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Tolerance assay of Pb resistant bacteria isolated from water of Ciujung River

Fajri Ikhsan [a,e,*], *Syarif Abdullah* [b], *Ahmad Shulhany* [c], *Dwi Isyana Achmad* [d], *Agusutrisno Agusutrisno* [f,g], *Hanif Fauzan Nadif* [a], *Ummul Mughfiroh* [a]

[a] *Department of Metallurgical Engineering, Faculty of Engineering, Universitas Sultan Ageng Tirtayasa, Jl. Jend. Sudirman Km. 03, Cilegon 42435, Indonesia*

[b] *Department of Mechanical Engineering, Faculty of Engineering, Universitas Sultan Ageng Tirtayasa, Jl. Jend. Sudirman KM. 03, Cilegon 42435, Indonesia*

[c] *Department of Civil Engineering, Faculty of Engineering, Universitas Sultan Ageng Tirtayasa, Jl. Jend. Sudirman KM. 03, Cilegon 42435, Indonesia*

[d] *Department of Agricultural Technology, Pontianak State Polytechnic, Jl. Jend. Ahmad Yani, Pontianak 78124, Indonesia*

[e] *Nanomaterial and Processing Technology Laboratory, Center of Excellence, Faculty of Engineering, Universitas Sultan Ageng Tirtayasa, Jl. Jend. Sudirman KM. 03, Cilegon 42435, Indonesia*

[f] *Department of Electrical Engineering, Faculty of Engineering, Universitas Sultan Ageng Tirtayasa, Jl. Jend. Sudirman KM. 03, Cilegon 42435, Indonesia*

[g] *Department of Electrical Engineering, Faculty of Information Science and Electrical Engineering, Kyushu University, 744 Motooka, Nishi-ku Fukuoka 819-0395, Japan*

[*] *Corresponding Author: fajri.ikhsan@untirta.ac.id*

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ABSTRACT

Bacteria are one group of some agents that are commonly used in biosorption. The Pb tolerance bacteria has been proved in many types of research. In the previous study, some bacteria were isolated from the Ciujung river, Serang, Banten water. This research studied the Pb maximum tolerance of those bacteria using the minimum inhibitory concentration (MIC) method. The isolates we assess in this research are IA4, IA_21, and IA_23 isolate. The bacteria inoculated on a nutrient agar (NA) plate medium that includes the various concentration of lead. The growth of bacteria is observed up to the minimum concentration of lead which bacteria cannot survive. The Pb maximum tolerance of IA4, IA_21, and IA_23 isolate is 30 ppm.

ABSTRAK

Bakteri adalah agen biologi yang sudah terbukti kemampuannya menyerap timbal (Pb) dalam proses biosorpsi. Pada penelitian sebelumnya sudah dilakukan isolasi beberapa bakteri yang mempunyai daya tahan terhadap Pb dari air sungai Ciujung. Pada penelitian ini, dilakukan pengujian kemampuan toleransi maksimum dari isolat-isolat bakteri tersebut terhadap logam timbal. Isolat-isolat bakteri yang diuji adalah isolat IA4, isolat IA_21, dan isolat IA_23. Pengujian kemampuan toleransi maksium dilakukan dengan metoda *Minimum Inhibitory Concentration* (MIC) dimana setiap isolat ditumbuhkan pada media yang mengandung variasi konsentrasi logam timbal. Pertumbuhan setiap isolat dipantau sampai pada konsentrasi timbal yang paling minimum dimana isolat-isolat tersebut tidak mampu lagi tumbuh pada konsentrasi tersebut. Kemampuan maksimum toleransi logam timbal untuk isolat IA4, IA_21, dan IA_23 yaitu 30 ppm.

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1. Introduction

In recent years, the industrial world has developed very rapidly. Various fields of industry are experiencing extraordinary changes. One of the many industrial fields that have progressed is the metal industry and the industry that involves metals in the manufacturing process. This situation has a dangerous impact on the environment. One of the negative impacts of industrial development involving metals is environmental pollution by heavy metals. Heavy metals are metals that have a density above 5 g/cm^3 [1]. In general, heavy metals in the environment come from two activities, namely geogenic activities and anthropogenic activities [2]. Geogenic activities can lead to metals in the environment, such as leaching and weathering of rock.

Meanwhile, anthropogenic activities are human activities that can increase the number of metals in nature, such as metal mining, pesticides, and smelting. In general, metals are divided into two major groups, namely essential metals and nonessential metals. Mn, Fe, Cu, and Zn are some examples of essential metals, while Cd, Pb, and Hg are some examples of nonessential metals [3]. Essential metals are needed in the body's metabolic processes in small amounts. Meanwhile, nonessential metals are not needed at all by the body. Even the presence of nonessential metals in the body could bring dangerous effects.

The body does not need nonessential metals because these metals are toxic. One of the nonessential metals harmful to the body is lead (Pb). Lead is a greyish, soft, highly malleable, and ductile metal with a density of lead is 11.34 g/cm^3. Based on its density, lead is a heavy metal. Lead is widely used in manufacturing processes in various industrial fields such as storage battery, manufacturing, printing pigments, fuels, photographic materials, explosive manufacturing, coating, automotive, aeronautical, and steel industries [4]. Although it has many benefits, lead also has harmful properties that could be dangerous to humans and other organisms. Lead is a toxic and persistent metal [5]. Lead accumulation in the body in high levels and long periods can cause various diseases included the nervous system, causing mental retardation and other nervous disorders. The accumulation of lead in a human's body will show symptoms if the lead level in this human's blood is higher than 1.93 μmol/L [6]. People suffering from lead poisoning may exhibit weakness, general disability, and eventual death. The effect is due to lead being able to inhibit the action of enzymes, change calcium metabolism in cells, bind to proteins in the liver, brain, and bones, and slow down nerve conduction [6-7]. The presence of lead in the environment must be addressed, and a mechanism for absorbing lead from a lead-polluted environment is required.

Several methods have been proposed to prevent lead contamination in the environment. In general, the method that has been proposed to restore lead polluted environment is divided into three parts, namely physical, chemical, and biological methods [8]. However, the methods that attracted the most attention among those three methods were the physical and chemical methods. The physical method is a method of recovering lead from the polluted environment by using physics principles. Several physical methods include ion exchange, membrane technology, reverse osmosis, evaporation recovery, and filtration. The chemical method is a method of removing lead from the polluted environment using chemical approaches. Some of the methods included in the chemical method are chemical precipitation, electrochemical treatment, and oxidation/reduction. On the other hand, it turns out that the physical and chemical methods still have several disadvantages, including not being eco-friendly, requiring high costs, and being inefficient for some contaminants. [9]. Some physical methods even require high costs for equipment installation, operation, and further processing of the sludge produced. Therefore, alternative methods that are eco-friendly, low in cost, and high efficiency are needed to solve those problems. The alternative method that is proposed is the biological method [10]. The biological method is a method of removing lead from the polluted environment using biological agents.

One of the biological agents that have been proven to absorb lead from the environment is bacteria. Every organism, including bacteria, that lives in an environment will adapt to the pressures of the environment. One of the factors that will press on the bacteria in an environment is the presence of heavy metals in that environment. Bacteria living in environments containing heavy metals will adapt with developing resistance to these metals. The level of resistance possessed by a bacterium living in a polluted environment against a heavy metal is highly dependent on the adaptability of the bacteria. Bacteria that have resistance to heavy metals are known to absorb these heavy metals. Thus, bacteria that are resistant to lead can absorb lead. Some bacteria that have been exhibited to be able to absorb lead are *Enterobacter cloacae* [11], *Bacillus sp, Pseudomonas sp, Micrococcus sp* [12], *Bacillus cereus* [13], *Bacillus Licheniformis* [14], *Stenotrophomonas maltophilia* [15]. Several heavy metal absorption mechanisms performed by bacteria have been reported. In general, the lead absorption mechanism of bacteria is divided into two ways, namely extracellular and intracellular mechanisms. The extracellular mechanism is an absorption mechanism performed by bacteria where the absorbed lead remains outside the cell. While the intracellular mechanism is an absorption mechanism performed by bacteria by converting lead into harmless species and accumulating lead in cells.

Previous studies isolated Pb-resistant bacteria from Ciujung River, Serang, Banten [16]. In this study, the maximum tolerance assay was performed on the three Pb-resistant isolates that had been isolated. The bacterial isolates tested in this study were IA4, IA$_2$1, and IA$_2$3 isolates. The maximum tolerance assay was conducted using the minimum inhibitory concentration (MIC) method. Minimum inhibitory concentration is usually used to evaluate antimicrobial potential [17]. The MIC value is defined as the lowest concentration of an antimicrobial capable of inhibiting microbial growth [18-19]. The MIC value will correlate with the maximum ability of a bacterium to tolerate the presence of an antimicrobial. Lead is a toxic metal not only to humans but also to microbes that live in an environment. A certain amount of lead in an environment can kill the microbes living in that environment. In this experiment, the lead will act as an antimicrobial used in the test performed. The MIC value of the lead obtained in this experiment will show the maximum ability of IA4, IA$_2$1, and IA$_2$3 isolate in tolerating lead.

2. Research Methodology

2.1. Material

Bacterium isolates in the glycerol stock form used in this research are collected from previous research. Chemical materials used in this research are lead (Pb) aqueous solution 1000 ppm, Nutrient Agar (NA) growth medium, and Nutrient Broth (NB) growth medium. A method that we used to make a lead aqueous solution refers to the stock solution manual of the American Chemical Society [20].

2.2. Bacteria solution stock

Bacteria solution stock was made by taking up IA4, IA$_2$1, and IA$_2$3 isolate from the glycerol stock using ose needle and inoculating it into the NB growth medium containing 10 ppm Pb. These inoculate were incubated in a shaker incubator at 37^{O}C for three days. The bacteria solution stock was used for a minimum inhibitory concentration (MIC) assay.

2.3. Minimum Inhibitory Concentration Assay

Minimum inhibitory concentration assay was undertaken by the modified Marzan method [21]. IA4, IA$_2$1, and IA$_2$3 isolate were inoculated on NA plate medium containing various lead concentrations by streaking technique. The lead concentration was started from 10 ppm. Inoculates were incubated in an incubator at 37^{o}C for 24 hours. The growth of isolates was detected up to the minimal concentration of lead at which these bacteria cannot live. The minimum concentration of lead in which the bacteria cannot survive is called MIC value, whereas the maximum concentration of lead in which the bacteria can still survive is the maximum ability of bacteria in tolerating lead.

3. Results and Analysis

3.1. Bacteria Solution Stock

Bacteria solution stock is made to make the IA4, IA_21, and IA_23 isolate viable. The viable cell is recommended to be used in minimum inhibitory concentration assay. In NB growth medium, IA4, IA_21, and IA_23 isolate gain their best growth in 3 days. This condition accords to the condition at the isolation step in the previous research [16]. On the first and second days after inoculation, the growth of bacteria was observed. However, the turbidity of the growth medium was very low even it could not be captured by a camera. On the third day after inoculation, the growth of bacteria was observed very clear. It was proved by the high turbidity of the growth medium.

3.2. Pb Maximum Tolerance of Bacteria

A minimum inhibitory concentration assay determines pb maximum tolerance of bacteria. IA4, IA_21, and IA_23 isolate were inoculated on an NA plate medium containing various lead concentrations and then determined the MIC value. MIC is defined as the minimum concentration of antimicrobial, it is lead in this case, that inhibits the growth of bacteria and this concentration is called MIC value [22]. The implication of this definition, the maximum concentration of antimicrobial in which bacteria still survive in this concentration is the maximum concentration that bacteria could tolerate. It also shows the maximum tolerance of bacteria. The MIC value and highest tolerance of IA4, IA_21, and IA_23 isolates found in this study are shown in Table 1.

Table 1. The MIC value and maximum tolerance of IA4, IA_21, and IA_23

No	Isolate	MIC Value (ppm Pb)	Maximum Tolerance (ppm Pb)
1	IA4	40	30
2	IA_21	40	30
3	IA_23	40	30

This result shows that all bacteria used in this research have the same maximum tolerance to lead. These maximum tolerances of bacteria are very low compared to some lead-resistant bacteria that have been studied. The comparing data of some lead-tolerant bacteria with IA4, IA_21, and IA_23 isolate has given in Table 2. Characterization of IA4, IA_21, and IA_23 isolate is an important thing to do to know more about these bacteria. Based on the gram of bacteria, gram-positive bacteria are more tolerant to heavy metals pollutants than gram-negative bacteria [23]. This is related to the cell wall of bacteria in which the cell wall of gram-positive bacteria has a higher capacity to absorb heavy metals than gram-negative bacteria. The cell wall of gram-positive bacteria is built from a 90% peptidoglycan layer, whereas the cell wall of gram-negative bacteria consists of lipids in the form liposaccharide and lipoprotein [24]. However, lead-resistant bacteria are both gram-positive and gram-negative. *Bacillus thuringiensis* and *Bacillus cereus* are gram-positive bacteria, whereas *Agrobacterium tumefaciens*, *Roseateles aquatilis*, *Pseudomonas psychrophila*, and *Pseudomonas sp*. are gram-negative bacteria. The other thing that is important to be observed is the optimum temperature and pH of the bacteria. Bacteria could adsorb more heavy metals in the proper condition than in the improper condition. *Corynebacterium glutamicum* will uptake lead best at pH of 5 and 20 $\pm$ $2^{O}C$ [25], *Symphortcarpus albus* will uptake lead best at pH of 5.5 and $45^{O}C$ [26], and *Pseudomonas putida* will uptake lead best at pH of 5.5 and $25^{o}C$ [27].

Table 2. The comparing data of some lead-tolerant bacteria

No	Bacteria	Maximum Tolerance (ppm Pb)	Reference
1	*Bacillus thuringiensis*	1,242	[28]
2	*Bacillus cereus*	1,242	[28]
3	*Bacillus cereus*	2,070	[23]
4	*Agrobacterium tumefaciens*	693.5	[29]
5	*Roseateles aquatilis*	693.5	[29]
6	*Pseudomonas psychrophila*	1,875	[30]
7	*Pseudomonas* sp	1,875	[30]
8	IA4 isolate	30	
9	IA_21 isolate	30	
10	IA_23 isolate	30	

The colonies of IA4, IA_21, and IA_23 isolate had been observed as brown colonies. Brown colonies indicate the mechanism of lead resistance of these bacteria is precipitation that is part of the extracellular mechanism to prevent lead movement on bacterial cell walls [23]. However, further study is needed to ensure the definite mechanism involved in lead resistance of IA4, IA_21, IA_23 isolate due to some lead-resistance mechanism has been suggested. The efflux mechanism is a bacterial process that prevents intracellular over-accumulation of heavy metals such as Pb by moving the heavy metals beyond the cell membrane through ATPase [31-32]. Intracellular bioaccumulation is a bacterial process for accumulating lead inside the cell-mediated by the metallothioneins protein [33]. Extracellular sequestration is a bacterial technique for accumulating lead outside the cell by employing an extracellular polymeric substance (EPS) known as exopolysaccharides [34-35]. Surface biosorption is a bacterium's extracellular method for absorbing lead through a siderophore generated by the bacteria [36]. Bioprecipitation is a bacterial process for precipitating lead within the cell in the form of Pb-phosphate [37-38]. Alteration in cell morphology is the mechanism involved when the bacteria prevent the heavy metals from coming into the cell by precipitation, and this mechanism also involves lead-resistance bacteria [36, 39]. In addition, some pigmented bacteria exhibits chelating agent secretion when it survives in the environment containing lead [40]. *Pseudomonas vesicular* and *Streptomyces secrete* red and brown-red pigment in the environment containing lead nitrate [41]. Some organisms exhibit the ability to degrade a lead compound when it exists in an environment containing lead [42].

4. Conclusion

IA4, IA_21, and IA_23 isolate are the Pb resistant bacteria that show absorption activity in the environment containing lead. Despite, its maximum tolerance is very low compared with reported lead-resistance bacteria, these isolates have a good prospect to be used as the alternative biosorption agents to absorb lead from the lead polluted environment. In this great expansion era of technology, it is possible to increase the maximum tolerance of IA4, IA_21, and IA_23 isolate using the synthetic biology approach. It would give a big expectation to use local strain bacteria to overcome environmental issues, specifically the heavy metals pollution.

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Reference

[1] Nies, D. H. (1999). Microbial heavy-metal resistance. *Applied Microbiology and Biotechnology*, vol. 51, no. 6, pp. 730-750.

[2] Ali, H., Khan, E., & Ilahi, I. (2019). Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation. *Journal of Chemistry*, vol. 2019, no. 6730305, pp. 1-14.

[3] Yılmaz, A. B., Sangün, M. K., Yağlıoğlu, D., & Turan, C. (2010). Metals (major, essential to non-essential) composition of the different tissues of three demersal fish species from Iskenderun Bay Turkey. *Food Chemistry*, vol. 123, no. 2, pp. 410-415.

[4] Bueno, B. Y. M., Torem, M. L., Molina, F. A. L. M. S., & De Mesquita, L. M. S. (2008). Biosorption of lead (II), chromium (III) and copper (II) by R. opacus: Equilibrium and kinetic studies. *Minerals Engineering*, vol. 21, no. 1, pp. 65-75.

[5] Check, L., & Marteel-Parrish, A. (2013). The fate and behavior of persistent, bioaccumulative, and toxic (PBT) chemicals: examining lead (Pb) as a PBT metal. *Reviews on Environmental Health*, vol. 28, no. 2-3, pp. 85-96.

[6] Lockitch, G. (1993). Perspectives on lead toxicity. *Clinical Biochemistry*, vol. 26, no. 5, pp. 371-381.

[7] Wani, A. Ara A. & Usmani JA (2015). Lead toxicity: A review. *Interdisciplinary Toxicology*, vol. 8, no. 2, pp. 55-64.

[8] Shamim, S. (2018). *Biosorption of Heavy Metals*. London: IntechOpen.

[9] Ratnawati, E., Ermawati, R., & Naimah, S. (2010). Teknologi biosorpsi oleh mikroorganisme, solusi alternatif untuk mengurangi pencemaran logam berat. *Jurnal Kimia dan Kemasan*, vol. 32, no. 1, pp. 34-40.

[10] Tiquia-Arashiro, S. M. (2018). Lead absorption mechanisms in bacteria as strategies for lead bioremediation. *Applied microbiology and biotechnology*, *102*(13), 5437-5444.

[11] Rani, M. J., Hemambika, B., Hemapriya, J., & Kannan, V. R. (2010). Comparative assessment of heavy metal removal by immobilized and dead bacterial cells: a biosorption approach. *African Journal of Environmental Science and Technology*, vol. 4, no. 2, pp. 77-83.

[12] Hussein, H., Ibrahim, S. F., Kandeel, K., & Moawad, H. (2004). Biosorption of heavy metals from waste water using Pseudomonas sp. *Electronic journal of Biotechnology*, vol. 7, no. 1, pp. 30-37.

[13] Babák, L., Šupinova, P., Zichova, M., Burdychova, R., & Vitova, E. (2012). Biosorption of Cu, Zn and Pb by thermophilic bacteria–effect of biomass concentration on biosorption capacity. *Acta Universitatis Agriculturae Et Silviculturae Mendelianae Brunensis*, vol. 60, no. 5, pp. 9-18.

[14] Samarth, D. P., Chandekar, C. J., & Bhadekar, R. K. (2012). Biosorption of heavy metals from aqueous solution using Bacillus licheniformis. *International Journal of Pure and Applied Sciences and Technology*, vol. 10, no. 2, pp. 12-19.

[15] Brooke, J. S. (2012). Stenotrophomonas maltophilia: an emerging global opportunistic pathogen. *Clinical Microbiology Reviews*, vol. 25, no. 1, pp. 2-41.

[16] Ikhsan, F., Herayati, H., Abdullah, S., & Rukmayadi, Y. (2020). Eksplorasi bakteri penyerap logam Pb dari air Sungai Ciujung. *Teknika: Jurnal Sains dan Teknologi*, vol. 16, no. 2, pp. 261-266.

[17] Zimmer, A. R., Bruxel, F., Bassani, V. L., & Gosmann, G. (2006). HPLC method for the determination of ecdysterone in extractive solution from Pfaffia glomerata. *Journal of pharmaceutical and biomedical analysis*, vol. 40, no. 2, pp. 450-453.

[18] Miranda, C. D., Rojas, R., Contreras-Lynch, S., & Vega, A. (2021). Evaluation of the correlation between minimum inhibitory concentrations (MIC) and disk diffusion data of Flavobacterium psychrophilum isolated from outbreaks occurred in Chilean salmonid farms. *Aquaculture*, vol. 530, no. 735811, pp. 1-7.

[19] Shao, L., You, C., Cao, J., Jiang, Y., Liu, Y., & Liu, Q. (2020). High treatment failure rate is better explained by resistance gene detection than by minimum inhibitory concentration in patients with urogenital Chlamydia trachomatis infection. *International Journal of Infectious Diseases*, vol. 96, pp. 121-127.

[20] Tyner, T., & Francis, J. (2017). Control, standard, and stock solutions. *ACS Reag. Chem.*, no. 3, pp. A-G.

[21] Marzan, L. W., Hossain, M., Mina, S. A. , Akter, Y., & Chowdhury, A. M. M. A. (2017). Isolation and biochemical characterization of heavy-metal resistant bacteria from tannery effluent in Chittagong city, Bangladesh. *Egypt. J. Aquat. Res.*, vol. 43, no. 1, pp. 65–74.

[22] Marzan, L. W., Hossain, M., Mina, S. A., Akter, Y., & Chowdhury, A. M. A. (2017). 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, no. 1, pp. 65-74.

[23] Utami, U., Harianie, L., Dunyana, N. R., & Romaidi, R. (2020). Lead-resistant bacteria isolated from oil wastewater sample for bioremediation of lead. *Water Science and Technology*, vol. 81, no. 10, pp. 2244-2249.

[24] Kurnia, K., Sadi, N. H., & Jumianto, S. (2015). Isolation and Characterization of Pb Resistant Bacteria from Cilalay Lake, Indonesia. *Aceh International Journal of Science and Technology*, vol. 4, no. 3, pp. 83-87.

[25] Choi, S. B., & Yun, Y. S. (2004). Lead biosorption by waste biomass of Corynebacterium glutamicum generated from lysine fermentation process. *Biotechnology letters*, vol. 26, no. 4, pp. 331-336.

[26] Akar, S. T., Gorgulu, A., Anilan, B., Kaynak, Z., & Akar, T. (2009). Investigation of the biosorption characteristics of lead (II) ions onto Symphoricarpus albus: batch and dynamic flow studies. *Journal of hazardous materials*, vol. 165, no. 1-3, 126-133.

[27] Uslu, G., & Tanyol, M. (2006). Equilibrium and thermodynamic parameters of single and binary mixture biosorption of lead (II) and copper (II) ions onto Pseudomonas putida: effect of temperature. *Journal of Hazardous Materials*, vol. 135, no. 1-3, pp. 87-93.

[28] Fatemi, H., Pour, B. E., & Rizwan, M. (2020). Isolation and characterization of lead (Pb) resistant microbes and their combined use with silicon nanoparticles improved the growth, photosynthesis and antioxidant capacity of coriander (Coriandrum sativum L.) under Pb stress. *Environmental Pollution*, vol. 266, no. 114982, pp. 1-11.

[29] Jebara, S. H., Abdelkerim, S., Fatnassi, I. C., Chiboub, M., Saadani, O., & Jebara, M. (2015). Identification of effective Pb resistant bacteria isolated from Lens culinaris growing in lead contaminated soils. *Journal of basic microbiology*, vol. 55, no. 3, pp. 346-353.

[30] Yongpisanphop, J., Babel, S., Kurisu, F., Kruatrachue, M., & Pokethitiyook, P. (2020). Isolation and characterization of Pb-resistant plant growth promoting endophytic bacteria and their role in Pb accumulation by fast-growing trees. *Environmental technology*, vol. 41, no. 27, pp. 3598-3606.

[31] Nies, D. H., & Silver, S. (1995). Ion efflux systems involved in bacterial metal resistances. *Journal of industrial microbiology*, vol. 14, no. 2, pp. 186-199.

[32] Rensing, C., Ghosh, M., & Rosen, B. P. (1999). Families of soft-metal-ion-transporting ATPases. *Journal of bacteriology*, vol. 181, no. 19, pp. 5891-5897.

[33] Gadd, G. M. (1990). Heavy metal accumulation by bacteria and other microorganisms. *Experientia*, vol. 46, no. 8, pp. 834-840.

[34] Bhaskar, P. V., & Bhosle, N. B. (2006). Bacterial extracellular polymeric substance (EPS): a carrier of heavy metals in the marine food-chain. *Environment international*, vol. 32, no. 2, pp. 191-198.

[35] Bramhachari, P. V., Kishor, P. K., Ramadevi, R., Rao, B. R., & Dubey, S. K. (2007). Isolation and characterization of mucous exopolysaccharide (EPS) produced by Vibrio furnissii strain VB0S3. *Journal of Microbiology and Biotechnology*, vol. 17, no. 1, pp. 44-51.

[36] Naik, M. M., & Dubey, S. K. (2011). Lead-enhanced siderophore production and alteration in cell morphology in a Pb-resistant Pseudomonas aeruginosa strain 4EA. *Current microbiology*, vol. 62, no. 2, pp. 409-414.

[37] Levinson, H. S., Mahler, I., Blackwelder, P., & Hood, T. (1996). Lead resistance and sensitivity in Staphylococcus aureus. *FEMS microbiology letters*, vol. 145, no. 3, pp. 421-425.

[38] Mire, C. E., Tourjee, J. A., O'Brien, W. F., Ramanujachary, K. V., & Hecht, G. B. (2004). Lead precipitation by Vibrio harveyi: evidence for novel quorum-sensing interactions. *Applied and Environmental Microbiology*, vol. 70, no. 2, pp. 855-864.

[39] Chakravarty, R., & Banerjee, P. C. (2008). Morphological changes in an acidophilic bacterium induced by heavy metals. *Extremophiles*, vol. 12, no. 2, pp. 279-284.

[40] Gilis, A., Corbisier, P., Baeyens, W., Taghavi, S., Mergeay, M., & Van der Lelie, D. (1998). Effect of the siderophore alcaligin E on the bioavailability of Cd to Alcaligenes eutrophus CH34. *Journal of Industrial Microbiology and Biotechnology*, vol. 20, no. 1, pp. 61-68.

[41] Zanardini, E., Andreoni, V., Borin, S., Cappitelli, F., Daffonchio, D., Talotta, P., ... & Cariati, F. (1997). Lead-resistant microorganisms from red stains of marble of the Certosa of Pavia, Italy and use of nucleic acid-based techniques for their detection. *International biodeterioration & biodegradation*, vol. 40, no. 2-4, pp. 171-182.

[42] Teeling, H., & Cypionka, H. (1997). Microbial degradation of tetraethyl lead in soil monitored by microcalorimetry. *Applied microbiology and biotechnology*, vol. 48, no. 2, pp. 275-279.