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Bioremediation of Heavy Metals Using *Staphylococcus* sp. in Shatt Al-Arab River

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Abstract

Many species are resistant to heavy metals in their surrounding polluted environment and *Staphylococcus* sp. is an example. This study aimed to isolate and characterize bacteria resistant to heavy metals in the Shatt Al-Arab River in southern Basra, Iraq. Based on the morphology and using Vitek II system, and due to their high resistance to heavy metals (mercury and chromium), two species of *Staphylococcus* (*Staphylococcus lentus* and *Staphylococcus lugdunensis*) were chosen and isolated. The minimum inhibitory concentration (MIC) of the isolates against Hg and Cr was determined after 72 h. of incubation in solid media. All isolates were resistant to Hg (2000 mgL^{-1}) and Cr (4000mgL^{-1}). Living biomass of *S. lentus* and *S. lugdunensis* was used to remove the heavy metal ions in various concentrations (5, 10 and 25 mgL^{-1}) of the solutions of aqueous metals. After 72 hours incubation, the removal percentage of *S. lugdunensis* was 98.91 and 78.78% for Hg and Cr respectively. That for *S. lentus* it was 77.83% for Cr after 72 hours, and 98.84% for Hg after 24 h. of incubation. The scanning electron microscope approved that the removal of these metals causes morphological changes in bacteria.

Key Words: Heavy metals, *Staphylococcus* sp., Scanning Electron Microscope

المعالجة الحيوية للعناصر الثقيلة باستخدام انواع من البكتريا العنقودية في مياه نهر شط العرب

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الخلاصة

هنالك العديد من الانواع البكتيرية التي لها القابلية على مقاومة العناصر الثقيلة في بيئاتها الملوثة، ومن ضمن تلك الانواع بكتريا المكورات العنقودية *Staphylococcus* sp. . هدفت الدراسة الحالية لعزل وتشخيص انواع من البكتريا التي لها القابلية على مقاومة العناصر الثقيلة في مياه شط العرب جنوب البصرة، العراق. حيث تم عزل نوعين من بكتريا المكورات العنقودية *Staphylococcus lentus* و *lugdunensis* اعتمادا على الصفات المظهرية *Staphylococcus* وباستخدام جهاز Vitek II ، وقد اختيرت لقابليتها العالية في مقاومة عنصري الزئبق والكروم.

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حدد التركيز الادنى للتحمل The minimum tolerance concentration للبكتريا وكانت جميع العينات مقاومة للزئبق (2000 ملغم/لتر) وللكروم (4000 ملغم/ لتر). استخدمت الكتلة الحية للبكتريا لإزالة العناصر الثقيلة بتراكيز مختلفة (10،5 و25 ملغم /لتر). وكانت نسبة الازالة لبكتريا 98.84%ـ *S. lugdunensis* للزئبق و78.78% للكروم بعد الحضن لمدة 72 ساعة. في حين بلغت لبكتريا 77.83% *S. lentus* للكروم بعد 72 ساعة حضن و.8498 % للزئبق بعد 24 ساعة من الحضن. أثبت استخدام المجهر الالكتروني الماسح Scanning Electron Microscope ان ازالة هذه العناصر سبب تغييراً مظهرياً في البكتريا.

Introduction

One of the most frequent and critical water pollutants are heavy metals. Due to their high toxicity, even at low concentrations, they persist in the environment and are harmful to species [1]. Metals differ from most organic pollutants because of their ability to accumulate in living tissues and their non-biodegradability, in addition to their possibility to enter the food chain where they would concentrate. Despite their importance as micronutrients and the presence of low dosages of heavy metals in plants and animals, growing heavy metals concentrations has negative consequences on the health of most living organisms [2].

The most prevalent sources of mercury pollution in wastewater are batteries, paint manufacturers, paper and pulp, oil refineries, and chlor-alkali production [3]. Some bacterial groups in areas polluted with mercury can exchange genes for mercury resistance due to constant exposure to toxic levels of mercury [4]. The most hazardous type being hexavalent chromium as it has a high oxidation potentiality, greater water solubility and its permeability across biological membranes at a high rate. Cr (VI) is recognized as a carcinogen and can cause lung, nasal and sinus cancers [5].

The physicochemical methods of remediation are costly and could be destructive to the environment [6]. As an alternative, bioremediation has been used as a friendly technology to the environment to remove the harmful pollutants [7]. It is an innovative technology to eliminate heavy metals in various areas [8]. Several studies have reported that bacteria are a potential means to remove heavy metals from the environment as an effective strategy, less expensive, their capability to receive largest amount of pollutants and being less harmful to the environment [8]. Numerous mechanisms have been developed by microorganisms in the presence of heavy metals and have gained genetic characteristics that mitigate the negative consequences of elevated metal ion concentrations [9, 10].

The bacterial cell wall is the first item that metal ions come in contact with, and solutes can accumulate on the cell wall's surface or inside the structure. Biosorption is strongly dependent on the cell wall's chemical functional groups such as carboxyl, hydroxyl, phosphonate and amine groups are among the functional groups found in the bacterial cell wall [11]. The use of microorganisms resistant to heavy metals in polluted water and soil has received an increasing attention due to various problems associated with the elimination of contaminants using conventional techniques [12].

The objectives of this study were to isolate and evaluate heavy metals resistant bacteria, determine the minimal inhibitory concentration (MIC) and use these resistant bacteria in the biological remediation of Shatt Al-Arab River.

Materials and Methods

Sample Collection

Water samples were collected in sterile 500 ml reagent bottles in 12 replicates from Shatt Al-Arab River in Basra, Iraq. The samples were transported to the laboratory using an ice box and stored at 4°C for later use.

Isolation and Identification of Bacteria

The bacterial species were isolated from the water samples on mannitol salt agar. They had been morphologically characterized based on gram staining and identified based on biochemical reactions by Vitek II system (VK2C8300, USA) [13].

Minimal Inhibitory Concentration (MIC)

The MIC of resistant bacteria to heavy metals was determined in stages by increasing the concentration of heavy metals on the Luria Bertani (LB) agar plate until they could no longer form colonies on the plate. The starting concentration of 50mgL^{-1} was prepared from 1M stock solution. The stock solution of $HgCl_2$ and K_2CrO_4 was prepared in sterile deionized water and sterilized by autoclaving at 121°C for 15 min [14]. MIC was detected when the isolates did not grow on the plates after incubation [15]. After the culture had grown to a certain concentration, it was transferred to a greater concentration. MIC was conducted at 37°C for 72 hours. All experiments were conducted in triplicates.

Removal of Heavy Metal Ions Assay

The living biomass of heavy metal-resistant bacteria was used to eliminate Cr and Hg. The isolated bacteria were cultured for 24 hours at 37°C in a nutrient broth. Centrifugation at 6000 rpm for 15 min was used to collect the cells and then it was rinsed three times with normal saline. Mixture of 50 mg of cells and 50 ml of nutrient broth with varying metal concentrations was prepared (25, 50 and 100 mgL^{-1}). Bacteria were cultured in a shaking incubator at 120 rpm for (24, 48 and 72 hours) at 30°C (pH7) [16, 17]. The cells were extracted by centrifugation for 15 min at 6000 rpm each time. Heavy metals were determined in supernatant using an atomic spectrophotometer (AA7000-Shimadzu) [18, 19]. The amount of heavy metal removed from the solution was calculated from

$$\%R = (A-B)/A*100$$

Where is the R = % of removal, A = Primary concentrations of heavy metals, B = Last concentrations of heavy metals [20].

Scanning Electron Microscopy (SEM)

SEM microscopy (Tescan Mera III, Czech Republic) was used to investigate the metal treated cells to see how it affected the characteristics of the cell surface.

Using double-sided tape, the samples were adhered to the brass pieces. With a secondary electron detector, the images were captured at a 200 kV acceleration voltage.

Statistical analysis was achieved using a Nova test using SPSS ver. 26.

Results

Isolation and Identification of Bacteria

Two gram-and catalase-positive bacterial species were isolated and identified presumptively as *Staphylococcus lentus* and *Staphylococcus lugdunensis* and subjected to identification by the Vitek II system (Table 1).

Minimal Inhibitory Concentration

From Table 2, the results of MIC show that after 72 hours of incubation, *Staphylococcus* sp. was more resistant to chromium (4000 mgL^{-1}) than mercury (2000 mgL^{-1}).

Table 1: The biochemical tests of *Staphylococcus* sp. by Vitek II system.

Biochemical Test	*S. lentus*	*S. lugdunensis*	Biochemical Test	*.S lentus*	*.S lugdunensis*
AMY	+	-	POLYB	+	+
PIPLC	-	-	dGAL	-	-
dXYL	+	-	dRIB	+	+
ADH1	+	+	ILATK	-	-
BGAL	-	-	LAC	-	-
AGLU	-	-	NAG	+	+
APPA	-	-	dMAL	+	+
CDEX	-	-	BACI	-	+
ASPA	-	-	NOVO	-	-
BGAR	-	-	NC6.5	-	+
AMAN	-	-	dMAN	-	-
PHOS	-	-	dMNE	+	+
LeuA	-	-	MBdG	-	-
ProA	-	-	PUL	-	-
BGURr	-	-	dRAF	+	+
AGAL	-	-	O129R	+	+
PYrA	-	+	SAL	-	-
BGUR	-	-	SAC	+	+
AlaA	-	-	dTRE	+	+
TYrA	-	-	ADH2S	-	-
dSOR	-	-	OPTO	+	+
URE	-	-			

Table 2: The MIC (mgL^{-1}) to heavy metals against *Staphylococcus* sp, after 72 hours of incubation

Heavy Metals	*Staphylococcus lentus*	*Staphylococcus lugdunensis*
Hg	2000	2000
Cr	4000	4000

Removal of Heavy Metal Ions by Staphylococcus sp.

The highest elimination percentage of Cr and Hg by *S. lentus* and *S. lugdunensis* was at a concentration of 10mgL^{-1} than 5 and 25mgL^{-1}. The capacity of *S. lentus* to eliminate heavy metals was 98.75% for Hg and 77.38% for Cr (Fig. 1). Meanwhile, the ability of *S. lugdunensis* was 98.63% for Hg and 80.48% for Cr (Fig. 2). The removal percentage of *S. lentus* was 77.83% for Cr after 72 h., while it was 98.84% for Hg after 24 h. of incubation (Figure 3). *S. lugdunensis* was 98.91% and 78.78 % for Hg and Cr respectively after 72 hours incubation (Figure 4).

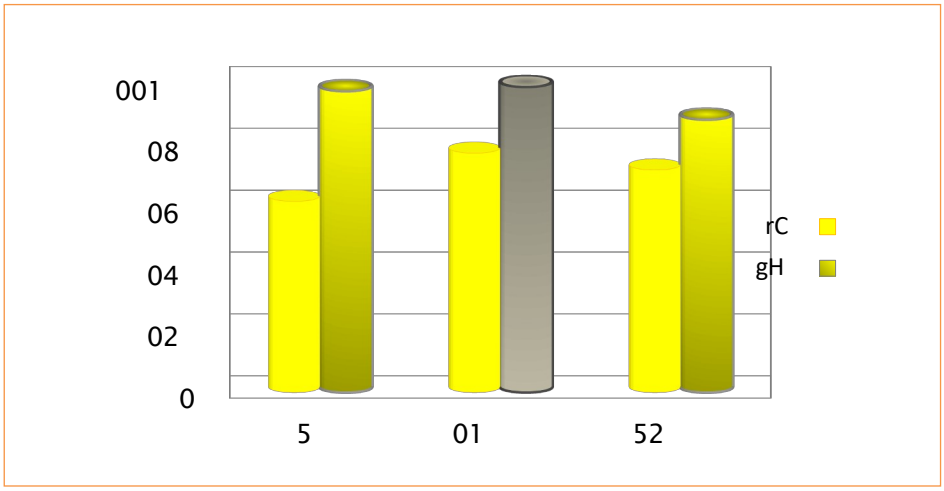


Figure 1: Heavy metal removal by *S. lentus* at different concentrations (5,10 and 25 mgL^{-1})

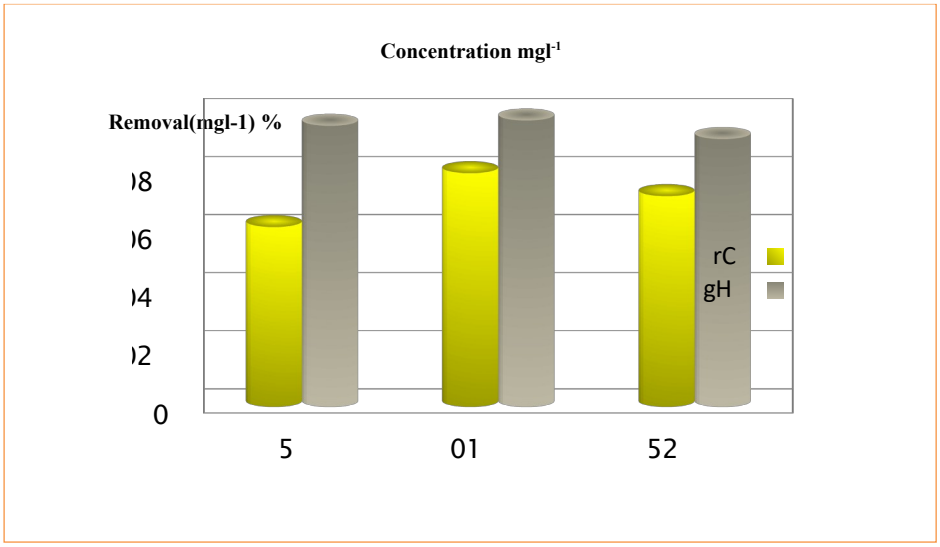
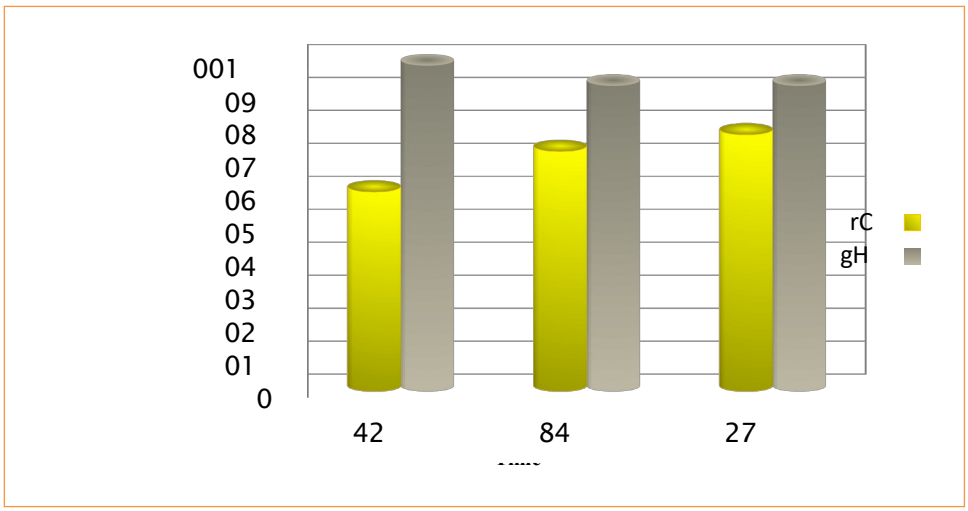


Figure 2: Heavy metal removal by *S. lugdunensis* at different concentrations (5,10 and 2 mgL^{-1})



.**Figure 3**: Heavy metal is removed by *S. lentus* in various times (24, 48 and 27 h.)

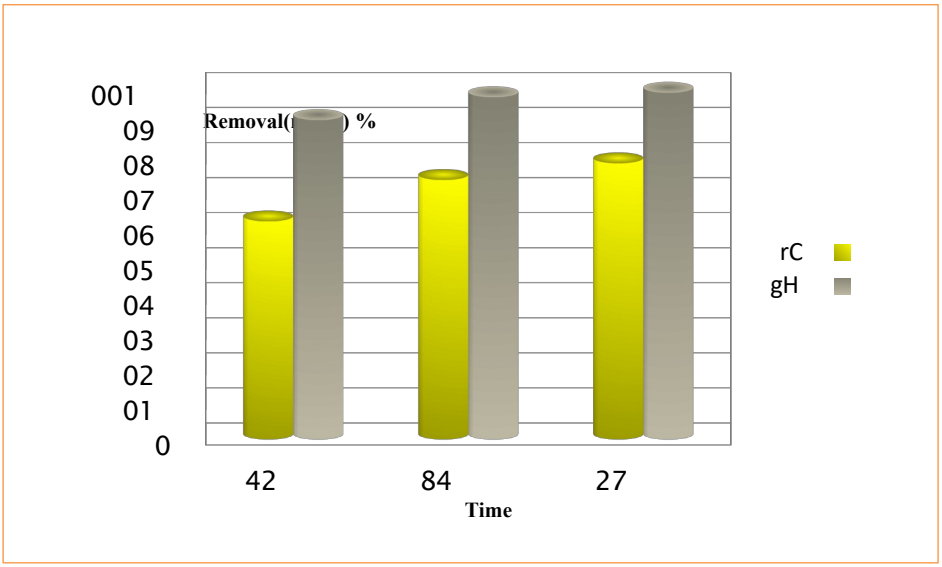


Figure 4: Heavy metal is removed by *S. lugdunensis* in various times (24, 48 and 27 h.)

Scanning Electron Microscopy

The SEM image of *S. lentus* treated with Cr and Hg at 10 mgL^{-1} concentration clearly showed a contraction of D1 and D2 cells (Fig. 5 B and C). The SEM image, on the other hand, demonstrated that the surface area of the Hg-treated D3 cells was greater than that of the untreated cells (Fig. 5 C). However, in comparison to the untreated cells, the surface area of the D1 and D2 cells treated with Cr increased in the SEM image of *S. lugdunensis* (Fig. 6 B). It can be seen as a cell attempt to acquire many metals. While the other cells treated with Cr and Hg clearly showed a contraction of the cells (Figure 6 B and C).

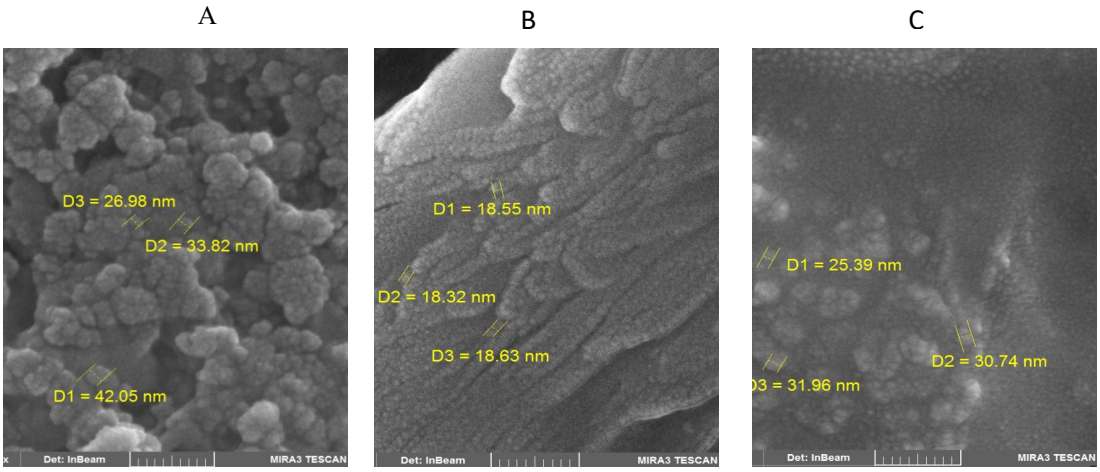


Figure 5: SEM microscopy *Staphylococcus lentus* 200 kx (A) in the control; (B) 10 mgL^{-1} of Cr; (C) 10 mgL^{-1} of Hg

The statistical analysis showed that there is no correlation coefficient between bacteria, metal concentrations and period of exposure (Table 3).

Table 3: Statistical tests between subjects' effects, dependent variable R %

Source	df	F	Sig.
Corrected Model	11	3.062	.011
Intercept	1	1268.740	.000
bacteria	1	.744	.397
metal	1	16.528	.000
TIME	2	1.121	.342
CON	2	4.181	.028
bacteria * metal	1	1.327	.261
bacteria * TIME	2	1.456	.253
bacteria * CON	2	.786	.467
Error	24		
Total	36		
Corrected Total	35		

a. R Squared = .584 (Adjusted R Squared = .393)

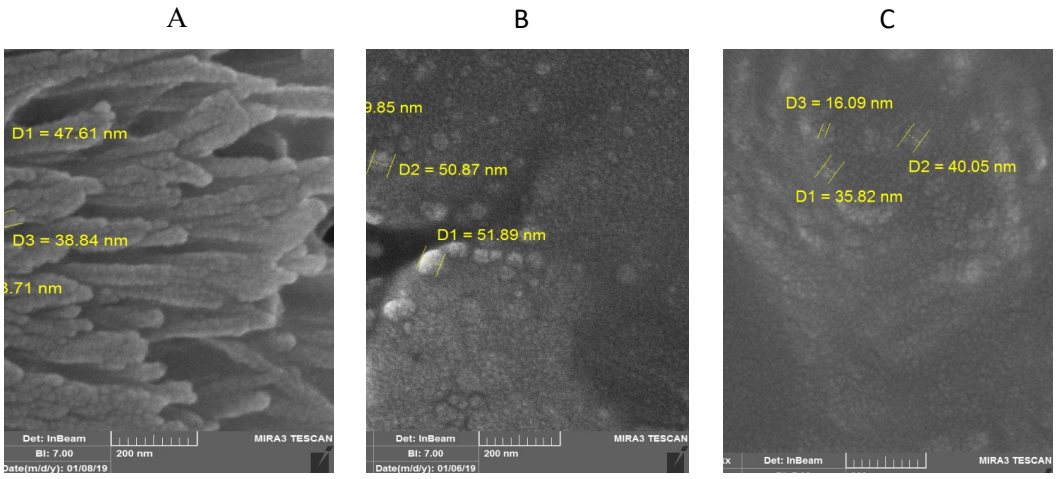


Figure 6: SEM microscopy *Staphylococcus lugdnensis* 200 kx (A) in the control; (B) 10 mgL^{-1} of Cr; (C) 10 mgL^{-1} of Hg

Discussion

The selected bacteria were characterized and identified using standard morphological tests and identified as *Staphylococcus lentus* and *Staphylococcus lugdunensis* by the Vitek II system. Precisely identified percentages of *S. lentus*, and *S. lugdunensis* were 95% and 90% respectively.

Minimal Inhibitory Concentration (MIC)

MIC completely inhibited bacterial growth by the lowest concentration of the heavy metals [21].

S. lentus and *S. lugdunensis* were able to tolerate Cr and Hg up to 4000 and 2000 mgL^{-1} respectively (Table 2). Gupta Mahendra *et al*. [22] observed that *Bacillus* sp. was resistant to heavy metals Cd, Cr, Ni and Co. Mercury resistance has been reported by *Staphylococcus* sp., *Pseudomonas* sp., *Proteus* sp., *Bacillus* sp., *Klebsiella* sp., *Salmonella* sp. and *Escherichia coli*. Sharma and Bansal [23] found that the resistance to heavy metals is species dependent.

The MIC of *Klebsiella* was 900gmL^{-1} for Zn and 700gmL^{-1} for *Staphylococcus*. While the MIC of *Bacillus* for copper after 24 h. and at 30 ℃ was 500 μgmL^{-1}. Tayang and Songachan [24] found that *Staphylococcus* sp. can remove Cd (44%) and Cu (34%) from soil.

Bacteria possess several resistance systems to tolerate heavy metals. One of these systems could be encoded by genes on chromosomes [25]. However, the loci that give resistance are more commonly seen on plasmids. Iyengar and Usha [26] and Abdelatey *et al*. [25] found that isolated *S. saprophyticus* sub sp. *bovis* strain has maximum tolerance for Cr, reaching up to 3000 μgmL^{-1}. While Rajbanshi [27] observed that the resistance of *Staphylococcus* sp. to Cr was 500 μgmL^{-1}. Adekanmbi and Falodun [28] found that about 95.5% of *S. aureus* were highly resistant to Cr and up to 72.7% tolerant to metal at 1500 μgmL^{-1}. Resistance to heavy metals is thought to be owing to a range of detoxifying mechanisms established by resistant bacteria. Exopolysaccharide complexation, bacterial cell envelope binding, metal reduction and metal flow are a few examples. Some of these methods are sometimes encoded in plasmid genes that can promote the transfer of hazardous metal resistance from one microorganism to another [29].

Removal of Heavy Metal Ions by Staphylococcus sp.

The elimination ability of *S. lentus* and *S. lugdunensis* for Cr and Hg was at 10mgL^{-1} concentrations (Fig. 1 and 2). These results are consistent with Baldiris *et al*. [5] who found that the percentage of removal of chromate by *Stenotrophomonas maltophilia* differed depending on the concentrations of this metal under optimum conditions (pH,7.0 and temp. 37°C).

The elimination efficiency is in inverse relation to the concentration of the impurities [30]. Adsorption has always been shown to decrease as the initial concentration of adsorbent in solution increases and the opposite is true [31]. The elimination rate raised over time and peaked through 72 h. of incubation (Fig. 3 and 4). Muneer *et al*. [32] observed that the removal of heavy metals depends on the type of microorganisms. *Bacillus* sp. was reported to be capable of reducing hexavalent chromium in its trivalent state, according to Rehman *et al.* [33]. After 96 h., these bacteria were able to eliminate 91% of the chromium in the medium. While Smrithi and Usha [34] found that *Bacillus* sp. can remove chromium with an increase in time. The removal of Hg by *S. lentus* after 24 h. was 98.84% and that accordance with Saranya *et al.* [35] who found that after 24 h. of incubation the removal of Hg by *Vibrio fluvialis* was 60% at a concentration of 100 μgmL^{-1}. *Staphylococcus aureus, Pseudomonas*

aeruginosa, Proteus vulgaris, Klebsiella pneumoniae and *Escherichia Coli* cultures were mixed. Chromium (33.4%), nickel (73.9%), zinc (90.1%) and cadmium (100%) were all eliminated from the oil refinery's wastewater [36].

The structure and elements of the cell wall, such as peptidoglycan, teichoic acids, lipoteichoic acids which are all required chemical components of bacterial surface structures, play a major role in metal biosorption by biomass [37]. The bacterial cells surfaces are covered in negatively charged phosphate and carboxyl groups, as well as positively charged amino groups. Based on pH, heavy metals can adsorb significantly on the bacterial surface [1].

Scanning Electron Microscopy

The exposure of *S. lentus* to Cr and Hg led to changes in the cells. This change in the properties of the surface can be explained as a negative reaction to a greater metal absorption by reducing the contact area with the metal. As seen in the case of these bacteria, which were treated with Cr and Hg, the degree of contraction could differ depending on the metal. This represents two different reactions to metallic stress. Also, the cells exposed to Hg differ from the untreated. This could indicate that the cell is attempting to gather more metals [38].
As compared to the untreated cells of *S. lugdunensis,* the surface area of the cells treated with Cr and Hg increased (Fig. 6B and C). This change in the properties of the surface can be explained as a negative reaction to a greater metal absorption by reducing the contact area with the metal [38].

The SEM images clearly showed morphological changes and deposition of heavy metals in the *Staphylococcus* sp. grown in a heavy metal state (Fig. 5 and 6). The bacterial cells in the control state were intact, transparent and had a smooth surface (Fig. 5a and 6a). Whereas in the presence of Cr and Hg and due to their toxic effects, the cells became distorted and adhered to each other. The adhesion and physical deterioration of the bacterial cells showed a reduction in the exposure of the total surface area to the toxicity of heavy metals. The cells loaded with heavy metals appeared dense, well filled and deposited on the surface of the cells [39]. The deposition of heavy metals on the cell surface describes the phenomenon of adsorption under load of heavy metals [40].

Conclusion

From results obtained by our investigation, we can conclude that *S. lugdunensis* and *S. lentus* can remove heavy metals from polluted water especially Cr and Hg. Furthermore, these bacteria were the habitants of polluted water and, hence, could be exploited through bioremediation.

References

[1] J. B. Sharma, S.; Patial, S.; Singh, S.; Bala, P. , ""“Multiple metal resistances and its correlation to antibiotic resistance in bacteria isolated from industrial effluent” " *International Journal of Current Research in Life Sciences, ,* vol. 7, pp. 1842-1847., 2018.

[2] Y. K. Zhao, D.; Chen, Z.; Zhan, J.; Wu, X. , "Removal of Chromium Using Electrochemical Approaches: A Review " *Int. J. Electrochem. Sci.,* vol. 13, pp. 1250 – 1259., 2018.

[3] Z. Khayyat Sarkar and V. Khayyat Sarkar, "Removal of Mercury (II) from Wastewater by Magnetic Solid Phase Extraction with Polyethylene Glycol (PEG)-Coated Fe3O4 Nanoparticles," *International Journal of Nanoscience and Nanotechnology,* vol. 14, pp. 65-70, 2018.

[4] A. M. Nascimento and E. Chartone-Souza, "Operon mer: bacterial resistance to mercury and potential for bioremediation of contaminated environments," *Genetics and Molecular Research,* vol. 2, pp. 92-101, 2003.

[5] R. Baldiris, N. Acosta-Tapia, A. Montes, J. HernándezR. Vivas-Reyes, "Reduction of hexavalent chromium and detection of chromate reductase (ChrR) in Stenotrophomonas maltophilia," *Molecules,* vol. 23, p. 406, 2018.

[6] Y. K. Leong and J.-S. Chang, "Bioremediation of heavy metals using microalgae: Recent advances and mechanisms," *Bioresource technology,* vol. 303, p. 122886, 2020.

[7] M. K. Choudhary, R.; Datta, A.; Nehra, V.; Garg, N. , "Bioremediation of Heavy Metals by Microbes," *In Bioremediation of Salt Affected Soils: An Indian Perspective,* pp. 233-255, 2017.

[8] A. Pambudiono, E. SuarsiniM. Amin, "The Potential of Indigenous Bacteria for Removing Cadmium from Industrial Wastewater in Lawang, East Java," *The Jurnal of Tropical Life Science,* vol. 8, pp. 62-67, 2017.

[9] S. Siddiquee, K. Rovina, S. A. Azad, L. Naher, S. SuryaniP. Chaikaew, "Heavy metal contaminants removal from wastewater using the potential filamentous fungi biomass: a review," *J Microb Biochem Technol,* vol. 7, pp. 384-395, 2015.

[10] T. Yang, M.-L. ChenJ.-H. Wang, "Genetic and chemical modification of cells for selective separation and analysis of heavy metals of biological or environmental significance," *TrAC Trends in Analytical Chemistry,* vol. 66, pp. 90-102, 2015.

[11] O. Abdi and M. Kazemi, "A review study of biosorption of heavy metals and comparison between different biosorbents," *J. Mater. Environ. Sci,* vol. 6, pp. 1386-1399, 2015.

[12] A. Mihdhir, A. Assaeedi, H. AbulreeshG. Osman, "Detection, identification and characterization of some heavy metals tolerant bacteria," *J Microb Biochem Technol [Internet],* vol. 8, pp. 226-30, 2016.

[13] A. M. Al-Taee, F. N. Alkanany, S. A. GmaisH. A. Alshawi, "Biodegradation of Aliphatic Hydrocarbons by Bacteria Isolated from Khor Al-Zubair Channel, Southern Iraq," *International Journal of Marine Science,* vol. 7, 2017.

[14] APHA, *Standard methods for the examination of water and wastewater / American Public Health Association, American Water Works Association, Water Environment Federation.*, 20th ed. ed. Washington, D.C: American Public Health Association, 1999.

[15] L. W. Marzan, M. Hossain, S. A. Mina, Y. AkterA. M. A. Chowdhury, "Isolation and biochemical characterization of heavy-metal resistant bacteria from tannery effluent in Chittagong city, Bangladesh: Bioremediation viewpoint," *The Egyptian Journal of Aquatic Research,* vol. 43, pp. 65-74, 2017.

[16] T. Tsuruta, "Separation of rare earth elements by microorganisms," *Journal of Nuclear and Radiochemical Sciences,* vol. 6, pp. 81-84, 2005.

[17] I. A. Stefanescu, L. Gavrila, R. D. Mocanu, R. I. OlariuC. Arsene, "Bioremediation perspective of Bacillus megaterium towards heavy metals in environments enriched with phosphogypsum," *Revista de Chimie,* vol. 62, pp. 245-249, 2011.

[18] B. Muneer, M. Iqbal, F. ShakooriA. Shakoori, "Tolerance and biosorption of mercury by microbial consortia: potential use in bioremediation of wastewater," *Pakistan Journal of Zoology,* vol. 45, pp. 247-254, 2013.

[19] L. Philip, L. IyengarC. Venkobachar, "ORIGINAL PAPERS Biosorption of U, La, Pr, Nd, Eu and Dy by Pseudomonas aeruginosa," *Journal of Industrial Microbiology and Biotechnology,* vol. 25, pp. 1-7, 2000.

[20] Y. Qin, B. ShiJ. Liu, "Application of chitosan and alginate in treating waste water containing heavy metal ions," 2006.

[21] R. Jaafar, A. Al-Sulaimi, A. Al-TaeeF. Aldoghachi, "Bioaccumulation of some heavy metals by metal resistant Bacillus thuringiensis isolated from soil in basra governorate-Iraq," *International Journal of Current Research,* vol. 7, pp. 16709-16713, 2015.

[22] K. Gupta Mahendra, K. Kiran, S. AmitaG. Shikha, "Bioremediation of heavy metal polluted environment using resistant bacteria," *J. Environ. Res. Develop,* vol. 8, pp. 883-889, 2014.

[23] J. Sharma and S. Bansal, "Shanu Patial, Shivani Singh and Preeti Bala, 2018.“," *Multiple metal resistances and its correlation to antibiotic resistance in bacteria isolated from industrial effluent” International Journal of Current Research in Life Sciences,* vol. 7, pp. 1842-1847.

[24] A. Tayang and L. Songachan, "Microbial bioremediation of heavy metals," *Current Science,* vol. 120, p. 1013, 2021.

[25] L. M. Abdelatey, W. K. Khalil, T. H. AliK. F. Mahrous, "HEAVY METAL RESISTANCE AND GENE EXPRESSION ANALYSIS OF METAL RESISTANCE GENES IN GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA PRESENT IN EGYPTIAN SOILS," *Journal of applied sciences in environmental sanitation,* vol. 6, 2011.

[26] C. A. Iyengar and M. S. Usha, "Removal of chromium by Staphylococcus saprophyticus subsp. bovis strain 1," *Biologija,* vol. 62, 2016.

[27] A. Rajbanshi, "Study on heavy metal resistant bacteria in Guheswori sewage treatment plant," *Our nature,* vol. 6, pp. 52-57, 2008.

[28] A. O. Adekanmbi and O. I. Falodun, "Heavy metals and antibiotics susceptibility profiles of Staphylococcus aureus isolated from several points receiving daily input from the Bodija abattoir in Ibadan, Oyo State, Nigeria," *Advances in Microbiology,* vol. 5, p. 871, 2015.

[29] N. Sindhu, R. K. Goyal, T. T. Pullan, T. RajanS. V. Madam, "Study on Al/TiB2 functionally graded metal matrix composites," *Materials Today: Proceedings,* vol. 44, pp. 2945-2951, 2021.

[30] O. Abdi and M. Kazemi, "A review study of biosorption of heavy metals and comparison between different biosorbents," *J Mater Environ Sci,* vol. 6, pp. 1386-1399, 2015.

[31] H. Agarwal, D. Sharma, S. K. Sindhu, S. TyagiS. Ikram, "Removal of mercury from wastewater use of green adsorbents–a review," *Electron. J. Environ. Agric. Food Chem,* vol. 9, pp. 1551-1558, 2010.

[32] B. Muneer, M. JIqbal, F. ShakooriA. Shakoori, "Tolerance and biosorption of mercury by microbial consortia: potential use in bioremediation of wastewater," *Pakistan Journal of Zoology,* vol. 45, 2013.

[33] A. Rehman, A. Zahoor, B. MuneerS. Hasnain, "Chromium tolerance and reduction potential of a Bacillus sp. ev3 isolated from metal contaminated wastewater," *Bulletin of environmental contamination and toxicology,* vol. 81, pp. 25-29, 2008.

[34] A. Smrithi and K. Usha, "Isolation and characterization of chromium removing bacteria from tannery effluent disposal site," *Int J Adv Biotechnol Res,* vol. 3, pp. 644-652, 2012.

[35] K. Saranya, A. Sundaramanickam, S. Shekhar, S. SwaminathanT. Balasubramanian, "Bioremediation of mercury by Vibrio fluvialis screened from industrial effluents," *BioMed Research International,* vol. 2017, 2017.

[36] E. E. Oaikhena, D. B. Makaije, S. D. Denwe, M. M. NamadiA. A. Haroun, "Bioremediation potentials of heavy metal tolerant bacteria isolated from petroleum refinery effluent," *Am J Environ Protect,* vol. 5, pp. 29-34, 2016.

[37] A. Pambudiono, E. SuarsiniM. Amin, "The Potential of Indigenous Bacteria for Removing Cadmium from Industrial Wastewater in Lawang, East Java," *Journal of Tropical Life Science,* vol. 8, 2018.

[38] V. Adarsh, M. Mishra, S. Chowdhury, M. Sudarshan, A. ThakurS. R. Chaudhuri, "Studies on metal microbe interaction of three bacterial isolates from East Calcutta Wetland," 2007.

[39] S. Sagar, S. Bajaj, D. Gola, A. Malik, P. Khankhane, R. KaurD. K. Singh, "A qualitative approach to nickel and lead uptake by heavy metal resistant bacteria Klebsiella sp. 10KN," *IJAR,* vol. 3, pp. 878-885, 2017.

[40] S. K. Das, M. MukherjeeA. K. Guha, "Interaction of chromium with resistant strain Aspergillus versicolor: investigation with atomic force microscopy and other physical studies," *Langmuir,* vol. 24, pp. 8643-8650, 2008.