48 hours, after which A central centrifugation was performed at a speed of 10,000 rpm for 15 minutes, the thin portion was discarded and the biomass collected under sterile conditions. 1 g of the biomass of the selected bacterial isolates was added separately to 100 ml of solutions of cadmium heavy metal salt at a concentration of 100 mg / liter. . The flasks were incubated in a vibrating incubator at a speed of (150 Rpm) and a temperature of (37 C°) for a period of (24, 48, 72) hours (Ray *et al.,* 2005; Stefanescu *et al.*, 2011). Then 10 ml is taken every day from the different concentrations for each mineral and for each bacterial species and centrifuged at a speed of 6000 rpm for 15 minutes to separate the filtrate, and then the concentrations of heavy metal ions in the filtrate are measured using a flame atomic absorption device (Philip *et al.* 2000). The removal percentage of the following heavy metals was calculated using the formula (Al-Enezi, 2014).

$$R\% = ((C0-C1) / C0) * 100$$

whereas : R = Percentage removal% C0 = concentration of heavy metal ions in the primary solution (mg / L). C1 = concentration of heavy metal ions in the final solution (mg / L).

Bioremediation of selective bacteria to wastewater samples were taken from the sewage station before the biological treatment of the water and the concentration of heavy metals in the samples was estimated before starting the biological treatment with a flame atomic absorption spectrometer, and then 100 ml of samples taken from the station were inoculated with (1 g) of the biomass from the bacterial isolates and the flasks were incubated in A vibrating incubator at a temperature of 37 C° and a number of cycles of 150 Rpm for a period of (24, 48, 72) and the results of the concentrations of heavy metals were taken as in the previous paragraph. Statistical analysis for all statistical analyses using the SPSS Version 9 program (IBM Corp., 2017). Significant differences among treatments were tested by using CRD analysis In all cases, under probability $P \leq 0.05$ was considered as significant.

Results and Discussion

Tolerance test of isolates selected for high cadmium concentrations 120 laboratories isolates were obtained by taking samples from the wastewater station in Al-Hindia district / Karbala governorate, by planting them in the medium of Nutrient agar broth. Screening was performed to distinguish the isolates (120 isolates) that have the ability to grow in agricultural media, containing concentrations of cadmium salts and concentrations of (25, 50, 100, 150, 200, 250, ... 600 mg /L) and the results of the screening showed obtained (6) bacterial isolates only, tolerant to a concentration of 600 mg /L of heavy metals. Table (1) shows the six selected isolates and their ability to withstand different concentrations of cadmium, including (800, 1000, 1200, 1400, 1600, 1800, 2000, 2200, 2400 and 2600) mg /L, as the selected isolates showed different growth behavior for the mentioned concentrations. , As it was observed that the highest concentration of isolate A35 was 2400 mg /L of cadmium metal, followed by isolates A15 and A49, which endured up to a concentration of 2200 mg /L, as for isolate A1, its growth was weak at concentrations 2000 and 2200 mg /L. As for isolates A10 and A32, their growth stopped at a concentration of 2,200 mg / L.

Table (1) The ability of the selected isolates to tolerate high concentrations of heavy metals

Cadmium concentrations mg / L										
Symbol of isolation	800	1000	1200	1400	1600	1800	2000	2200	2400	2600
A1	+++	+++	+++	+++	+++	++	+	+	-	-
A10	+++	+++	+++	+++	++	++	+	-	-	-
A15	+++	+++	+++	+++	+++	+++	++	+	-	-
A32	+++	+++	+++	+++	++	++	+	-	-	-
A35	+++	+++	+++	+++	+++	+++	+++	++	+	-
A49	+++	+++	+++	+++	+++	+++	++	+	-	-

Key: +++ intensive growth ++ medium growth + weak growth - no growth

Emenike *et al.* (2018) indicates that the toxicity of heavy metals varies depending on many factors, including isolation and the site of isolation, as some isolates tolerate high concentrations, while other isolates react negatively even at low concentrations of metals, and this explains the importance of the isolation site in obtaining the required isolation. Nupur *et al.* (2020) that a decrease in the level of growth with an increase in the concentration of heavy metals is an indication of the toxic effect of heavy metals on the growth of microorganisms.

Many researchers have also confirmed in different studies that bacteria isolated from metal-contaminated areas are more tolerant of heavy elements than non-contaminated environments (Yin *et al.*, 2019). Also, Liu *et al.* (2021) The response of isolates to heavy metals depends on many factors, including what concerns the isolation itself, and some of it depends on the type of metal that was chosen and the concentration of the mineral in the medium.

Diagnosis of Selected Isolates the six selected isolates were subjected to the culture and phenotypic tests. The results of the microbial culture of the broth

medium fed after (24 hours at a temperature of 37 C°) resulted in the emergence of bacterial colonies and according to what is shown in Table (2). As for the microscopic characteristics, after staining with a Gram stain, the bacterial isolates appeared after 24 incubation hours. They are all Gram negative. Table (2) shows the phenotypic and biochemical tests of the bacterial isolates isolated in this study.

Table (2) Physical and biochemical tests of bacterial isolates

Isolation Code	Gram stain	catalase test	oxidase test	lactose fermentation	cell form
A1	-	+	-	-	Red short Rod
A10	-	+	-	+	Rod colonies are white in color
A15	-	+	-	-	Short orange Rod
A32	-	+	-	+	Shiny floral olonies
A35	-	+	-	-	Short orange Rod
A49	-	+	-	-	Short orange Rod

(+) A positive result for the test, (-) a negative result for the test

Diagnosis of selected bacterial isolates using the Api20E diagnostic kit the Api 20 E diagnostic kit was used, which is characterized by its ease and accuracy of diagnosis, as the results were for the diagnosis of bacterial isolates as shown in Table (3) as the results showed that each of the isolates (A1, A15, A35, A49) belong to the genus *Serratia marcesens* with a diagnostic ratio (98.5 and 97, 97 and 95.9%), respectively. As for the isolate A10, it was found that it belongs to the genus *Enterobacter aerogenes*, with a diagnosis rate of 82%. The results of the examination of isolate A32 showed that it belongs to the genus *Klebsiella pneumonia*, with a diagnosis rate of 99.3%.

Table (3) results of biochemical examinations using Api 20E

Isolates						Test type	Test
A49	A35	A32	A15	A10	A1		
+	+	+	+	-	+	Beta-galactosidase	ONPG
-	-	-	-	-	-	Arginine dihydrolase	ADH
+	+	+	+	+	+	Lysine decarboxylase	LDC
+	+	-	+	+	+	Orinithine decarboxylase	ODC
+	+	+	+	+	+	Citrate utilization	CIT
-	-	-	-	-	-	H2S production	H_2S
-	-	+	-	-	+	Urea hydrolysis	URE
-	-	-	-	-	-	Deaminase	TDA

-	-	-	-	-	-	Indole production	IND
+	+	+	+	+	-	Acetoin production	VP
+	+	-	+	-	-	Gelatinase	GEL
+	+	+	+	+	+	fermentation of glucose	GLU
+	+	-	+	+	+	fermentation of mannose	MAN
-	+	+	+	-	-	fermentation of inositol	INO
+	+	+	+	+	+	fermentation of sorbitol	SOR
-	-	+	-	+	-	fermentation of rhamnose	RHA
+	+	+	+	+	+	fermentation of sucrose	SAC
+	+	+	+	+	+	fermentation of melibiose	MEL
-	-	-	+	+	+	fermentation of amygdalin	AMY
-	-	+	-	+	-	fermentation of arabinose	ARA
%95.9	%97	%99.3	%97	%82	%98.5	The percentage of diagnosed isolates	

(+) A positive result for the test, (-) a negative result for the test

Diagnosis of selected bacterial isolates using the Vitek2 device the results of the diagnostic tests for the selected isolates using the Vitek2 device, which included 64 tests, gave a diagnostic rate of the isolates as follows: -

- The isolates A1, A15, A35 and A49 belong to Serratia marcesens with a probability of 99% for each.
- A10 isolate belongs to Enterobacter aerogenes with a probability of 85%.
- A32 isolate belongs to Klebsiella pneumonia, with a probability of 99%.

Evaluation of the efficiency of the selected isolates in removing cadmium metal

Table (4) shows the percentage of removing cadmium metal after (24, 48 and 72) hours from a medium containing 1 gm of the live mass of bacterial isolates to 100 ml of saline solution of cadmium metal at a concentration of 100 mg / liter. The results showed in Table (4) that isolation A35 gave the highest removal rate after 72 hours, reaching 74.399%, while isolation A10 gave the lowest removal rate after 72 hours, which was 42.856%. Table (4) also shows that isolations A15 and A49 converged in the rate. Removal after 72 hours and reached (71,462 and 71,390) %, respectively, and no significant difference was observed between them.

Table (4) The percentage of cadmium removal by bacterial isolates (initial concentration is 100 mg / L)

Isolation	% Removal rate after time period			Average%
	24 Houres	48 Houres	72 Houres	
A1	9.213	23.262	42.856	25.110
A10	18.914	31.738	51.773	34.141
A15	23.303	45.170	71.462	46.645
A32	20.914	40.716	71.390	44.34
A35	23.657	40.821	74.399	46.292
A49	11.843	31.754	66.348	36.648
Average%	17.974	35.576	63.038	

• L.S.D for isolation at the level of 0.05 = 1.088

• L.S.D for the time at the level of 0.05 = 0.769
• L.S.D for the overlap between isolation and the time period at the level of 0.05 = 1.884

It was noticed from Table (4) that the rate of removal of cadmium after 24 hours was low and reached 17.974%, then it increased to (35,576 and 63,038)% after 48 and 72 hours, respectively. It was also observed that there was a high significant difference between the time periods of the experiment. It is noticed from Table (4) that there were no significant differences in the removal rate for isolating A35 and A15, while a highly significant difference was observed in the removal rate between isolates A35 and A15 on the one hand and the rest of the isolates on the other hand.

That the difference in levels of resistance between bacterial isolates may be due to the difference in the concentrations of different heavy metals in the environment and in the wastewater station water, which is one of the places contaminated with heavy metals, which may give the bacteria an opportunity to adapt to the environment either by developing resistance mechanisms or transmitting resistance genes through the plasmid The reason for the different levels of resistance is due to the different mechanisms such as the difference in absorption or the transfer of toxic metals, or in some cases that the metal is enzymatically transformed by means of oxidation and reduction reactions into chemicals that are less toxic than the original compound, or the reason may be due to the difference in the genetic diversity of the microorganisms as well as the nature of Interactions between microorganisms and minerals (Yin *et al.*, 2019 ; Wang *et al.*, 2021).

The results of the evaluation of the six isolates selected in removing the cadmium metal indicated that the isolates A35 and A49 belonging to the genus of bacteria *Serratia marcesens* were the most efficient in removing heavy metals, followed by the isolates A1 and A15 of the same genus, while the isolates A10 and A32 of the two genuses *Enterobacter aerogenes* and *Klebsiella pneumonia* They were less efficient at adsorbing heavy metals compared to *S. marcesens.*

Several studies have indicated the efficiency of bacterial isolates under study in removing heavy metals. Ramya & Boominathan (2017) showed that *Serratia* sp. Of the heavy metal-resistant species that were isolated from the sample collected from industrial effluents that contained metallic contaminants such as lead, zinc, copper, silver and mercury. Amrik Bhattacharya *et al.* (2018), he indicated the efficiency of *S. marcescens* isolate in bioremediation of cadmium, as the removal rate reached 96% after 96 hours.

And between Kumar *et al.* (2019) that bacterial isolates collected from industrial wastewater were tolerant to the cadmium metal that was traced back to the two genuses *Serratia sp.* and *Klebsiella pneumonia* had tolerated high concentrations of cadmium and the removal rate was (44.46 and 40)% for each of the two isolates, respectively, and when mixed together, the removal rate was 50.92%. Chen *et al.* (2019) a mechanism for absorption of cadmium by *S. marcescens* bacteria, as it was able to obtain isolation from soil contaminated with heavy metals in Hunan Province, China, and used it in treating cadmium from

wastewater, as the removal rate reached 80%. Cells and then intracellular uptake occur and it was found that a heavy metal binding protein (especially iron-binding protein), amino acid, histidine-binding proteins, and redox enzymes were responsible for removing cadmium. The results of the aforementioned studies were similar to what was obtained in our current study.

Bioremediation of selective bacteria to wastewater

Table (5) shows the rate of cadmium metal concentration and the percentage of its removal from the wastewater station water, as the results showed that the isolated A35 was significantly superior to the rest of the other isolates after 24 and 48 hours, as it reached (52.646 and 89.255)%, respectively. Whereas, A32 isolation was the lowest removal efficiency after 24 and 48 hours, as it reached (37,440 and 60,382)%, respectively. The six isolates excelled in removing the cadmium metal after 72 hours, and at a high rate, with no significant differences between the isolates.

It is also noted from Table (5) that the rate of cadmium removal reached 80.620% for isolating A35, followed by isolating A49 and A15, with a removal rate of (78.102 and 77.164)%, respectively, and no significant difference was observed among isolates (A35, A49, A15) while it outperformed the rest Other isolates. It is noticed from Table (5) that there is a high significant difference between the time periods, as the highest rate of removal of cadmium metal from wastewater reached 99.415% after 72 hours, and it significantly exceeded the rest of the time periods, while the rate of removal rate reached 78.482% after 48 hours. An hour, which was significantly higher than the 24-hour treatment, in which the percentage of cadmium removal from wastewater reached 47.38%.

Table (5) Cadmium removal% (initial concentration 1.883 mg /L) in the field experiment by bacterial isolates

Isolation	% Removal rate after time period			Average%
	24 Houres	48 Houres	72 Houres	
A1	48.079	78.509	99.363	75.317
A10	37.440	60.382	99.239	65.687
A15	48.752	83.643	99.097	77.164
A32	49.673	84.847	99.786	78.102
A35	52.646	89.255	99.961	80.620
A49	47.690	74.261	99.044	73.665
Average%	47.38	78.482	99.415	

• L.S.D for isolation at the level of 0.05 = 3.793
• L.S.D for the time at the level of 0.05 = 2.682
• L.S.D for the overlap between isolation and the time period at the level of 0.05 = 6.570

The results of the evaluation of the six isolates selected for removing heavy metals (cadmium) from the wastewater station water indicated that the isolates A35 and A49 belonging to the genus *S. Marcesens* and the isolate A10 belongs to the genus *E. aerogenes* were the most efficient in removing cadmium from Wastewater after the passage of (24, 48 and 72) hours. The remaining isolates of *S. marcesens*

varied in removing the mixture of heavy metals, while the isolate A32 of *K. pneumonia* was the least efficient in the absorption of cadmium and lead minerals compared to the rest of the other isolates. The isolate A15 of the genus *S. marcesens* is less efficient in removing nickel from wastewater.

It was observed through the results that the efficiency of the bacterial isolates differed in removing heavy metals from the sewage station water compared to their efficiency in the water containing the brine of heavy metals at a concentration of 100 mg / liter. This may be due to the fact that the bacteria were isolated from the wastewater and thus are adapted to heavy metals In it, or it may be due to the occurrence of some kind of interaction with wastewater as a result of its containing micro-organisms that interfere with the isolates under study. Bioremediation with the use of bacteria is one of the most effective management tools to get rid of environmental hazards such as heavy metals and it also works to break down and remove harmless pollutants using natural biological activity cost (Ojuederie and Babalola, 2017).

Bacteria are used as the most important microorganism in treating water contaminated with heavy metals, and the introduction of a mixture of bacteria or local bacteria could provide a potential bioremediation process for water contaminated with heavy metals without disturbing the target environment (Kang *et al.*, 2016). Application of mixtures of bacterial isolates may also be more efficient for heavy metal biological treatment compared to single strain cultures (Kang *et al.,* 2016). The highly toxic form of heavy metals can be changed to less toxic ones by microorganisms that are resistant to heavy metals through the reactions of their metabolic processes such as bioaccumulation and bio-transport, which work to detoxify heavy metals by microorganisms (Verma & Sharma, 2017).

The heavy metal bioremediation strategy depends mainly on the metabolic capabilities of the bacterial cells and many bacteria require different amounts of heavy metals as primary and essential micronutrients for their growth and development. The interactions between the bacterial cells and the heavy metal ion can be active or inactive depending on the metabolism process. Heavy metals in that a low concentration of the mineral promotes bacterial growth. Nevertheless, a high concentration harms the integrity of the cell membrane, cell organelles, and genetic material (Singh & Prabha, 2020). Several studies have indicated the efficiency of bacterial isolates under study in removing heavy metals. Concórdio-Reis *et al.* (2020) The genus *Enterobacter sp.* They removed lead from heavy water with a ratio of (91.6-93.9)%, and they indicated that this genus can be used in biological treatment and is safe to treat wastewater contaminated with lead.

Chen *et al.* (2019) that the living cells of *S. Marcescens* removed cadmium from 97.12%, while the dead cells had a percentage of removing cadmium 86.1% of wastewater. Irawati & Tahya (2021) demonstrated the ability of three bacterial isolates isolated from the Sukolilo River in Indonesia, is *Enterobacter cloacae* 1, *Enterobacter cloacae* 4a, and *Serratia nematodiphila* to remove copper by (96.68, 98.31 and 99.03%), respectively, and showed that the bacteria isolated from contaminated water Heavy metals are more efficient in removing heavy metals.

They recommend using bacteria isolated from wastewater in treating heavy metal pollution because it is environmentally adapted to it.

Conclusions

The application of biological treatment technology to remove pollutants from the water, being environmentally friendly and low in cost. The possibility of obtaining a local bacterial isolates that have the ability to remove heavy metals from wastewater, as the isolates A35 and A49, which belong to the genus of bacteria *Serratia marcesens*, were distinguished in the removal of heavy metals, followed by isolation A10 of the genus *Enterobacter aerogenes*. Conducting more isolation and diagnostic procedures to reach highly efficient microbial isolates to remove various heavy metals. Conducting more studies on the use of isolates selected in this study in wastewater treatment in other areas.

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