Biosaintifika 11 (2) (2019) 289-295

Biosaintifika

Journal of Biology & Biology Education

http://journal.unnes.ac.id/nju/index.php/biosaintifika

Biosorption of Heavy Metal Pollution by *Enterobacter agglomerans*

Nur Kusuma Dewi, Ibnul Mubarok, Ari Yuniastuti✉

DOI: http://dx.doi.org/10.15294/biosaintifika.v11i2.20471

Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang, Indonesia

History Article

Submitted 15 June 2019
Revised 17 July 2019
Accepted 22 August 2019

Keywords

Biosorption; Heavy Metals; *Enterobacter agglomerans*

Abstract

Biosorption is a new waste treatment technology that can eliminate toxic heavy metals. Biosorption can be considered as an environmentally friendly alternative technology to treat industrial liquid waste that is economically proper to use. One of them is biosorption that utilizes the microorganisms' absorption ability, especially bacteria that can absorb heavy metals in waters, such as *Enterobacter agglomerans*. This research aimed to determine the ability of *E. agglomerans* in reducing heavy metals pollution in local river. The research employed the measurement of the effect of lead (Pb) to *E. agglomerans* growth using Optical Density (OD) at wavelength 600 nm. The colony numbers were calculated using a standard curve. While the ability of *E. agglomerans* to reduce heavy metals concentration in liquid media was measured using AAS with a wavelength of 240 nm. The results showed that lead affected the growth of *E. agglomerans*. The OD value has a negative relationship with the concentration level of Pb. The ODs were decreased from 2.867 to 1.242, using Pb level from 0 ppm to 20 ppm. Therefore, it proved that *E. agglomerans* could reduce heavy metals concentration in local river in Central Java Province. This research was the first report on *E. agglomerans* activity on heavy metal in contaminated water. This result can be used as a reference for industrial sites near the river to treat their wastewater before discharging it to the river body to preserve its water purity.

How to Cite

Dewi, N. K., Mubarok, I., & Yuniastuti, A. (2019). Biosorption of Heavy Metal Pollution by *Enterobacter agglomerans*. *Biosaintifika: Journal of Biology & Biology Education*, 11(2), 289-295.

✉ Correspondence Author:
Sekaran, Gunungpati, Semarang 50229
E-mail: ari_yuniastuti@yahoo.co.id

p-ISSN 2085-191X
e-ISSN 2338-7610

INTRODUCTION

The growth of industrial activities bring some positive impacts, but there are also some negative impacts in the form of pollution due to improper waste disposal. Heavy metal pollution issues are increasing in accordance with the development of various studies which have been directed towards various technological applications to treat environmental pollution caused by heavy metals. Several heavy metal pollutants in industrial waste are mercury, copper, lead, chrome, zinc, cadmium, uranium, selenium, and nickel. According to Tchounwou *et al.* (2012), heavy metals in waste are usually in various conditions, such as not dissolved, dissolved, reduced, oxidized, and forming a complex.

Some heavy metal ions such as lead (Pb), cadmium (Cd) and chrome (Cr) are hazardous for human health and the continuity of natural environment life. Even at low concentrations, heavy metal ions effects can influence directly or until they are accumulated in the food chain as studied by Singh *et al.* (2011). Cd, Pb, and Hg can contaminate water, air, and soil. These contaminants eventually end up in the water. Therefore, the water environment is the most profound concern in environmental monitoring. In river waters, Cd, Pb, and Hg can accumulate in sediments, water, or river biota (Withgott & Brennan, 2007; Soemirat, 2005; Wardhana 2004).

The treatment of wastes containing heavy metals that have been conducted so far is chemically and physically by oxidation and reduction as well as by coagulation and sedimentation, filtration, ion exchange, reverse osmosis, electrochemical processing, and evaporation (Baik *et al.* 2002). Physical chemistry methods that are now widely used have several disadvantages, including high installation and operational costs, require many chemicals, and produce a Gram-negative of sludge. Thus, it will be a problem because these methods require further processing and high costs in its disposal.

Therefore, it is necessary to develop a wastewater treatment method with an environmentally friendly alternative technology that is low in cost and has high efficiency in identifying pollutants while processing the waste. It can be done by utilizing the microorganisms ability to absorb heavy metals called biosorption. The other thing that makes biosorption to be a right choice is the availability of various biosorbent materials such as fungi, bacteria, yeast, algae, and biopolymers such as alginate and chitosan which are the side products of the fishing industry.

Generally, the advantages of using microorganisms as biosorbents are low operational costs, high efficiency, have a metal-binding ability, produce minimum amount of sludge, have a desorption mechanism that allows metal recovery, have a regeneration mechanism that allows metal reused, many raw materials are available and easily obtained, and does not require added nutrition by using dead microbes (Javanbakht *et al.*, 2014). Bacteria have high metals affinity and can accumulate heavy metals. According to Kang *et al.* (2016), bacteria can grow in an environment with heavy metals contamination and often resistant to heavy metal ions because they produce biosurfactant/emulsion compounds that can absorb various types of heavy metals such as cadmium (Cd), chromium (Cr), lead (Pb), copper (Cu), and zinc (Zn) from contaminated soil. Bacteria that resistant to heavy metals can be used as biosorbents and bio accumulators. As found by Zulaika *et al.* (2012), resistance characteristics of bacteria on heavy metals can be utilized as the bioremediation agent for heavy metal pollution.

The objective of this research was to determine the ability of the bacterial species of *Enterobacter agglomerans* for reducing the heavy metal pollution as a biosorption agent. Bioremediation purpose was expected to help the environmental problems. The results of the study can be used as a reference for industries or companies near local river in Central Java to treat their wastewaters before discharging it to the river body.

METHODS

Research Location

Water sampling was done at three sites of local river in Central Java, i.e., the upstream, middle, and downstream points. The analysis and determination of *Enterobacter agglomerans* in the absorption of heavy metal were performed at Microbiology Laboratory, Food and Nutrition PAU of Gadjah Mada University in Yogyakarta.

Sampling Techniques

The sampling technique used was purposive sampling by choosing river sites, which were estimated to contain heavy metal lead (Pb). The water and sediment samples were taken using Ekman Bottom Grab. The Ekman Bottom Grab was inserted into the water column until it reached the canal bottom and was pulled. The sterile bottles were inserted into the sediment pile and were opened inside it. Then, the sediment was put into a sterile bottle until it was full. The sterile bottle was closed while still inside the sediment.

Preparation of Bacterial Growth Medium

Each of the sediment samples was taken and was used as the source to find the bacteria which were able to accumulate lead with a concentration of 250 ppm. Isolation of bacteria that accumulate lead found in sediment in local river in Central Java was performed using the serial dilution and purification. From the dominant colony of bacteria, the single territory was taken to be purified. The pure culture was later grown in a new fresh NA medium.

Nutrient Broth (NB) and Nutrient Agar (NA) was used as growth media for microorganisms. The media were sterilized by putting them in an autoclave at 121 °C for 15 minutes. Next, the NB and NA were incubated for 24 hours at 30 °C to ensure that there was no contamination. The growth media were supplemented by 0.3996 gr/L $PbNO_3$ with a concentration of 250 ppm in a petri dish. One isolate of dominant bacteria was morphologically characterized by observing the characteristics of the colony's shape and color, as well as observing the morphological characteristics of the bacterial cells using Gram staining. Molecular identification using 16S rRNA was performed to identify the species of the bacterial isolate.

The colony was characterized based on its morphological characteristics. The macroscopic features of purified bacteria that were observed, including observation of shape, size, color, surface, edge, and elevation of bacterial colonies. The first step of identification of microorganisms the characterization by observing the morphological characteristics of bacteria. Next, the biochemical test was done as performed by Smith *et al.* (2017) on the microbial communities and by Fibriana *et al.* (2017) on the identification of microorganisms from pigment-producer bacteria from potato peel waste.

Absorption of Pb by Microorganism

Determination of the lead absorbed by bacterial isolate was carried out following Hussein *et al.* (2004). Examination of Pb heavy metal absorption by bacterial strain using AAS (atomic absorption spectrophotometry) was taken from the highest growth of bacteria in media mixed with heavy metal Pb. One ml bacterial isolate was inoculated in 20 ml of the media. Bacterial culture was incubated in a rotary shaking incubator at 28 °C at a speed of 120 rpm. Then, the culture was centrifuged at a rate of 5000 rpm for 15 minutes. The supernatant was separated to measure its heavy metals concentration by using Atomic Absorption Spectrometry (AAS) method at a wavelength of 270 nm. The growth phase of microorganisms was measured using spectrophotometry method every four hours using OD_{600} to measure the cell density.

RESULTS AND DISCUSSION

Isolation of bacteria that accumulate heavy metal

This study used sediment deposits that were found at three different sample points in local river in Central Java, where at each point, the deposited sediment contained lead based on the preliminary tests that had been conducted (Central Java Environmental Agency, 2010). The highest lead was at the upstream part of the river, whereas the lowest was in the downstream part, as seen in Table 1.

Table 1. The concentration of Pb in local river in Central Java

River area	Pb concentration (mg L^{-1})
Upstream	1.252
Middle	1.143
Downstream	0.358

The appearance of bacterial colonies that grew on agar plate media showed typical shape and size. Isolation results showed that there were bacterial colonies that grew on agar media enriched with 0.3996 gr/L $PbNO_3$ with a concentration of 250 ppm in a petri dish. The characteristics of the bacteria and identification results are presented in Table 2.

The identification results showed that *Enterobacter agglomerans* was the most abundant species which occupied the sediment of the river. Characteristics of *E. agglomerans* are medium size, circular shape, milky white color, rough surface, entire edge, and elevation convex. The results of microscopic observation showed that *E. agglomerans* cells have bacilli (stem) form, Gram-negative (-), and no endospores. Gram staining of bacterial isolates was conducted to classify this bacteria as Gram-positive or Gram-negative bacteria. The Gram staining is proven to be valid to differentiate those bacterial species. Nath *et al.* (2018) performed the Gram staining to identify the toxic metal-tolerant bacteria from soil. Liang *et al.* (2015) performed the purification of Gram-negative Strains by single colony isolation: influence on strain colony characteristics. The colony characterization has been proven to be effective to identify the bacterial strain. Therefore, it was assumed that this species had potential as a biosorbent of lead. The further process was the determination of lead influence on bacterial growth.

Table 2. Morphological characteristics of one most dominant bacterial isolate obtained from sediment samples

Morphology parameters	Characteristics
Colony shape	Circular
Edge	Entire
Elevation	Convex
Size	Medium
Surface	Rough
Odor	Odorless
Transparency (opacity)	Turbid
Pigmentation	Milky white color
Catalase test	Catalase positive
Gram staining	Gram-negative
Cell shape	Bacilli (stem)
Endospores	No
Results of 16S rRNA identification	Enterobacter agglomerans
Conclusion	Enterobacter agglomerans

The effects of heavy metal lead (Pb) to the growth of *Enterobacter agglomerans*

Growth is defined as an increase of cell quantities and organisms structure that can be expressed by size, followed by an increase of number, cell size, weight or mass, and other parameters. The growth of microbial groups refers more to the increase in cells number, not referring to the individual development of cellular organisms. Growth is expressed as an increase in amount or mass of inoculum higher than initial condition (Maier & Pepper, 2015). The relationship between cells number and growth time can be shown using the growth curve. In this study, the growth curve of *Enterobacter agglomerans* was made through turbidity levels observation (Optical Density) using a spectrophotometer in every 4 hours. The observation results showed that the adaptation phase was not - visible at concentrations containing lead of 0, 5, 10, and 15 ppm. It is like bacterial growth directly to the log (exponential) phase. However, in the treatment containing a lead concentration of 20 ppm, the adaptation phase was slow; this was due to the high lead concentration. The exponential phase is the cell division phase, where cells will divide until the maximum number of cells is reached (a very rapid growth period) (Maier & Pepper, 2015). In this study, the log phase occurred at 0^{th} until 16^{th} hours. The optimal phase of *E. agglomerans* occurred at 16^{th} hour, as can be seen in Figure 1.

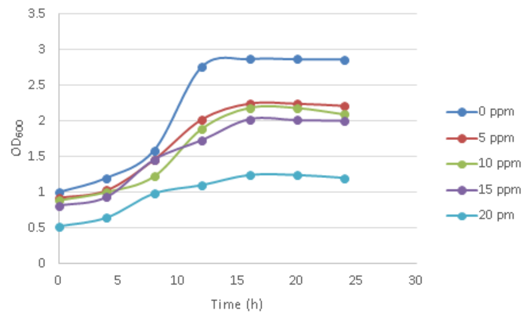

Figure 1. Growth curve of *Enterobacter agglomerans* under the treatment of lead in various concentration (0-20 ppm) for 24 h

Based on the results of this research, the treatment of 0 ppm showed the highest growth, whereas the highest treatment concentration at 20 ppm resulted in the lowest growth of *E. agglomerans* with 1 (Table 3).

Table 3. Growth number of cells after treatment using Pb in the various concentration

Pb concentration	OD_{600}	Growth Number ($CFU\ mL^{-1}$)
0	2.867	38.85×10^{11}
5	2.243	88.50×10^{11}
10	2.183	76.77×10^{11}
15	2.025	74.86×10^{11}
20	1.242	33.00×10^{11}

Bacterial growth can be hampered by the presence of heavy metals in the environment. Lead is a toxic heavy metal that can block the enzymes work. Heavy metals are naturally occurring elements which have a high atomic weight and density of at least five times greater than water. They are various industrial, domestic, agricultural, medical, and technological applications that have led to wide distribution in the environment. Their toxicity depends on several factors, including dosage, route of exposure, and chemical species, as well as age, sex, genetics, and nutritional status of exposed individuals. Because of the high levels of toxicity, arsenic, cadmium, chromium, lead, and mercury among priority metals that have a public health significance. This metal element is considered a systemic poison that is known to cause multiple organ damage, even at lower levels of exposure. Tchounwou *et al.*

(2012) analyzed heavy metal toxicity to the biotic agents and the environment. It affected the plants and animals' organs even at lower levels of exposure by bioaccumulation.

The most optimal growth of *E. agglomerans* in all treatments, which were given different lead concentrations for 28 hours incubation was at 0 ppm treatment. The optimal growth that is almost similar to the control was found in the treatment of 5 ppm lead concentration. Growth decrease of *E. agglomerans* along with the increase of lead concentration in media indicates that Pb toxicity is high enough to inhibit bacterial growth.

Microorganisms generally have a protective mechanism against toxic metals to maintain their life. This mechanism involves the formation of metal complexes with cell membranes proteins so that metals can be accumulated in cells without interfering their growth. If the metal concentration is too high, this accumulation can inhibit cell growth because the organism's protection system is unable to compensate for metals toxic effects (Ayangbenro & Babalola, 2017). Dixit *et al.* (2015) stated that low levels of heavy metals such as Hg, Ag, Cu, Au, and Pb could be toxic. This is because heavy metals have oligodynamic power, which is the killing power of heavy metals at low levels. The next bacterial growth phase is the stationary phase. At the maximum stationary phase, the number of dead cells increases until the number of living cells or division results are the similar as the number of dead cells; therefore, the living cells number is constant, this is like there is no growth anymore (zero growth) (Maier & Pepper, 2015; Mathis & Ackermann, 2016).

In this study, the stationary phase of bacterial growth with 0, 5, 10, and 15 ppm Pb concentrations occurred after 16^{th} to 24^{th} hours. However, in the treatment containing 20 ppm lead concentration, no stationary phase was found. It is because OD observations were conducted every 4 hours. However, after experiencing the optimal phase, it was like bacteria in that group directly experience the death phase. In this study, the mortality phases of 0, 5, 10, 15, and 20 ppm treatment began after the 24^{th} hours.

The death phase of *E. agglomerans*, which is influenced by lead metal, can be observed through the change of media color from clear to be turbid, media smell from odorless to be odorous, and from clear to brown mucus color. It is in accordance with Namgyal *et al.* (2017) study which showed that the change of media color could be seen at 0 ppm treatment, which was bright yellow, while treatment with lead addition caused more turbid and darker color in line with incubation time increase. It could be seen the decrease of bacterial growth. In addition, strong acid smell and darkening mucus color also indicate that the bacteria have reached the death phase (Rajesh & Rajesh, 2018). The death phase of *E. agglomerans* began after 24^{th} hour. Achieving a longer optimal phase shows that *E. agglomerans* is a bacterium whose growth is relatively slower compared to the other bacteria. It also proved that *E. agglomerans* is a bacteria that can live longer in environments with lead contamination.

Bacterial growth can be influenced by several factors, such as nutrients availability in the media which consists of macronutrients (C, H, O, N, S, P, K, Ca, Mg, Fe) and micronutrients, pH, temperature, nutrients, oxygen availability and other factors (Basu *et al.*, 2015). The optimal temperature for *E. agglomerans* growth is 28 °C.

Based on visual observations, the higher concentration of heavy metal lead (Pb) in the media, the more dark mucus was produced. The mucus of *E. agglomerans* is a metabolic result produced in the log phase. Some of the compounds produced by bacterial cells in multiplication phase are ethanol, lactic acid, organic acids, amino acids, fatty acids, and others. The absorption and immobilization have occurred through different mechanisms such as ion exchange, complexation, and sedimentation (De Philippis *et al.*, 2011; Wang *et al.*, 2010).

Dixit *et al.* (2015) stated that heavy metals occur naturally in the environment from the weathering of the parent material pedogenetic processes and also through anthropogenic sources. The most significant natural sources are mineral weathering, erosion and volcanic activity, whereas anthropogenic sources depend on human activities such as mining, smelting, steel plating, use of pesticides and disposal of phosphate fertilizers, as well as biosolid (for example, livestock manure, compost, and municipal sewage sludge), and atmospheric deposits.

Liquid waste containing heavy metal lead (Pb) can cause adverse effects on the environment, such as water pollution. Although the removal of toxic heavy metals (Pb) from industrial waste has been practiced for decades, the cost-effectiveness of the most common physical-chemical processes such as oxidation and reduction, chemical deposition, screening, electrochemical processing, evaporation, ion exchange and reversal of osmosis processes are limited. High reagent requirements and unpredictable metal ion removal are some of the other disadvantages associated with the technique. Furthermore, strong and polluting reagents are used for desorption, producing toxic sludge and secondary environmental pollution. These losses can become clearer, and further aggravate the process costs in the case of contaminated groundwater, mine tailings and other industrial wastes due to liquid wastes containing complicated organic matter and low metal contamination. The biotechnology approach can be successful in these fields and is designed to cover that niche. Microorganisms have developed various steps to respond to heavy metal pressure through processes such as transportation across cell membranes, biosorption to cell walls and trapped in extracellular capsules, precipitation, complexation, and oxidation-reduction reactions (Malik, 2004). In this regard, a solution to water pollution that contains heavy metals is biosorption. Biosorption is a technology that utilizes absorption by microorganisms, such as bacteria (Volesky & Holan, 1995; Gavrilescu, 2004; Suryani *et al.*, 2017; Sasi *et al.*, 2018). There are the facts that there are two effective biotic methods used are biosorption and bioaccumulation. Based on the research on heavy metal removal process, a comparison between biosorption and bioaccumulation showed that biosorption is better than bioaccumulation. It is because of the intoxication of heavy metal is performed by inhibiting heavy metals entry into the microbial cell (Hansda & Kumar, 2016). In this research, a bacterium that is resistant and able to accumulate heavy metals is *Enterobacter agglomerans*. In this research, *E. agglomerans* was employed in the determination of bioabsorption activity on heavy metal Pb in sediments and water of Local River in Central Java, Semarang, Indonesia.

Biosorption has attracted the attention and also included as a promising alternative to replace or add to the process of removing heavy metals from water (Bahadir *et al.*, 2007; Carolin *et al.*, 2017). There are many mechanisms involved in biosorption; some are still not fully understood. The biosorption mechanism can be classified based on dependence on cell metabolism called metabolic dependence or according to the location where metals removed from solution are found which are called non-metabolic dependents/independent metabolism such as extracellular accumulation/precipitation, absorption of cell surfaces and intracellular accumulation/ precipitation (Mustapha & Halimoon, 2015). This research is the first to report the application of *E. agglomerans* on biosorption activities. Therefore, this research can be a reference to support the effort to recover water by biosorption and bioremediation.

CONCLUSION

According to the research results, *E. agglomerans* can reduce heavy metals concentration in the contaminated waters by biosorption method. Further research is needed by using different types of bacteria and heavy metals. The use of a consortium of various species is also necessary with the choice of consortium and carbon/nutrient source must depend on the nature of the waste to save the cost.

REFERENCES

Ayangbenro, A. & Babalola, O. (2017). A new strategy for heavy metal polluted environments: a review of microbial biosorbents. *International journal of environmental research and public health*, *14*(1), 94.

Bahadir, T., Bakan, G., Altas, L., & Buyukgungor, H. (2007). The investigation of lead removal by biosorption: an application at storage battery industry wastewaters. *Enzyme and Microbial Technology*, 41(1-2), 98-102.

Baik, M. H., & Friesner, R. A. (2002). Computing redox potentials in solution: Density functional theory as a tool for rational design of redox agents. *The Journal of Physical Chemistry A*, 106(32), 7407-7412.

Basu, S., Bose, C., Ojha, N., Das, N., Das, J., Pal, M., & Khurana, S. (2015). Evolution of bacterial and fungal growth media. *Bioinformation*, *11*(4), 182.

Carolin, C. F., Kumar, P. S., Saravanan, A., Joshiba, G. J., & Naushad, M. (2017). Efficient techniques for the removal of toxic heavy metals from aquatic environment: A review. *Journal of environmental chemical engineering*, *5*(3), 2782-2799.

Central Java Environmental Agency (Badan Lingkungan Hidup Propinsi Jawa Tengah). (2010). *Laporan Pemantauan Kualitas Air Semarang*. BLH Jateng. Semarang

De Philippis, R., Colica, G., & Micheletti, E. (2011). Exopolysaccharide-producing cyanobacteria in heavy metal removal from water: molecular basis and practical applicability of the biosorption process. *Applied microbiology and biotechnology*, *92*(4), 697.

Dixit, R., Malaviya, D., Pandiyan, K., Singh, U., Sahu, A., Shukla, R., Singh, B, Rai, J., Sharma, P., Lade, H., & Paul, D. (2015). Bioremediation of heavy metals from soil and aquatic environment: an overview of principles and criteria of fundamental processes. *Sustainability*, 7(2), 2189-2212.

Fibriana, F., Amalia, A. V., & Mubarok, I. (2017). Isolasi dan Karakterisasi Mikroorganisme Penghasil Pigmen dari Limbah Kulit Kentang. *Jurnal Mipa*, *40*(1), 7-13.

Gavrilescu, M. (2004). Removal of heavy metals from the environment by biosorption. *Engineering in Life Sciences*, *4*(3), 219-232.

Hansda, A., & Kumar, V. (2016). A comparative review towards potential of microbial cells for heavy metal removal with emphasis on biosorption and bioaccumulation. *World Journal of Microbiology and Biotechnology*, 32(10), 170.

Hussein, H., Ibrahim, S. F., Kandeel, K., & Moawad, H. (2004). Biosorption of heavy metals from wastewater using Pseudomonas sp. *Electronic Journal of Biotechnology*, 7(1), 30-37.

Javanbakht, V., Alavi, S. A., & Zilouei, H. (2013). Mechanisms of heavy metal removal using microorganisms as biosorbent. *Water Science and Technology*, 69(9), 1775-1787

Kang, C. H., Kwon, Y. J., & So, J. S. (2016). Bioremediation of heavy metals by using bacterial mixtures. *Ecological Engineering*, *89*, 64-69.

Liang, J., De Bruyne, E., Ducatelle, R., Smet, A., Haesebrouck, F., & Flahou, B. (2015). Purification of Gram-negative suis Strains From Biphasic Cultures by Single Colony Isolation: Influence on Strain Characteristics. *Helicobacter*, *20*(3), 206-216.

Maier, R. M., & Pepper, I. L. (2015). *Bacterial growth*. In Environmental Microbiology (pp. 37-56). Academic Press.

Malik, A. (2004). Metal bioremediation through growing cells. *Environment International*, 30(2), 261-278.

Mathis, R., & Ackermann, M. (2016). Response of single bacterial cells to stress gives rise to complex history dependence at the population level. *Proceedings of the National Academy of Sciences*, 113(15), 4224-4229.

Mustapha, M. U., & Halimoon, N. (2015). Microorganisms and biosorption of heavy metals in the environment: a review paper. *J. Microb. Biochem. Technol*, 7, 253-256.

Namgyal, D., Chandra, A., Reddy, G., & Kumar, K. (2017). Biosorption of Heavy Metal Using Bacteria Strain and Its Optimization. *Indian journal of pharmacology*, 43(3), 246

Nath, S., Deb, B., & Sharma, I. (2018). Isolation of toxic metal-tolerant bacteria from soil and examination of their bioaugmentation potentiality by pot studies in cadmium-and lead-contaminated soil. *International Microbiology*, 21(1-2), 35-45.

Sasi, F. A., Kusumaningrum, H. P., & Budiharjo, A. (2018). Molecular Characterization of Zinc (Zn) Resistant Bacteria in Banger River, Pekalongan, Indonesia. *Biosaintifika: Journal of Biology & Biology Education*, *10*(3), 622-628.

Singh, R., Gautam, N., Mishra, A., & Gupta, R. (2011). Heavy metals and living systems: An overview. *Indian journal of pharmacology*, 43(3), 246.

Smith, W. P., Davit, Y., Osborne, J. M., Kim, W., Foster, K. R., & Pitt-Francis, J. M. (2017). Cell morphology drives spatial patterning in microbial communities. *Proceedings of the National Academy of Sciences*, 114(3), E280-E286.

Soemirat, S. (2005). *Epidemiologi Lingkungan*. Yogyakarta: UGM Press

Suryani, Y., Cahyanto, T., Sudjarwo, T., Panjaitan, D. V., Paujiah, E., & Jaenudin, M. (2017). Chromium Phytoremediation of Tannery Wastewater using Ceratophyllum demersum. *Biosaintifika: Journal of Biology & Biology Education*, 9(2), 233-239.

Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). *Heavy metal toxicity and the environment. In Molecular, clinical and environmental toxicology* (pp. 133-164). Springer, Basel.

Volesky, B., & Holan, Z. R. (1995). Biosorption of heavy metals. *Biotechnology progress*, *11*(3), 235-250.

Wang, H., Bi, N., Saito, Y., Wang, Y., Sun, X., Zhang, J., & Yang, Z. (2010). Recent changes in sediment delivery by the Huanghe (Yellow River) to the sea: causes and environmental implications in its estuary. *Journal of Hydrology*, 391(3-4), 302-313.

Wardhana, W. A. (2004). *Dampak Pencemaran Lingkungan (Edisi Revisi).* Yogyakarta: Penerbit Andi.

Withgott, I., & Brennan, S. (2007). *Environment: The science behind the stories*. 699p+ appendices. San Francisco: sn.

Zulaika, E., Luqman, A., Arinda, T., & Sholikah, U. (2012). Bakteri Resisten Logam Berat yang Berpotensi Sebagai Biosorben dan Bioakumulator. *Seminar Nasional. Waste Management for Sustainable Urban Development*.