Baghdad Science Journal

Volume 22 | Issue 8 Article 13

8-15-2025

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How to Cite this Article

Kader, May A. Abdul and Mohammad, Ghada A. (2025) "Ability of *Staphylococcus epidermidis* and *Escherichia coli* to Eliminate Cadmium, Lead and Copper from Tigris River in Mosul City," *Baghdad Science Journal*: Vol. 22: Iss. 8, Article 13.
DOI: https://doi.org/10.21123/2411-7986.5024

BAGHDAD SCIENCE JOURNAL 2025;22(8):2612–2618

RESEARCH ARTICLE

Ability of *Staphylococcus Epidermidis* and *Escherichia Coli* to Eliminate Cadmium, Lead and Copper From Tigris River in Mosul City

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ABSTRACT

The heavy metals are non-biodegradable and could be toxic and carcinogenic, thus, their improper concentration in water could result in critical health issues for living organisms. Therefore, this study aimed to remove three heavy metals (Cadmium, lead and copper) by biological treatment especially using environmental nonpathogenic bacteria such as *Staphylococcus epidermidis* and *Escherichia coli*. Eight samples were collected from Tigris River from different regions of Mosul city. Two containers were used for each sample to measure the concentration of minerals before and after treatment by bacteria. The results showed that *E. coli* was more efficient in removing two heavy metals (cadmium and lead), while the *Staph. epidermidis* was more capable of removing copper. This may benefit the sustainable development of the environment in the future by using these bacteria to remove hazardous metals if they are present in large concentrations in the water.

Keywords: Bacteria, Bioremediation, Heavy metals, Sustainable development, Water

Introduction

Heavy metals (HMs) toxicity is considered the most destructive pollution comes to the world. They not only damage the environment and health but also constitute a large threat to all forms of life. Some microorganisms are a cost-effective, eco-friendly and effective alternative to remediating HMs contamination. Microorganisms may successfully remove and/or recover HMs from their environment in a variety of ways that are both efficient and simple to use.[1]

Since pollution poses a danger to ecosystems, human health, and the ability to pursue sustainable development, the use of microbiological treatment to remove HMs contamination has gained attention from scientists.[2] Due to the great diversity in the living world, bacteria provide a suitable source for the bioremediation of many pollutants. There are many materials (living or dead biomass) that can be continuously inspected to detect the effectiveness of bioremediation of HMs.[3] Generally, it has been confirmed that HMs biosorption offers a great sorption of capacity compared with bioaccumulation.[4]

The existence of HMs in wastewater has been growing with an increase in human activities and different industries e.g. batteries, electroplating industries, pesticides, textile industries, paper manufacturing and so on. They derive from many sources such as dyes, fertilizer and pesticides, some fixing agents that might be added with dyes to improve their adsorption with fibers, pigments, cleaning agents and bleaching agents.[5] The presence of large quantities of HMs in water leads to critical health issues for living organisms. The most common HMs are zinc (Zn), lead (Pb), cadmium (Cd), mercury (Hg), arsenic (As), chromium (Cr), Nickel (Ni), and copper (Cu). Although some

Received 4 November 2023; revised 26 April 2024; accepted 28 April 2024.
Available online 15 August 2025

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https://doi.org/10.21123/2411-7986.5024

Table 1. The sites of samples and dating.

No. of sample	Site	Date/time	
1	Al-khusar River in Al-Rifaee district.	1/10/2021	5:20 pm
2	Industrial district behind the Dekor factory for paint /at right.	4/11/2021	11:15 am
3	Industrial district behind the Dekor factory for paint /at left.	3/11/2021	11:20 am
4	Barmaid pours in valley pours in yoke Tigris in Rifaee district.	1/12/2021	10:00 pm
5	Barmaid in the Hawee Al-Kaneesa under the third bridge.	2/3/2022	12:30 pm
6	Al-Khausar river in Al-Rifaee district.	2/3/2022	11:30 pm
7	Barmaid in the Hawee Al-Kaneesa under the third bridge.	22/3/2022	6:15 pm
8	Al-Khusar river in 17Tamooz district.	22/3/2022	6:30 pm

others are detected in traces but they are considered as a serious agents. All of them are necessary and need to be removed.[6]

In particular, HMs cause different and many problems for the aquatic environment.[7] Any processes that occur through the action of microorganisms or their enzymes are defined as bioremediation which can eliminate pollutants and return the environment to its natural conditions, while biosorption results from the immobilization of any soluble metals sequestered by the cells of microorganisms. The development and progress in the various industrial fields and the corresponding increase in the quantities of waste significantly changed the toxic complexity of waste effluents. All these pollutants can be bio remediated by different microorganisms and plants.[8] Changes in the physiology and morphology of cells were observed for accumulation by increasing the concentration of metal ions. In addition, any organisms able to accumulate HMs could tolerate one or more metals at a higher concentration.[9] For this result, the toxic ions of HMs are transformed and detoxified into stable, nontoxic and inactive forms.[10] HMs may damage the membranes of microorganisms, but the bacteria possess properties of enzymatic activity which is required to beat these toxic effects.[11]

Due to the importance of using eco-friendly bacteria in the sustainable development of water generally, the research aimed to utilize non-pathogenic bacteria (*Staphylococcus epidermidis* and *E. coli*) to remove some HMs which include (cadmium, lead and copper) from water.

Materials and methods

Bacterial isolates

Two different bacterial species isolated from the environment (water) obtained from the College of Science/Department of Biology (one of them is *Staphylococcus epidermidis* presents gram-positive and *Escherichia coli* presents gram-negative bacteria) were used to test whether they were able to remove HMs.

Sample collection

Eight samples were collected from the Tigris River from different regions in Mosul city (as shown in Table 1) using sterilized containers of 50 ml.

Sample processing

After sampling, water was preserved immediately by acidifying with 1.5 ml concentrated nitric acid (HNO_3) (1M) to pH $<$ 2. Depending on (APHA,1985).[12] After the acidification, samples were stored in a refrigerator at approximately 4°C until the measurement to prevent changes in volume due to evaporation.

The experiment

Firstly, the concentrations of minerals were measured before any treatment by Atomic Absorption Spectrophotometer 210 VGP (USA) in the environmental research center/Mosul University, then the autoclaving was done to cancel any bacterial species effects presented in the sample. After that two containers were used for each sample; for the first container: 9 ml of water sample was mixed with one ml of overnight bacterial broth (*S. epidermidis* which was obtained from the Department of Biology/Collage of Science) in a concentration equal to 1.5×10^8 colony forming units/ml with a comparison of No. 0.5 MacFarland tube; in a second container, the process was repeated with (*E. coli* which was obtained from Department of Biology/Collage of Sciences), both containers were left for 5 days in shaking incubator at 37°C. Centrifugation was done at 10000 g for 10 minutes. After that the concentrations of minerals (cadmium, lead and copper) were measured by Atomic Absorption Spectrophotometer 210 VGP (USA) to notice the bacterial effect on the removal of dissolved minerals.[12]

Results

Our results generally have revealed a decrease in readings of almost all samples with both Staph.

Table 2. The HMs concentrations (ppb) before and after bacterial treatment.

No of sample	Cd (ppb)			Pb (ppb)			Cu (ppb)		
	Before	*Staph.*	*E.coli*	Before	*Staph.*	*E.coli*	Before	*Staph.*	*E.coli*
1	3.7	3.3	3	2.2	2	2	14	8.6	13
2	7	6.1	6.6	3	2.2	3	16.7	13.2	15
3	5.5	4.7	5	3.3	3.1	2	9.6	6.1	6.6
4	3.7	3.3	3	2	2	2	8.6	ND	ND
5	6.3	5.8	5.4	9	8	2.4	9.6	1.7	0.5
6	7.5	7	7	13.7	8.7	10	11	1.5	0.8
7	6.8	5.8	6.1	19.5	15.3	16.4	3.2	1.8	2.4
8	0.24	0.23	0.2	0.7	0.5	0.5	0.4	0.3	0.3
Mean	5.09	4.53	4.54	6.67	5.22	4.79	9.14	4.15	4.84
SD	2.43	2.18	2.30	6.78	5.04	5.51	5.30	4.72	6.06

Purple color = the treatment is better in *Staph. epidermidis*.
Pink color = the treatment is better in *E. coli*.
ND = Not Done.

epidermidis and E. coli bacteria for cadmium, lead and copper before and after bacterial treatment (Table 2). It observed that *Staph. epidermidis* (indicant in purple color) was quantitatively the most HMs remover from *E. coli* (indicant in pink color) where it was effective in Cadmium and lead in three sites while in copper in four sites.

However, to notice the amount of HMs decreased and which bacteria was the best at removing these three HMs, percentages were calculated and cleared up in (Table 3). In which, the three HMs (cadmium, lead and copper) had the highest reducing percentage by *E. coli* more than *Staph. epidermidis* and to resume the calculations we have subtracted the largest percentage minus the least percentage for all measurements.

The lead metal occupied the first rank of removing (95.55)% by *E.coli* as a total decrease, while copper had the second when gained a value equal to (66.16) % by *staph. epidermidis*, and finally the cadmium was the latest one as a reducible metal with (35.05) % using *E. coli*. Then the summation for all HMs studied by both bacteria showed that *E. coli* had approximately 150 % removal compared with *Staph. epidermidis* which had only 125 %.

In consequence, the E. *coli* was the more efficient in removing two HMs (cadmium and lead), while the *Staph. epidermidis* was the more capable of removing copper.

Discussion

The appearance of different removal percentages by the same bacteria (although it is used in the same concentration) is due to the different sources of the sample, i.e. different qualitative and quantitative pollution. The removal percentages of Pb by *E.coli* from the sites 1, 3 and 5 were past 9, 39 and 73% respectively, while for copper at sites 1, 3, 5 and 6 the removal was higher than 7, 31, 94 and 92% respectively, it may due to differences in pollution quality sources of these sites.[13]

The bacteria in their removal of HMs are affected by the degree of contamination of the sample through the content of organic and/or inorganic substances that could be affected positively or negatively on the bacterial treatment of these HMs,[14] the first site was wastewater effluent, hence the removal value for lead in this site was less; however, the lead removal value increased in site 3 by *E. coli* because this site is in an industrial district and contains inorganic substances that enhance bacteria to utilize Pb. For site 5 the bacteria exceeded in removing this metal because of mixing our sample with river water resulting in sample dilution and increasing the bacterial ability to remove this metal as mentioned by.[15] As well as the affinity of HMs to bind to bacterial species is also influenced by some factors such as biological, chemical, and physical including the ability of ion exchange, organic and clay content, ligands presence, HMs concentration, and bacterial species.[16]

Our results come by the results of Khanafari and co-workers[17] who used another species of *Bacillus* to remove lead from sewage water, the uptake after 72 hrs. was 65% in pH 7, while Kumar[18] found that *Staphylococcus* spp. was the best at removing of lead than *Bacillus* spp. and *Pseudomonas* spp.

Bacterial populations are often capable of surviving environmental stresses (including the presence of HMs),[15] this may explain having the bacteria for some genes which responsible for HMs resistance, Our results could be explainable by Kumar,[18] who revealed that bacterial mobile genetic elements, such as

Table 3. The total percentages of HMs decreased by both bacterial treatment.

No of sample	Cd (ppb)			Pb (ppb)			Cu (ppb)		
	***Staph.* %**	***E.coli* %**	**Largest -Least (substrate)**	***Staph.* %**	***E.coli* %**	**Largest -Least (substrate)**	***Staph.* %**	***E.coli* %**	**Largest -Least (substrate)**
1	**10.81**	**18.91**	**8.1**	**9.09**	**9.09**	**0**	**38.57**	**7.14**	**31.43**
2	**12.85**	**5.71**	**7.14**	**26.66**	**0**	**26.66**	**20.95**	**10.17**	**10.78**
3	**14.54**	**9.09**	**5.45**	**6.06**	**39.39**	**33.33**	**36.45**	**31.25**	**5.2**
4	**10.81**	**18.91**	**8.1**	**0**	**0**	**0**	**ND**	**ND**	**ND**
5	**7.93**	**14.28**	**6.35**	**11.11**	**73.33**	**62.22**	**82.29**	**94.79**	**12.5**
6	**6.66**	**6.66**	**0**	**36.49**	**27**	**9.49**	**86.36**	**92.72**	**6.36**
7	**14.7**	**10.29**	**4.41**	**21.53**	**15.89**	**5.64**	**43.75**	**25**	**18.75**
8	**4.16**	**16.66**	**12.5**	**28.57**	**28.57**	**0**	**25**	**25**	**0**
Mean	**10.31**	**12.57**		**17.44**	**24.16**		**47.62**	**40.87**	
SD	**3.80**	**5.34**		**12.72**	**24.32**		**26.30**	**37.13**	
Sum. of subtractions of *Staph. epidermidis*			17			41.79			66.16
Total sum. of substruction of *staph. epidermidis*			-125						
Sum. of subtractions of *E. coli*			35.05			95.55			18.86
Total sum. of substruction of *E. coli*			-150						

Purple color = the treatment is better in *Staph. epidermidis*.
Pink color = the treatment is better in *E. coli*.
ND = Not Done.

plasmids or transposons, can carry multiple genes encoding metal resistance. Thus exposure to one agent selected for microbial resistance to toxicants, the organisms may be important in performing biological processes in contaminated habitats.[19]

The impact of metals in the range of water and wastewater from beneficial is to seriously toxic through troublesome. Some of these metals are essential, while others could affect water consumers, wastewater treatment systems, and are receiving water. Depending on the concentration of these metals they could be either beneficial or toxic.

As the challenge of heavy metals removal from water and wastewater, they may need large amounts of bio adsorbents and some extra chemicals to keep a pH that gives suitable conditions for adsorption.[20] To control the biological growth copper salts are used in water supply systems in distribution pipes and reservoirs and to catalyze the oxidation of manganese. Lead is a hazardous cumulative body toxic. Original waters rarely contain more than 20 microgram/L, although the values as high as 400 microgram/L have been installed. Lead metal in the water supply may come from mine, industrial, and smelter discharges or from the dissolution of some old lead plumbing. The tap waters that are acidic, soft, and may be not suitably treated contain lead that results from an attack on lead service pipes.[12] Cadmium is significantly highly toxic and has been presented in some cases of poisoning through eating. Cadmium also causes some generalized cancers in many laboratory animals and has been linked epidemiologically with some human cancers.

The microorganisms' activity and metabolic diversity are considered to have tremendous potential in different waste treatment fields through metabolic pathways via cells. Some toxic compounds are used as energy sources for cellular processes e.g. respiration, fermentation, and metabolism.[21] Different types of microorganisms like some bacteria, fungi, yeast and algae can be able to efficiently accumulate HMs and bio-sorbents. Different types of functional groups which include carboxyl, carbonyl, sulfhydryl, sulfate, phosphate, hydroxyl and amino are identified on the cell walls of microorganisms, they are responsible for the binding of metals through different mechanisms, ion exchange, precipitation, adsorption, and coordination.[1] Some bacteria and cyanobacteria can remove HMs because their cell wall can capture these HMs. After all, the groups of negatively are charged within their fabric.[22]

Bioaccumulation and Biosorption are not as same, and they should not be used interchangeably. bioaccumulation (as done in our study) is a process metabolically–active where the microorganisms uptake HMs into the intracellular space by using importer complexes that create a translocation pathway through the lipid bilayer. Once inside the intracellular space, the HMs can be sequestered by proteins and peptide ligands (i.e. storage system).[23] In contrast, biosorption is the adsorption of some particles to the biological matrix by using some physical interactions (electrostatic forces), chemical interactions (ion or proton displacement), chelation, or complexion. At the original pH, the extracellular surface of microorganisms contains anionic moieties that provide binding sites for cationic HMs.[24]

Conclusion

This study clarified the role of bacteria in removing some heavy metals from the Tigris River in Mosul city. The bacterium *E.coli* was more efficient in removing cadmium and lead, while *Staph. epidermidis* was more capable of removing copper. We conclude from the above, that there is an important role of bacteria in the sustainable development of water due to their metabolic activity, which helps in overcoming some major challenges in the environment.

Acknowledgment

The authors' sincerest thank goes to the University of Mosul, College of Science for the provision of facilities that strengthened the quality of this research exertion.

Authors' declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Tables in the manuscript are ours. Any images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- No animal studies are present in the manuscript.
- No human studies are present in the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at the University of Mosul.

Authors' contribution statement

M.A.A. contributed and design of the study, performed microbiological analysis and G.A.M. was responsible for field sampling, data collection. Both authors read and approved the final version of the manuscript.

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قدرة بكتيريا Staphylococcus epidermidis و Escherichia coli على ازالة الكادميوم، الرصاص والنحاس من مياه نهر دجلة في مدينة الموصل

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المستخلص

تعد المعادن الثقيلة مواد غير قابلة للتحلل ومن الممكن ان تكون سامة و مسرطنة عند تواجدها في المياه بتراكيز كبيرة وتسبب العديد من المشاكل الصحية التي تؤثر على جميع الكائنات الحية. تهدف الدراسة الحالية الى ازالة ثلاثة انواع من المعادن الثقيلة (كادميوم ، رصاص ، نحاس) باستخدام طرائق معالجة حيوية وبشكل خاص استخدام انواع من البكتيريا غير الممرضة والتي تتواجد في البيئة ومنها Staph. epidermidis و E. coli . جمعت ثمان عينات ماء من مناطق مختلفة من نهر دجلة في مدينة الموصل . ثم تم معالجة العينات وخفض الحامضية باستخدام حامض النتريك. وقد استخدام مكررين لكل عينة لقياس تركيز العناصر قبل وبعد المعالجة بالبكتيريا. أظهرت النتائج ان بكتيريا E. coli كانت الاكثر كفاءة لإزالة كل من عنصري الكادميوم والرصاص فيما تفوقت بكتيريا Staph. epidermidis في قدرتها على ازالة عنصر النحاس، مما قد يفيد مستقبلا في التنمية المستدامة للبيئة من خلال استعمال هذين النوعين من البكتريا في ازالة هذه المعادن الخطرة اذا تواجدت في المياه بتراكيز عالية.

الكلمات المفتاحية: البكتريا، الإصلاح الحيوي، المعادن الثقيلة، التنمية المستدامة، الماء.