Bioremediation Of Soil Polluted With Copper (Cu^{2+}) By Mixed Culture Bacteria Thiobacillus Sp. And Clostridium Sp.

Anna Kristina Rosa Vernans, Bambang Iswanto, Astri Rinanti

Abstract: This research was conducted to reduce the presence of heavy metals such as Cu^{2+} on polluted lands using mix culture Thiobacillus sp. and bacteria Clostridium sp. as bioremediation substances. Currently, soil contamination due to heavy copper metals is a huge global responsibility. It is, therefore, necessary to innovate a promising, cost-effective, and environmentally friendly technique without secondary pollution to combat these metals. This is a follow-up research after Thiobacillus sp. and Clostridium sp. was found to have adequately lived on artificial media, such as Stone Mineral Salt solution containing metal polluters weighing about 100 ppm, with growth speed of cells/mL 21.580. Furthermore, both bacteria was used to describe the sterile soil containing 100 ppm heavy metal Cu^{2+}, with pH 7, 27% average humidity, under a temperature of $\pm$ 25°C, from 48 to 312 hours. The final concentration was measured using the Atomic Absorption Spectrophotometric method. The results therefore showed that bacterial culture was able to mix the Cu^{2+} in 24, 72, 120, 168, 216, 264 and 312 hours with each having 18.67%, 27.05%, 39.64%, 40.84%, 56.15%, 36.97% 32.64% respectively. However, the highest removal efficiency occurred for 216 hours, which was above 50%. Therefore, this research is expected to scientifically contribute to the environment, particularly in biotechnology, in order to provide an alternative eco-friendly atmosphere. Management-related efforts were also achieved, which was able to handle the contaminated media using the effective bacteria.

Index Terms: Bioremediation, Clostridium sp., effective bacteria, heavy metal (Cu^{2+}), removal efficiency, soil polluted, Thiobacillus sp.,

1. INTRODUCTION

Population growth and development rapidly increased during the industrial revolution, which later influenced the quality of the environment in an ecosystem [1]. However, a decline in environmental quality was due to a variety of industrial waste which accumulated with the incidence of various diseases, thereby, leading to the death of living beings, including humans [2]. The use of heavy metals as the main raw material in the production process is carried out in a variety of industries such as mining, metal smelting, electroplating, and in the field of energy production. Similarly, heavy metal waste is also obtained from agricultural activities such as the production of fertilizer and application of pesticide to based metal [3]. Heavy metal has become one of the contaminants that affects environmental pollution [4] such as copper (Cu) in the form of Cu^{2+} ions [5]. When it comes in contact with living beings, its contaminant becomes toxic and inorganic polluters due to the nature of xenobiotic [6], the non-biodegradable [7] substance that quickly accumulates food chain or environmental cycle [8]. Although copper (Cu) is one of the essential elements used in regulating energy metabolism [9] which act as metal ions and antioxidant, it is toxic at high concentrations [10].Due to its characteristics it has high toxicity and is able to accumulate biological cells, thereby giving rise to toxic effects, carcinogenic, mutagenic and Teratology in human beings or organisms, thereby posing a threat to environmental sustainability [11]. Various results of staple food cultivated on land were contaminated with heavy metals and adverse to human health whenever it is consumed [12]. Plants that accumulated it originally responded to varying types of plant, ecotype, as well as the specific tolerance against the metal [13]. However, assuming the plant has a tolerance of one type of metal, it is sensitive to others [14]. There are a variety of techniques and methods used to the remove copper content of an environment, such as physical chemistry, mitigation or biology [15]. However, the application of these methods is still considered an additional cost. Various countermeasures were chemically and physically considered inefficient due to its high cost. Therefore, the reagent needs high potentiality led to secondary pollution. The methods used in biological remediation (bioremediation) are considered ecological [16] with innovative technology [17] that promises to overcome the pollution of heavy metals in the soil, thereby making it environmentally friendly, efficient and able to permanently reduce pollution [18]. Bioremediation is the process of lowering or detoxifying a contaminated environment using physiological capabilities of microorganisms such as bacteria [22], [24], [25], [26], fungi [27], microalgae [28], and plant [29]. Some microorganisms are known to require a varied amount of energy to reduce heavy metal essential for growth and development. However, bioremediation is conducted by relying on indigenous microbe or improving the addition of microbial exogenous, either in the form of a single or mixed culture. Based on the description, this research was conducted to eliminate the heavy metal Cu^{2+} contained in the ground by utilizing a mixed culture of bacteria Thiobacillus sp. and Clostridium sp. which needs to be tested for its potential as an absorbent (biosorbent) heavy metal Cu^{2+}.

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2 RESEARCH METHODOLOGY

This research uses a mix of culture bacteria comprising of Thiobacillus sp. and Clostridium sp. as bioremediation. In the previous research, bioremediation was able to grow adequately on the growth medium of artificial Stone Mineral solution. This research uses a mix of culture bacteria comprising of Thiobacillus sp. and Clostridium sp. as bioremediation. In the previous research, bioremediation was able to grow adequately on the growth medium of artificial Stone Mineral Salt solution (SMSs) with a speed of cells/mL 21.580 (Figure 1)

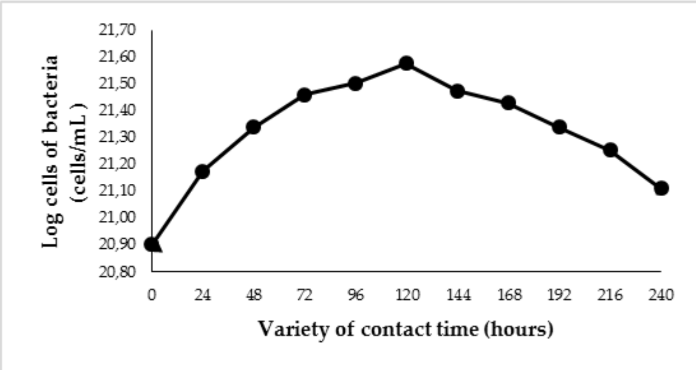

Fig. 1. *Growth curve of mixed cultures of Thiobacillus sp. bacteria. and Clostrisium sp. in liquid media Stone Mineral Salt solution (SMSs)*

The liquid media contained 10 to 100 ppm of heavy metal Cu^{2+}, under temperatures of 20 to 40 °C, pH 7 and with a stirring speed of 150 rpm. However, within 48 hours, the average allowance (removal) of heavy metal Cu^{2+} was 92.00%. In a previous research in liquid media, a sensitivity test of Thiobacillus sp. and Clostrisium sp. The Cu^{2+} heavy metals were carried out to determine whether the mixed culture of bacteria was resistant or not by varying the concentration of Cu^{2+} (ppm) heavy metals which were tested at 10 to 100 (Figure 2). The results showed that no inhibition zone was formed for 48 hours. This proves that the mixed culture of Thiobacillus sp. and Clostrisium sp. insensitive or resistant to the presence of heavy metals Cu^{2+} and mixed cultures it has a high resistance to heavy metals Cu^{2+} which are pollutants of xenobiotics and are toxic but are suitable substrates for the growth of mixed bacterial cultures. Based on the previous research, this current study uses bioremediation test to analyze the ability to eliminate 100 ppm Cu^{2+} heavy metals contained in soil sterile, pH 7, humidity average of 27% and an average temperature of $\pm$ 25°C.

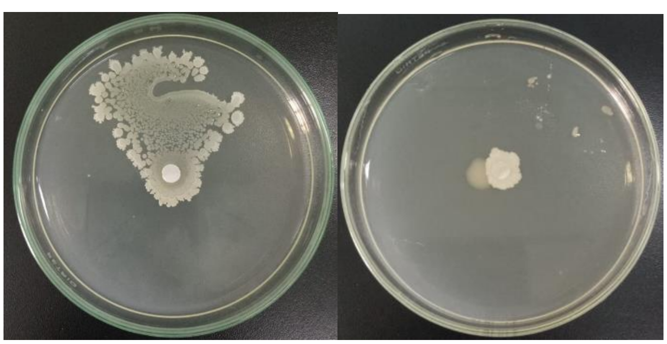

Fig. 2*. Sensitivity test of Thiobacillus sp. and Clostrisium sp. with heavy metals Cu^{2+} at concentrations displayed only 10 ppm (left) and 100 ppm (right)*

2.1 Review Stage Preparation of Sterile Soil Medium and Heavy Metal Polluters Cu^{2+}

Solid media is the land used for organic farming activities sterilized using an autoclave temperature of 125°C for approximately one-half hour and revised using an oven with a temperature of 200°C. The soil is sifted to obtain a size smaller to the surface area to facilitate mixed culture bacteria degradation of the heavy metal polluters Cu^{2+}. Artificial pollution was created by making the parent solution of 1000 ppm in advance, using dissolving 1 gram $CuSO_4.5H_2O$ into 1liter equates. Furthermore, 100 ppm solution of $CuSO_4.5\,H_2O$, regarded as polluters are presented to the sterile soil.

2.2 Preparation of Growth Medium Stone Mineral Salt solution (SMSs) and The Cultivation of Bioremedian (Bioremediation Cultivation)

Liquid growth media in a sterile state is also used in advanced research as the complete media in the ratio Carbon: Nitrogen: Phosphor to determine the right fit for growth. One liter Stone Mineral Salt solution (SMSs) containing 0.5 gr calcium carbonate ($CaCO_3$); 2.5 gr ammonium nitrate (NH_4NO_3); 1 gr sodium hydrogen phosphate ($Na_2HPO_4.7H_2O$); 0.5 gr mono potassium nitrate KH_2PO_4); 0.5 gr magnesium sulfate ($MgSO_4.7H_2O$); and 0.2 gr magnesium chloride ($MnCl_2.7H_2O$). Addition of molase is done as a carbon source because Stone Mineral Salt solution (SMSs) do not contain carbon (Figure 3).

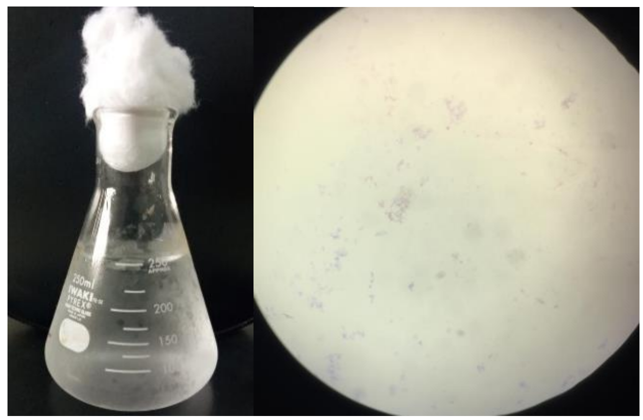

Fig. 3*. Stone Mineral Salt solution (left) and molase (right)*

Bioremediation Thiobacillus sp. is gram-negative while Clostridium sp. is a gram-positive bacterium (Figure 4)

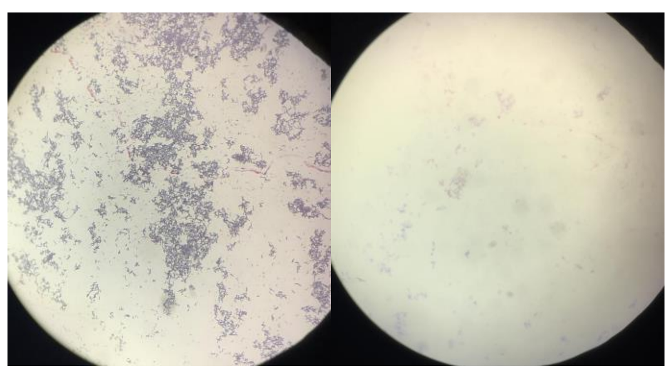

Fig. 4. *Mixed Culture Bacteria Thiobacillus sp. and Clostridium sp.*

Also, data were collected from the Microbiology/Biology Laboratory, Department of environmental engineering, Universitas Trisakti, Jakarta, Indonesia. The result of the previous research proves the exponential growth phase occurred in the bioremediation 5 days, at pH 7.

2.3 Removal Efficiency Cu^{2+} in Polluted Soil

The research lasted 312 hours, which is approximately 13 days, and sampling conducted daily. Analysis of Atomic Absorption Spectrophotometry (AAS) was carried out to determine the concentration of heavy metals Cu^{2+} at the beginning and end of the study. Percentage of heavy metal Cu^{2+} separation was calculated using the following equation:

$$\text{Removal efficiency (\%)} = \frac{C(a) - C(b)}{C(a)} \times 100\%$$

Where:
C (a): initial concentration of heavy metal Cu^{2+} in solid media (ppm)
C (b): final concentration of heavy metal Cu^{2+} in solid media (ppm)

3 RESULT AND DISCUSSION

The removal efficiency of heavy metal Cu^{2+} (%) on a wide variety of contact time (hours) for 312 hours of observation is shown in Table 1 and Figure 6. While the measurement value of various environmental factors such as the pH value and humidity for 312 hours can be seen in Table 2

TABLE 1
REMOVAL EFFICIENCY OF HEAVY METAL Cu^{2+} (%) ON A WIDE VARIETY OF CONTACT TIME (HOURS)

Variety of Contact Time (Hours)	Removal Efficiency (%)	Standard Deviation
24	18.67	0.03
72	27.05	0.12
120	39.64	0.16
168	40.84	0.05
216	56.15	0.06
264	36.97	0.04
312	32.64	0.08

TABLE 2
HUMIDITY AND PH VALUES IN THE DEGRADATION OF Cu^{2+} HEAVY METALS

Variety of Contact Time (Hours)	Humidty (%)				pH			
	X_1	X_2	X_3	∑X	X_1	X_2	X_3	∑X
24	28	28	28	28.00	7.7	7.6	7.7	7.67
72	28	27	27	27.33	7.6	7.7	7.7	7.67
120	27	28	27	27.33	7.7	7.6	7.6	7.63
168	28	28	27	27.67	7.6	7.6	7.6	7.60
216	28	26	27	27.00	7.5	7.5	7.4	7.47
264	26	27	27	26.67	7.3	7.3	7.2	7.27
312	25	25	26	25.33	7.2	7.1	7.2	7.17

Based on the results of Cu^{2+} heavy metal removal on the variation of contact time (hours) shown in Table 1 and Figure 3, the highest allowance was carried out by mixed cultures of Thiobacillus sp. and Clostrisium sp. the contact time for 216 hours was 56.15% with an average humidity of 27% and pH of 7.47 (Table 2) with a speed of cells/mL 21.792 (Figure 5). The study proved that bioremediation has the capability to function synergistically in utilizing heavy metals as a source of nutrient to expand its allowance by 50% (Figure 6). The culture ability of a mixture of Thiobacillus sp. and Clostrisium sp. which is synergistic in reducing Cu^{2+} heavy metals as a substrate seen in the increase of Cu^{2+} heavy metal degradation from contact times (hours) 24, 72, 120, 168 and 216 that is equal to 18.67%, 27.05%, 39.64%, 40.84% and 56.15%. And then the decline in allowance at 264 hours and 312 hours with a allowance of 36.97% and 32.64% The decrease in removal of Cu^{2+} heavy metals (%) at the contact time of 216 hours to the contact time of 264 hours can be caused by various environmental factors which do not support the mixed culture of the bacteria Thiobacillus sp. and Clostrisium sp. such as less availability of nutrients, the two cultures can be antagonistic, or changes in temperature (^{o}C), availability of oxygen, pH or moisture level, resulting in a mixture of cultures of the bacteria Thiobacillus sp. and Clostrisium sp. can not reduce heavy metals optimally.A good pH value for the growth of microorganisms in the bioremediation process is 5.5-8.8 with humidity increasing 25%-28% [21]. While the temperature is good for mixed cultures of the bacteria Thiobacillus sp. and Clostridium sp. is involving 30^{o}C [22] because it belongs to the species of mesophilic microorganisms [23].

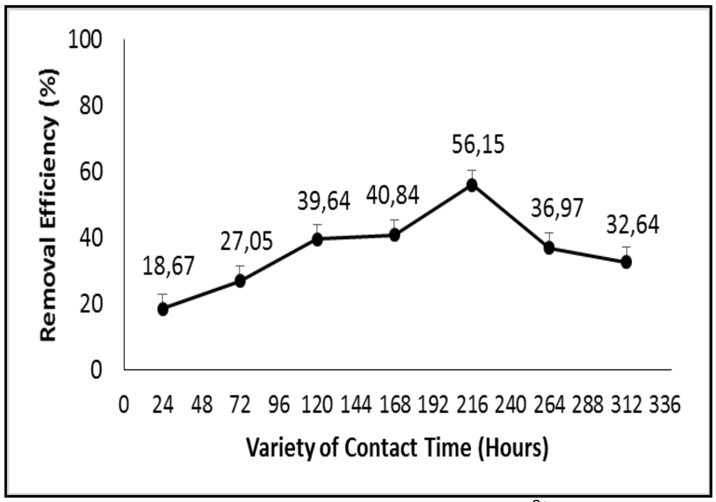

***Fig. 6**. Removal efficiency of heavy metal Cu^{2+} (%) on a wide variety of contact time (hours)*

Furthermore, the efficiency was higher at a contact time of 48 hours compared to the use of increasingly mixed bacterial cultures which utilized Viridibacillus arenosi B-21, soli Sporosarcina B-22, Enterobacter cloacae KJ-46 and E.cloacae KJ-47. This was only able to reduce heavy metals by 5.6% for 48 hours, with a pH of 7 and temperature of 30^{o}C [24]. This also resulted in the use of bacterial mixed culture chemolithotrophic, especially Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans dan Leptospirillum ferrooxidans species, to reduce soils polluted with heavy metals by 62% after its remediation reached 5.90 [25]. Also, in this research [26], a single culture bacteria known as Pseudomonas aeruginosa was utilized with a tolerance range of 1000 ppm and allowance amounting to 0.67 mg/g. The successful application of the bioremediation method depends on the ability of active metabolic microorganisms, such as the use of the heavy metal as a source of nutrient [19]. External and internal factors also influenced bioremediation. These external factors include the chemical environment, such as humidity, temperature, pH, soil type, and the presence of oxygen, while internal factors are metabolic activity by leveraging the growth of microbes [20], [24]. Other research [24], [25], [26] also stated that the removal of heavy metals from the environment is active and is influenced by microorganisms with different physiological characteristics. Similarly, any microbes that synergize the varied amount of energy required to reduce heavy metal is essential for growth and development.

4 CONCLUSION

In a tropical environment, at neutral pH, 27% humidity, with temperature ranging from ± 25 ° C, mix culture the bacteria Thiobacillus sp. and Clostridium sp. plays an active role as a bioremediation to extract heavy metal Cu^{2+} from the soil. Its efficiency increases from 24 to 216 hours and reducing beyond this time. However, the highest removal efficiency achieved was 50%, due to the bioremediation utilization of alternative methods which potentially overcame the pollution of heavy metals in the soil.

REFERENCES

[1] Zhao, Q., Yue, S., Bilal, M., Hu, H., Wang, W., & Zhang, X. (2017). “Comparative genomic analysis of 26 Sphingomonas and Sphingobium strains: Dissemination of bioremediation capabilities, biodegradation potential and horizontal gene transfer”. Science of The Total Environment, 609, 1238–1247. (http://dx.doi.org/10.1016/j.scitotenv.2017.07.249)

[2] Bilal, M., Rasheed, T., Nabeel, F., Iqbal, H.M., Zhao, Y., 2019b. “Hazardous contaminants in the environment and their laccase-assisted degradation–a review”. J. Environ. Manag. 234, 253–264. (https://doi.org/10.1016/j.jenvman.2019.01.001)

[3] Shaheen, N., Irfan, N. M., Khan, I. N., Islam, S., Islam, M. S., & Ahmed, M. K. (2016). “Presence of heavy metals in fruits and vegetables: Health risk implications in Bangladesh”. Chemosphere, 152, 431–438. (http://dx.doi.org/10.1016/j.chemosphere.2016.02.060)

[4] Nanda, M., Kumar, V., & Sharma, D. K. (2019). “Multimetal tolerance mechanisms in bacteria: The resistance strategies acquired by bacteria that can be exploited to “clean-up” heavy metal contaminants from water”. Aquatic Toxicology, 212, 1–10. doi:10.1016/j.aquatox.2019.04.011

[5] Jones, A. S., Marini, J., Solo-Gabriele, H. M., Robey, N. M., & Townsend, T. G. (2019). “Arsenic, copper, and chromium from treated wood products in the U.S. disposal sector”. Waste Management, 87, 731–740. (https://doi.org/10.1016/j.aquatox.2019.04.011)

[6] Hu, Y., Zhou, J., Du, B., Liu, H., Zhang, W., Liang, J., Zhang, W., You, L., Zhou, J. (2019). “Health risks to local residents from the exposure of heavy metals around the largest copper smelter in China”. Ecotoxicology and Environmental Safety, 171, 329–336. (https://doi.org/10.1016/j.ecoenv.2018.12.073)

[7] Khan, A. H. A., Nawaz, I., Yousaf, S., Cheema, A. S., & Iqbal, M. (2019). “Soil amendments enhanced the growth of Nicotiana alata L. and Petunia hydrida L. by stabilizing heavy metals from wastewater”. Journal of Environmental Management, 242, 46–55. (https://doi.org/10.1016/j.jenvman.2019.04.040)

[8] Gustafsson, J. P. (2019). “Vanadium geochemistry in the biogeosphere –speciation, solid-solution interactions, and ecotoxicity”. Applied Geochemistry. (https://doi.org/10.1016/j.apgeochem.2018.12.027)

[9] Latorre, M., Troncoso, R., & Uauy, R. (2019). “Biological Aspects of Copper”. Clinical and Translational Perspectives on Wilson Disease, 25–31. (https://doi.org/10.1016/B978-0-12-810532-0.00004-5)

[10] Argüello, J. M., Raimunda, D., & Padilla-Benavides, T. (2013). “Mechanisms of copper homeostasis in bacteria”. Frontiers in Cellular and Infection Microbiology, 3.doi:10.3389/fcimb.2013.00073

[11] Liu, L., Bilal, M., Duan, X., & Iqbal, H. M. N. (2019). “Mitigation of environmental pollution by genetically engineered bacteria — Current challenges and future perspectives”. Science of The Total Environment. (https://doi.org/10.1016/j.scitotenv.2019.02.390)

[12] Zhou, J., Du, B., Hu, Y., Liang, J., Liu, H., Fan, X., Zhang, L., Cui, H., Liu, X., Zhou, J., 2019a. “A new criterion for the health risk assessment of Se and Pb exposure to residents near a smelter”. Environmental Pollution .244, 218–227. (https://doi.org/10.1016/j.envpol.2018.10.038)

[13] Thakur, S., Sharma, S.S., 2016. “Characterization of seed germination, seedling growth, and associated metabolic responses of Brassica juncea L. cultivars to elevated nickel concentrations”. Protoplasma 253, 571–580. doi:10.1007/s00709-015-0835-0

[14] Sharma, B., Singh, P., Chauhan, P. S., Singh, S., & Singh, R. P. (2019). “Microbes-Assisted Remediation of Metal Polluted Soils”. New and Future Developments in Microbial Biotechnology and Bioengineering, 223–232. (https://doi.org/10.1016/B978-0-444-64191-5.00016-X)

[15] Kanadasan, J., & Abdul Razak, H. (2015). “Engineering and sustainability performance of self-compacting palm oil mill incinerated waste concrete”. Journal of Cleaner Production, 89, 78–86.doi:10.1016/j.jclepro.2014.11.002

[16] Dixit, R., et al. (2015). “Bioremediation of Heavy Metals from Soil and Aquatic Environment: An Overview of Principles and Criteria of Fundamental Processes”. Sustainability, 7(2), 2189–2212.doi:10.3390/su7022189

[17] De Freitas, E. D., de Almeida, H. J., de Almeida Neto, A. F., & Vieira, M. G. A. (2018). “Continuous adsorption of silver and copper by Verde-lodo bentonite in a fixed bed flow-through column”. Journal of Cleaner Production, 171, 613–621. doi:10.1016/j.jclepro.2017.10.036

[18] De Freitas, E. D., de Almeida, H. J., de Almeida Neto, A. F., & Vieira, M. G. A. (2018). “Continuous adsorption of silver and copper by Verde-lodo bentonite in a fixed bed flow-through column”. Journal of Cleaner Production, 171, 613–621. doi:10.1016/j.jclepro.2017.10.036

[19] Dangi, A. K., Sharma, B., Hill, R. T., & Shukla, P. (2018). “Bioremediation through microbes: systems biology and metabolic engineering approach”. Critical Reviews in Biotechnology, 1–20. (https://doi.org/10.1080/07388551.2018.1500997)

[20] Kumar, P., Kim, K.-H., Bansal, V., Lazarides, T., & Kumar, N. (2017). “Progress in the sensing techniques for heavy metal ions using nanomaterials”. Journal of Industrial and Engineering Chemistry, 54, 30–43. (http://dx.doi.org/10.1016/j.jiec.2017.06.010)

[21] Kensa,V.M. (2011). Bioremediation - An Overview. Journal of Industrial Pollution Control 27 (2): 161-168.

[22] Sunaryo, T., Widyatmoko, H., & Rinanti, A. (2018). Chlorpyrifos removal by Thiobacillus sp. and Clostridium sp. in liquid medium. MATEC Web of

Conferences, 197, 13012.doi:10.1051/matecconf/201819713012
[23] Supaphol, S., Jenkins, S. N., Intomo, P., Waite, I. S., & O'Donnell, A. G. (2011). Microbial community dynamics in mesophilic anaerobic co-digestion of mixed waste. Bioresource Technology, 102(5), 4021–4027.doi:10.1016/j.biortech.2010.11.124
[24] Kang, C.-H., Kwon, Y.-J., & So, J.-S. (2016). "Bioremediation of heavy metals by using bacterial mixtures". Ecological Engineering, 89, 64–69.doi:10.1016/j.ecoleng.2016.01.023
[25] Nicolova, M., Spasova, I., Georgiev, P., & Groudev, S. (2017). "Microbial removal of toxic metals from a heavily polluted soil". Journal of Geochemical Exploration, 182, 242–246. doi:10.1016/j.gexplo.2016.11.003
[26] Nagashetti V, Mahadevaraju GK, Muralidhar TS, Javed A, Trivedi D, Bhusal KP. "Biosorption of heavy metals from soil by Pseudomonas aeruginosa". International Journal of Innovative Technology and Exploring Engineering (IJITEE). 2013; 2(6):9-17
[27] Ririhena, S. A. J., Astuti, A. D., Fachrul, M. F., Silalahi, M. D. S., Hadisoebroto, R., & Rinanti, A. (2018). "Biosorption of heavy metal copper (Cu^{2+}) by Saccharomyces cerevisiae". IOP Conference Series: Earth and Environmental Science, 106, 012090. doi:10.1088/1755-1315/106/1/012090
[28] Rinanti, A., Fachrul, M. F., Hadisoebroto, R., & Silalahi, M. (2017). "Improving biosorption of Cu (II)-ion on artificial wastewater by immobilized biosorbent of tropical microalgae". International Journal of GEOMATE, 13 (36), 6-10 (http://dx.doi.org/10.21660/2017.36.2744).
[29] Mahardika, G., Rinanti, A., & Fachrul, M. F. (2018). "Phytoremediation of heavy metal copper (Cu^{2+}) by sunflower (Helianthus annuus l.)". IOP Conference Series: Earth and Environmental Science, 106, 012120. doi:10.1088/1755-1315/106/1/012120