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Decrease of Heavy Metal Using Effective Microorganism 4 (EM4) As the Soil Bioremediation Effort.

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ABSTRACT

Research has been conducted on the use of effective microorganism 4 (EM4) as bioremediator on soil contamination. Heavy metals observed include cadmium (Cd), chromium (Cr) and lead (Pb). Added EM4 by 5%, 10%, 15%, 20% and 25% carried out for 7 days and observed decrease of heavy metals. The decrease in heavy metals was measured using the Perkin Elmer AAS instrument. Based on the results obtained it can be said that EM4 can be used as bioremediator agent. The greatest heavy metal decline in lead was 100%, followed by chromium up to 94.611% in use of 5% EM4 and cadmium 49.213% on 25% EM4 usage.

Keywords: heavy metal, EM4, bioremediation of soil

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INTRODUCTION

Contamination of heavy metals in the environment becomes very important to note. At one chemical laboratory location in Surabaya, East Java, Indonesia is not accompanied by a wastewater treatment plant. This led to a preliminary analysis of the possibility of heavy metal pollution on the ground. Based on the results of previous research it is known there are several types of heavy metals in the soil around the laboratory. Heavy metals include cadmium (Cd), chromium (Cr) and Lead (Pb). The concentrations of heavy metals were identified with maximum levels of Cd 0.254 ppm, Cr 1,8 ppm, and Pb 4.97 ppm. The Agency for the Toxic Substances and Disease Registry (ATSDR) has shown it as lead, cadmium and chromium as the three most toxic weights. The toxicity (heavy toxicity) of heavy metals depends on the type, degree, synergy-antagonist effect and the nature of the chemical physique. The greater the heavy metal content, the greater the toxicity. These three metals for long periods of time, which are naturally present in the soil, can accumulate in humans and cause serious health problems. Unlike organic pollutants, heavy metals do not undergo chemical changes or microbial damage and persist in place for longer durations after release to the soil [1, 2, 3, 4]. Thus the three heavy metals should be handled before accumulating even more.

Cadmium (Cd) causes central nervous disorders, and peripheral nerves. In certain doses may cause symptoms of neuropathy, muscle atrophy and myelin degeneration in mice. In acute hemorrhagic doses causes lesions in the sensory ganglion. Cadmium also inhibits the chemical transmission of the neuromuscular junction. This effect leads to inhibition of the function of calcium (Ca) in the nerve terminals and causes a decrease in the clearance of presynaptic acetyl choline as neurotransmitters of the nerve endings. Cd toxicity after birth also causes less disturbance of the reflex response, hypoactivity, impaired coordination and decline in learning activities. Effects on the respiratory system: chronic bronchitis, progressive fibrosis in the lower respiratory tract and septal rupture between the alveoli caused by emphysema [2, 5].

Lead (Pb) although naturally present in nature but can accumulate in humans and can cause serious health problems. Lead can lead to poisoning of the nervous system, hematologic, hematotoxic, and affect the kidneys work. Lead also causes disruption of genito urinary system, nervous system, gastric intestinal tract, hematopoetic system, and skin [2, 3, 5].

Chromium (Cr) can be harmful to the environment, although only in small amounts, chromium is stable and accumulates in the body, so over time can trigger cancer cells (carcinogenic) money can harm health. In the soil chromium is found in the form of Cr (III) and Cr (VI). Chromium also causes disruption of the genito system of urinary, nervous system, liver, respiratory tract, and skin [2, 5, 6, 7].

Efforts to overcome the pollution caused by heavy metals, among others, chemically, physics and biology. The chemical way with the addition of certain chemical compounds is also possible to add contamination. How to physics by adding adsorbent. This is commonly done for water pollution cases. This method is easy and the results are also good but still have to cultivate the soil first in liquid form. So the most effective way to deal with heavy metal pollution in the soil is by biologically using microbes or called bioremediation.

Bioremediation is a process of degrading biologically harmful organic pollutants with the help of microorganisms, into other compounds such as carbon dioxide (CO₂), inorganic compounds and / or other organic compounds[8, 9]. So bioremediation is one of the alternative technologies to solve environmental problems by utilizing the help of microorganisms. These microorganisms are yeast, fungi (mycoremediation), yeast, lipolytic mold algae, earthworms, and bacteria that act as bioremediator agents [10, 11, 12, 13, 14, 15].

In this study will be tested the use of effective microorganism 4 (EM4) to overcome the heavy metal pollution in the soil around the laboratory. EM4 is a multi-bacterial product that has been sold in Indonesia. EM4 is widely used in composting. The bacteria content in EM4 is expected to be utilized as degradation of heavy metals in the soil around the laboratory. It is useful to provide policy on the management of heavy metal waste resulting from the laboratory chemical process that uses heavy metals.

MATERIALS AND METHODS

This research was conducted in Inorganic Chemistry Laboratory, Chemistry Department, FMIPA Universitas Negeri Surabaya. The materials used include land taken from the sample location, EM4 and HNO₃ 1%. Equipment used include glassware, Whatman 42 filter paper, and Perkin Elmer AAS instrument.

Research steps

Sample Preparation

One hundred grams of soil samples were known to initially mixed EM4 with variations of 5%, 10%, 15%, 20% and 25% (v / w) are inserted into polybags. Prepared also the land without treatment as a control. After 7 days, the soil measured levels of Cr, Cd, and Pb. 10 grams of soil taken up plus 50 mL HNO₃ 1% soaked and filtered. The filtrate was measured with AAS to determine the levels of Cr, Cd and Pb remaining. Then the calculated percent decrease in the weight of the metal content.

Preparation of standard solution

Preparation of standard solution is made by diluting 1000 ppm of standard solution of Cd, Cr and Pb to 500 ppm. The solution was diluted again to 100 ppm then diluted again to the standard concentration variation. For standard solution of Cd 1, 1.5, 2, 3.5 and 5 ppm. standard solutions of Cr 2, 4, 6, 10, 14 ppm and standard solutions of Pb 10, 20, 40, 60 and 80 ppm.

Instrumentation

Measurement of sample using Perkin Elmer AAS instrument. The data obtained in the form of absorbance data. The absorbance data is then converted to concentration data. The measured concentration is the residual metal concentration of the sample.

RESULTS AND DISCUSSION

The decrease in heavy metals can be calculated by reducing the initial metal concentration by residual concentration calculated based on absorbance data of AAS results. The difference in concentration lost compared to the initial concentration to be calculated as the concentration of heavy metal drop. Data percentage of heavy metal decline can be seen in table 1.

Table 1: Data of heavy metal drop

Concentration of bioremediation agent	Percent of Heavy Metal Decrease		
	Cd	Cr	Pb
5%	37,795	94,611	100
10%	38,189	94,167	100
15%	42,126	93,389	100
20%	47,244	93,111	100
25%	49,213	93,111	100
control	37,008	83,667	74,145

Based on the data in table 1 it can be seen that there is a decrease of all three heavy metals. Pb metal has the greatest decline, furthermore chromium metal and cadmium metal. Effective microorganism 4 (EM4) is a multi-bacterial product that has been circulating in the Indonesian market. This EM4 product contains many microorganisms applied to agriculture. One of them is used as an inoculation to increase the diversity and population of microorganisms. Microorganisms contained in EM4 can be used to describe the process of composting waste and organic waste. In EM4 there is a mixture of several types of microorganisms both aerobic and anaerobic that live arbitrarily with each other. The composition of microorganisms composing EM4 is lactic acid bacteria, yeast, actinomycetes and photosynthetic bacteria [10].

The process of loss of heavy metals by microbes through heavy metal bonding by bacteria. When heavy metal ions are bound by the cell wall ion exchange occurs where the ions in the cell wall are replaced by heavy metal ions and the second is a complex formation between heavy metal ions with functional groups such as carbonyl, amino, thiol, hydroxy, phosphate, and hydroxy-carboxyl located on the cell wall [16]. Some microbial mechanisms adapt to metal contaminated conditions such as microbes utilizing metals as energy sources, precipitating metals in the form of insoluble metal salts, immobilizing metals in cell walls, producing chelating agents, changing the permeability of microbial cell membranes to metals, and reducing metals to non-toxic forms [17]. Figure 1 is a hypothetical reaction between heavy metals and microbial cells. Microbial cells secrete lactic acid which functions as a heavy metal chelating agent (eg Cd).

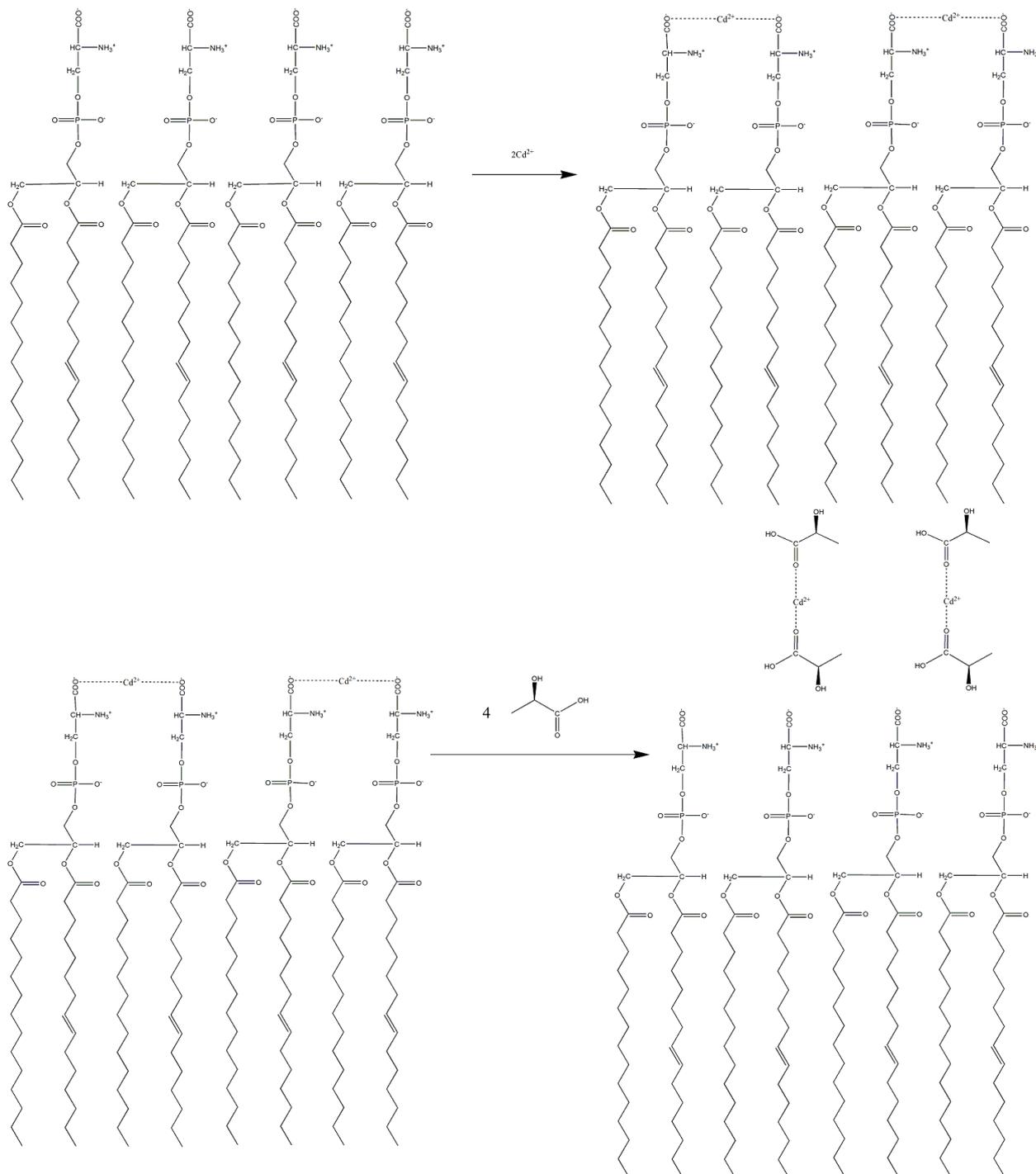


Figure 1: Hypothetical reaction between heavy metal Cd and microorganisms

Based on the reaction in FIG. 1 it shows the cell wall membrane when encountered with heavy metals releasing lactic acid which serves as a heavy metal chelating agent. In the cell wall there are carbonyl functional groups. This carbonyl group can bind to heavy metals. Through the metabolism process bacteria secrete lactic acid. Lactic acid then chelating heavy metals form heavy metal complexes and loose from cell walls.

Based on the data in Table 1 it can be said that generally the percentage of cadmium decrease is lower than that of chromium metal. This can be explained using the theory of HSAB (hard soft acid base). According to HSAB theory Cr is classified as hard acid and Cd as soft acid, and Pb intermediates. The involved microorganism releases lactic acid and hydroxyl which is a hard base. The bond between the hard acid and the hard base is the ionic bond. Meanwhile, the bond between soft acid and hard base is a covalent bond. Then the rate of reaction between compounds with ionic bonds is faster than compounds with covalent bonds. This is why chromium decreases faster than cadmium.

Lead as intermediate compound has the greatest decrease up to 100% higher than chromium. This is because in the soil the chromium is in the form of Cr (III) which is maintained on the surface of the silicate oxide of the soil and forms a stable chemical even in the presence of acidic pH. This stability prevents the reaction rate of Cr as a hard acid when it encounters lactic acid or hydroxyl from microorganisms in the soil. The more EM4 used is the more availability of lactic acid in the media. Increased lactic acid causes soil pH to get more acid and chromium more stable. Thus, the resulting increase in the amount of EM4 used further decreases the amount of heavy metals that can be remediated. This is different from cadmium where more and more lactic acid is available, the more cadmium metal that can be tied so that the percentage of heavy metal decline is increasing.

In general, in the control sample also decreased heavy metals Cd, Cr and Pb. The percentage decrease in control was lower than the sample by treatment of EM4 administration. This is because giving EM4 will increase the amount of lactic acid bacteria that play the role of releasing lactic acid. Naturally these bacteria exist in the soil. The addition of EM4 increases the amount of lactic acid bacteria. Decreasing of heavy metals is higher than without the addition of EM4. Then it can be said that EM4 can act as bioremediation agent.

CONCLUSION

Based on the results of the analysis can be concluded that the effective microorganism 4 (EM4) can be used as a bioremediatory agent. The decline of heavy metal Cd biggest 49.213%, Cr 94, 611% and Pb metal of 100%

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REFERENCES

- [1] Nieto-Márqueza, A., Pinedo-Flores, A., Picassoc, G., Atanesa, E., and Koub, R. S. *Journal of Environmental Chemical Engineering* 2017; 5: 1060–1067
- [2] Soemirat J., *Toksikologi Lingkungan*. Reference Publications, Gadjah Mada University Press, Yogyakarta. 2009, pp. 37-40
- [3] Ok Y S, Lee H, Jung J, Song H, Chung N, Lim S, Kim J G. *Agr Chem Biotechnol* 2004; **47**: 143–146
- [4] Mahar, A., Wang, P., Ronghua, L., Zhang, Z., *Pedosphere* 2015; 25(4): 555–568
- [5] Radojević M., and Bashkin, V.N. *Practical Environmental Analysis*. Reference Publications, Royal Society of Chemistry, Cambridge, 1999, pp. 359-361
- [6] Haryani, K., Hargono, dan Budiyati. *JReaktor* 2007; 11:2
- [7] I. Reijonen, H. Hartikainen, *Appl. Geochem* 2016; 74: 84–93
- [8] Brown L. D., Cologgi D.L., Gee. K. F., and Ulrich A.C. *Bioremediation of Oil Spills on Land* University of Alberta. Reference Publications, Edmonto AB Canada, 2017, pp. 699-729
- [9] Mohammed N., Allayla R.I., Nakhla G.F., Faroaq S., and Husain T., *Journal of Environmental Science and Health* 1996; A31: 1547



- [10] Hayati, N., Uji Efektivitas Wastetreat untuk Bioremediasi Logam Berat dalam Sludge Pabrik Kertas Deinking, Thesis, Sumberdaya Lahan Department, IPB, 2011.
- [11] Priadie, Bambang, J. Ilmu Lingkungan 2012; 10(1): 135-145.
- [12] Prasetyono, Eva, J. Akuatik Sumberdaya Perairan, 2013; Vol 7, No 1
- [13] Puspitasari, D. J., dan Khairuddin J. KOVALEN, 2016; 2(3):98-106
- [14] Mukharomah E., Munawar, and Widjajanti H., Jurnal Ilmu Lingkungan, 2015; Volume 13 issue 1: 19-26
- [15] A.B. Azizi, M.P.M.Lim, Z.M.Noor, Noorlidah Abdullah, J. Ecotoxicology and Environmental Safety; 2013; 90: 13–20
- [16] Suhendrayatama, Heavy Metal Bioremoval by Microorganism; a Literature Study, <http://www.istecs.org/Publication/japan/010211.2001>.
- [17] Widyati, E. J. Info Hutan, 2008;2: 151-160