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Cultivation of Microalgae Mixed Culture from Mahoni Small Lake UI Campus Depok using Zeolite Substrate with Varying Pb Concentration

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Abstract: Research on the cultivation of mixed cultures of Situ (small lake) Mahoni UI Campus Depok has been done. This research was a preliminary study to measure and compare the growth of Situ Mahoni mixed cultures microalgae grown in Bold's Basal Medium (BBM) with addition of zeolite and without addition of zeolite, and varying concentrations of heavy metal Pb. The concentrations of heavy metal Pb used were 0 ppm, 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm for two treatments. The results of this research were that the growth of mixed cultures with the addition of zeolite had the highest abundance of 8.800 cells/mL on day 5 (T5) at a Pb concentration of 40 ppm. Meanwhile, the growth of mixed culture without the addition of zeolite had the highest abundance of 21.700 cells/mL on day 10 (T10) at a Pb concentration of 60 ppm. The growth of microalgae mixed cultures in the medium without addition of zeolite was higher than using zeolite. The activation of zeolite pores causes the microalgae growth is relatively lower. This work aimed to study the growth of a mixed cultures microalgae in varying concentrations of Pb with and without the addition of zeolite.

Keywords: BBM; Lead (Pb); Microalgae mixed culture; Situ Mahoni; Zeolite

Introduction

Microalgae are photosynthetic microorganisms and can assimilate several types of heavy metals (Geremia *et al*., 2021). Microalgae have resistance to heavy metals by making protein complexes without reducing the function of these proteins and the amount of heavy metal ions is stored in vacuoles (Priya *et al*., 2022). Microalgae that are exposed to toxins such as Plumbum (Pb) and Hydrargyrum (Hg) will produce enzymes such as ascorbate peroxide, glutathione reductase, and catalase to fight free radicals (Priya *et al*., 2022). Mixed cultures microalgae consist of two or more organism in the inoculum (Prihantini *et al*., 2021). Mixed cultures microalge can occur naturally, such as in the lake.

Microalgae has the ability to remediate metals (Ajayan *et al*., 2015). *Scenedesmus* sp. is an example of green algae which has a wide distribution in all aquatic environments and is easy to obtain (Mirghaffari *et al*., 2015). *Scenedesmus* may have a high tolerance to adverse environmental conditions and have a maximum adsorption capacity exceeding 50 mg g^{-1} for Pb (Morillas-España *et al*., 2021). As another example, microalgae from the Chlorophyta division, namely *Chlorella vulgaris*, can absorb ammonium content of 30% of 878 mg/L and phosphorus of 43% of 154 mg/L in liquid livestock waste (You *et al*., 2021). Microalgae from the Cyanophyta division, namely *Leptolyngbya* sp. can absorb 90.14% of ammonium and 72.62% of phosphate in liquid waste from the brewing industry (Papadopoulos *et al*., 2020). The use of microalgae biomass in heavy metal bioremediation greatly saves costs and has a high level of efficiency (Manzoor *et al*., 2019).

Heavy metals can be defined as metals and metalloids with a density of more than 5 g/cm^3 (Leong & Chang, 2020). They tend to persist in nature and can cause environmental and health problems because heavy metals cannot be decomposed naturally (Yang *et al*., 2015). Living creatures that come into direct contact with heavy metals will experience serious threats to physical health, genetic damage, mutations, damage to

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the central nervous system, and increased risk of cancer. At very low concentrations, heavy metals can be toxic, carcinogenic, mutagenic, and teratogenic (Leong & Chang, 2020). One of the most toxic heavy metals is Plumbum (Pb) or lead (Chugh *et al*., 2022).

Plumbum (Pb) or lead is very dangerous heavy metal for human health and the environment, especially in water. In various industries. Pb is used for the production of coal, batteries, and pipelines (Leong & Chang, 2020). Various industrial activities such as battery production, electroplating processes, and manufacturing of microelectronic products result in the release of Pb into the environment (Chugh *et al*., 2022). The maximum limit for Pb pollution in the environment is 0.015 mg/L (Yang et al., 2015). Apart from causing health problems in humans, Pb can disrupt the function of human organs and even cause death (Das *et al*., 2016).

Accumulation of heavy metals by microalgae is environmentally friendly (Das et *al*., 2016) have been shown to tolerate toxic heavy metals such as Pb (Kumar *et al*., 2015). One example of microalgae is *Scenedesmus* sp. which shows an absorption rate of the heavy metal Pb of 75.3-99% (Karla *et al*., 2021). Absorption of the heavy metal Pb by *Scenedesmus* sp. has the highest removal efficiency approaching 100% (Kalra *et al*., 2021). The use of *Scenedesmus* microalgae species as a Pb^{2+} biosorbent has shown good results in reducing the heavy metal Pb in polluted environments (Gu & Lan, 2021).

Zeolite or $CaAl_2Si_4O_{12}{\cdot}nH_2O$ is a hollow aluminosilicate mineral derived from alkali metals (Taamneh & Sharadqah, 2021). Zeolites are used to remediate environmental pollution, especially heavy metal. The synthesis of zeolite materials has been extended to remove heavy metals (Irannajad & Haghighi, 2021). The use of zeolites for catalysis, adsorption, energy purposes, soil remediation, heavy metal remediation, and agricultural purposes has been carried out and is efficient in removing contaminants (Ugwu *et al*., 2022). The toxic nature of heavy metals accumulated in the environment poses a serious threat to human life and the environment (Elboughdiri, 2020). The adsorption method using zeolites is considered an appropriate method for treating wastewater due to its ease of use and cost-effectiveness (Scharnberg *et al*., 2020).

Therefore, this preliminary study was conducted to find out the microalgae mixed cultures using zeolite and without zeolite with varying concentration of Pb. The research objective was to measure and compare the growth of microalgae mixed cultures grown in Bold's Basal Medium (BBM) with addition of zeolite and without addition of zeolite, and varying concentrations of heavy metal lead (Pb).

Method

This research was carried out at the Department of Biology, Universitas Indonesia, from August until October 2023. The equipment used in this study includes a microscope [Olympus], autoclave [TOMY SX-500], analytical balance [Precisa], 50 and 500 mL Erlenmeyer flask [Iwaki], 2L medium bottle [Duran], 12 mL amber glass bottle, pipettor, volumetric pipette [Iwaki], Pasteur pipette [Normax], glass object, 18x18 mm coverslip, and universal pH indicator [Merck]. The materials used were microalgae samples for enrichment obtained from Situ Mahoni UI Depok, zeolite, and Bold's Basal Medium (BBM) for microalgae growth. Supporting chemicals are also used, such as 0.5 N HCl, 0.25 N NaOH, 70% alcohol, aquades, and spirtus.

The process of making Bold Basal Medium (BBM) is carried out by first making a stock solution with several ingredients, such as macronutrients consist of $NaNO_3$, KH_2PO_4, $MgSO_4.7H_2O$, $CaCl_2.2H_2O$, K_2HPO_4, NaCl; micronutrients consist of KOH, $FeSO_4.7H_2O$, H_3BO_3, $ZnSO_4.7H_2O$, $MnCl_2.4H_2O$, MOO_3, $CuSO_4.5H_2O$, $CO(NO_3)_2.6H_2O$; and aquades (distilled water) as solvent.

The $PbCl_2$ metal stock solution was prepared by dissolving 0.1 g of lead chloride in 100 mL of aquades. After the material has dissolved, the solution is transferred into a 125 mL amber glass bottle. The stock solution is used for the medium mixture during growth tests.

Zeolite sterilization is carried out in two ways, namely washing and activation. Washing is carried out using distilled water to remove dirt contained in the zeolite. After that, the zeolite was dried using dry sterilization in an oven for three hours at 150^{o}C. Zeolite activation is carried out using the dealumination and decaionization stages. Dealumination or acidification of zeolite is a method to increase the Si or Al ratio by immersing the zeolite in an acid solution. Soak the acid solution using 0.25 N HCl with 15 g of zeolite for 24 hours.

After that, the zeolite was washed using distilled water to remove the remaining acid solution. Decationization was carried out to increase the catalytic ability of zeolite, namely by soaking the acidified zeolite in a 0.5 N NaOH solution for 24 hours. After that, the zeolite was rinsed with distilled water to remove the remaining NaOH solution and then drained until dry.

The sample was transferred to a 12-well microplate filled with fuel in each well at a rate of 3/4 of the volume of the microplate well. After that, the sample solution is given which will be enriched by 4-5 drops using a Pasteur pipette. All stages are carried out aseptically in the transfer box. Next, the microplate well was glued using parafilm and placed on a culture rack for a period of one month (Prihantini, 2020).

Microalgae samples resulting from enrichment were able to grow well, then grown in 50 mL of BBM in a 100 mL Erlenmeyer with a concentration ratio of microalgae and BBM, that is 1: 2. The samples were placed on a culture rack and left for 7 days. Then stored in the algae culture room at room temperature 25°C.

Inoculation of the test culture begins by preparing 12 Erlenmeyer flasks with a size of 100 mL and each flask is given 40 mL of fuel and 10 mL of inoculum. In the first treatment, 6 Erlenmeyer flasks were given a solution of the heavy metal Pb with concentrations of 0 ppm (0 mL), 20 ppm (1 mL), 40 ppm (2 mL), 60 ppm (3 mL), 80 ppm (4 mL), and 100 ppm (5 mL). In the second treatment, the same Pb concentration was used with 2.5 grams of zeolite substrate added. The light intensity used was 800 lux. The culture room temperature ranges from 27 – 28°C.

Cell abundance was calculated by counting the number and type of cells from each treatment. During the data collection period, cell abundance calculations were carried out every 24 hours starting from inoculation (T_0) until the last day of observation (T_{14}). Counting the number and type of cells uses the subsampling method, namely 1 drop of mixed culture is taken using a pasteur pipette of approximately 0.04 mL, then dropped onto a preparation glass, and covered with a cover glass measuring 18 x 18 mm. Cell enumeration was carried out using a light microscope [Olympus] at a magnification of 40 x 10. Cell abundance was calculated using Equation 1.

$$N = \frac{C \, x \, At}{As \, x \, S \, x \, V} \quad (1)$$

The N value indicates cell abundance. C shows the number of cells counted. It shows the slipcover area is 18x18 mm. As is the area of one strip on the microscope, S is the number of strips observed, and V is the volume of the sample dropped on the preparation glass. The results of this calculation will obtain a cell abundance value/mL (APHA, 2005).

The conditions of the culture room were measured using temperature and light intensity parameters. Thermometers are used to measure temperature and lux meters to measure light intensity. The environmental parameter measured in mixed culture is pH using universal pH indicator paper. Based on the results of measuring the light intensity and temperature of the culture room for 14 days, the culture room had an average light intensity of 800 lux and the temperature of the culture room for 14 days had an average value of 26.6°C. The pH value of the mixed culture did not change for 14 days in both treatments, namely 5.5 without zeolite substrate and 8.5 with zeolite. The pH value increased from the first day of growth (T_0) in cultures using zeolite substrates.

Result and Discussion

Based on the enrichment process, it was found that 3 dominant genera were able to grow well on BBM, namely *Scenedesmus*, *Coenochloris*, and *Kirchneriella* which belonged to the *Chlorophyceae* class. The abundance of microalgae cells is obtained based on observations starting from the first day of inoculation (T_0) to the 14th day (T_{14}) and then the cells will be counted to obtain cell abundance. In Figure 1, a microalgae mixed culture is presented.

The cell abundance growth curve of the mixed culture of microalgae is shwon in Figure 2. At the time of inoculation (T_0), the abundance of cells in the mixed culture without zeolite with a Pb concentration of 0 ppm was 4.400 cells/mL, a concentration of 20 ppm was 4.300 cells/mL, a concentration of 40 ppm was 4.300 cells/mL, a 60 ppm concentration of 4.000 cells/mL, an 80 ppm concentration of 3.900 cells/mL, and a 100 ppm concentration of 4.000 cells/mL. The growth of the mixed culture in the control treatment with a Pb concentration of 0 ppm showed that the abundance of cells undergoing the adaptation phase starting from day 2 was 8.600 cells/mL to day 6, namely 6.200 cells/mL.

The abundance growth of mixed cultures using zeolite substrates shown on Figure 3. The abundance of cells in mixed cultures using zeolite substrate at the time of inoculation (T_0) with a Pb concentration of 0 ppm and 20 ppm was 4.300 cells/mL, a concentration of 40 ppm was 3.900 cells/mL, a concentration of 60 ppm and 80 ppm was 3.800 cells/mL, and a concentration of 100 ppm is 3.900 cells/mL. In the control treatment with a Pb concentration of 0 ppm, the mixed culture experienced a decrease on day 1, namely 3.900 cells/mL.

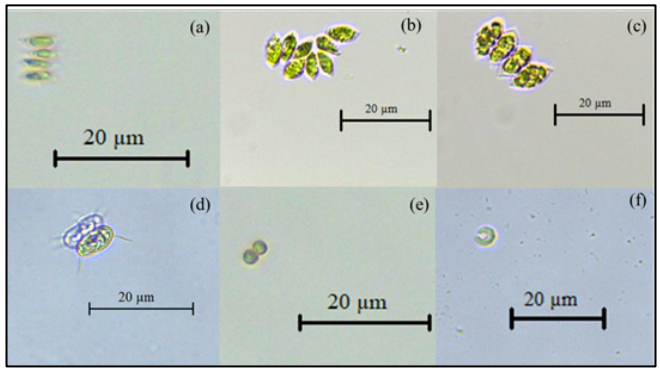

Figure 1. Microalgae mixed culture of Situ Mahoni: (a), (b), (c), and (d) *Scenedesmus*, (e) *Coenochloris*, and (f) *Kirchneriella.* Bar = 20µm. Magnification = 400.

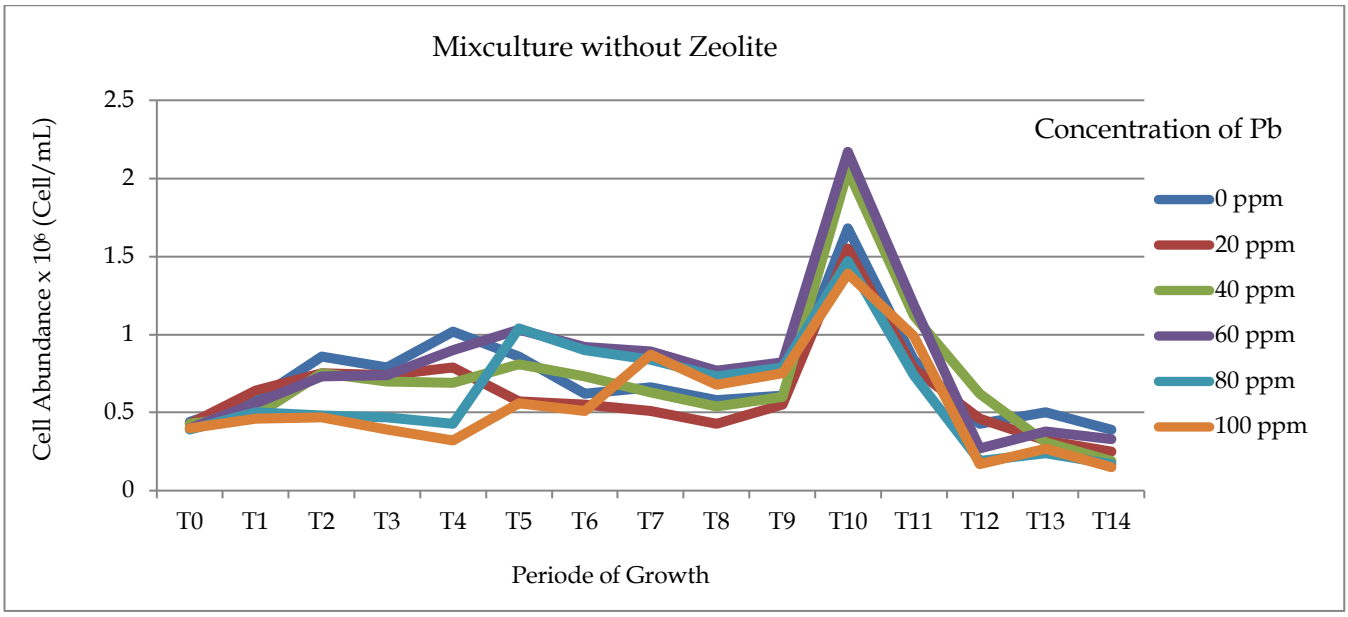

Figure 2. Abundance of mixed culture cells without zeolite

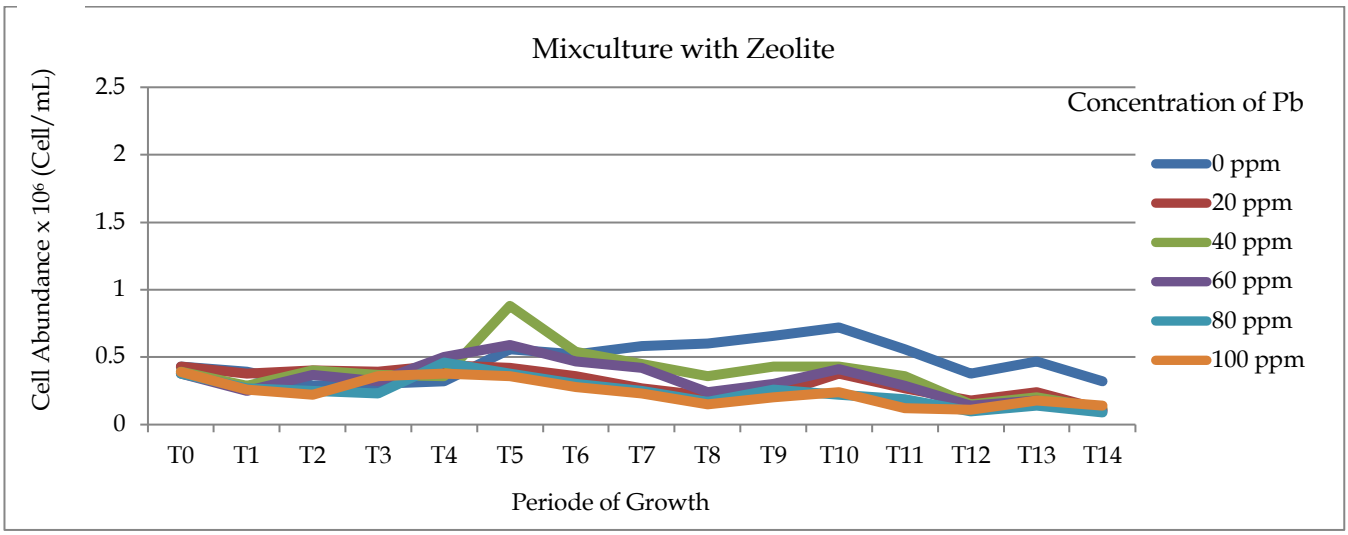

Figure 3. Abundance of mixed culture cells with zeolite

The peak growth of mixed culture microalgae without zeolite was occurred on day 10. The cell abundance was 21.700 cells/mL. The decrease in growth occurred from day 11, namely 11.900 cells/mL to day 14, namely 3,300 cells/mL. Growth with a Pb concentration of 80 ppm showed an adaptation phase on day 2 of 5.000 cells/mL to day 5 of 10.400 cells/mL. On day 10, peak growth occurred with a cell abundance of 14,700 cells/mL. The decrease in growth occurred from day 11 at 7.400 cells/mL to day 14 at 1.700 cells/mL. Growth with the largest concentration, namely 100 ppm, on day 2 experienced an increase in cell abundance of 4.700 cells/mL until day 5, namely 5.600 cells/mL. The growth peak was on day 10, namely 13.900 cells/mL, then on day 11, there was a decline with a number of 9.900 cells/mL until day 14 at 1.500 cells/mL.

The highest cell abundance in mixed culture growth was found in Pb with a concentration of 60 ppm, namely 21.700 cells/mL on day 10 (T10). The lowest cell abundance was found on day 14 (T14) with a Pb concentration of 100 ppm, namely 1.500 cells/mL. Mixed cultures microalgae consisting of *Scenedesmus*, *Coenochloris*, and *Kirchneriella* can grow well on BBM medium with various concentrations of the heavy metal Pb, especially at a concentration of 60 ppm. Microalgae can assimilate several types of heavy metals in their bodies. Heavy metals are absorbed and accumulated in microalgae cells by absorption and adsorption during the growth period (Perez-Rama

et al., 2002). However, heavy metal treatment with excessive concentrations can inhibit cell growth for microalgae (Kemer *et al*., 2020).

The adaptation of mixed culture microalgae with zeolite phase starts from day 2 at 2.900 cells/mL to day 6 at 5.200 cells/mL. On day 10, peak growth occurred with a cell abundance of 7.200 cells/mL. On days 13 to 14, there was a decrease of 4.700 cells/mL and 3.200 cells/mL. The number of cell abundances decreased at a Pb concentration of 20 ppm on day 1, namely 3.800 cells/mL, then fluctuated on day 2, amounting to 4.000 cells/mL until day 3, namely 3.900 cells/mL. Peak growth occurred on day 4, namely 4,400 cells/mL. The decrease occurred from day 13, namely 2.400 cells/mL to day 14, amounting to 1.100 cells/mL. The growth of mixed culture with a Pb concentration of 40 ppm decreased on day 1 by 2.900 cells/mL and the growth adaptation phase began on day 2 at 4,000 cells/mL until day 4, namely 3.600 cells/mL. On day 5, a peak occurred with a cell abundance of 8.800 cells/mL. The decrease occurred on days 13 to 14, namely 2.000 cells/mL and 1.200 cells/mL.

At a Pb concentration of 60 ppm, the mixed culture decreased on day 1 by 2.500 cells/mL then an adaptation phase occurred on day 2, namely 3.700 cells/mL to day 4, namely 5.000 cells/mL. Peak growth occurred on day 5 at 5.900 cells/mL. On day 13 and day 14, there was a decrease of 1.800 cells/mL and 1.000 cells/mL. The decrease in growth at a Pb concentration of 80 ppm occurred on day 1 at 2.700 cells/mL. There was an adaptation phase on day 2 of 2.500 cells/mL until day 3 of 2.300 cells/mL. Peak growth occurred on day 4 at 4.600 cells/mL. There was a decrease in growth on day 13 of 1.400 cells/mL and day 14 of 900 cells/mL. The growth of mixed cultures with a Pb concentration of 100 ppm decreased on day 1 by 2.600 cells/mL. On day 2 there was an adaptation phase of 2.200 cells/mL until day 3 it was 3.600 cells/mL. On day 4, there was a peak growth in cell abundance of 3.800 cells/m. There was a decrease in growth on days 13 to 14 with a number of 1.800 cells/mL and 1.400 cells/mL.

In mixed culture growth with a zeolite substrate, the highest cell abundance was found in Pb with a concentration of 40 ppm of 8.800 cells/mL on day 5 (T5). On day 14 (T_{14}) with a concentration of 80 ppm, there was a low cell abundance of 900 cells/mL. Mixed cultures using zeolite substrates had lower growth compared to mixed cultures that did not use zeolite. This occurs because the open surface of the activated zeolite pores causes nutrients from the medium and heavy metals to be absorbed into these pores, resulting in low cell growth in mixed cultures (Hong et al., 2019). Zeolite can inhibit the growth of microalgae, but zeolite is effective in absorbing the heavy metal Pb (II) in wastewater (Hamidpour et al., 2010).

The heavy metal lead is about 2.7 times more toxic and dangerous than cadmium (Zamani-ahmadmahmoodi & Beygi, 2020). Lead is very dangerous even in low concentrations (He & Chen, 2014). Based on research Dewi & Nuravivah (2018), it was explained that the highest cell growth of the microalgae Chlorella occurred in lead treatment with a concentration of 3 ppm (Dewi & Nuravivah, 2018). Meanwhile, the lowest cell growth occurred at a lead concentration of 5 ppm. High of the heavy metal lead concentrations can inhibit the growth of microalgae (Dewi & Nuravivah, 2018). This research reported that mixed cultures microalgae growth well at Pb concentrations of 40 ppm (with addition of zeolite) and 60 ppm (without addition of zeolite). The mixed cultures microalgae has good adaptability to the heavy metal lead. Based on research Tao *et al*., (2021), zeolite with low concentration was used to increase the growth of microalgae, especially their biomass (Tao *et al*., 2021). Meanwhile, zeolite with high concentration can inhibit the growth of microalgae. Previous research about the addition of zeolite to the growth of mixed cultures microalgae with varying concentrations of the heavy metal lead has not been found. Therefore, this research is an important initial study to carry out.

Conclusion

The microalgae mixed culture of Situ Mahoni was able to grow well on BBM medium with varying concentrations of the heavy metal Pb without the addition of zeolite. The highest cell abundance in the mixed culture without a zeolite substrate with a Pb concentration of 60 ppm was 21.700 cells/m while the highest mixed culture with a zeolite substrate with a Pb concentration of 40 ppm was 8.800 cells/mL. The growth of mixed cultures with zeolite showed a decrease in cell growth in the initial growth period. This is because the zeolite substrate undergoes activation can effect the absorption of the heavy metal Pb content in the BBM medium as well as the nutrients contained in the

medium. The growth of mixed cultures microalgae using zeolites can inhibit cell growth.

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Author Contributions
Conceptualization, SM and NBP.; methodology, SM and NBP.; software, SM and NBP.; validation, NBP.; formal analysis, SM and NBP.; investigation, NBP.; resources, SM and NBP.; data curation, SM and NBP.; writing—original draft preparation, SM and NBP.; writing—review and editing, SM and NBP.; visualization, SM and NBP.; supervision, NBP.; project administration, SM and NBP.; funding acquisition, SM and NBP. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest
The authors declare no conflict of interest.

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