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# UTILIZATION OF ALGAE *SPIRULINA* sp. AS A TOXICITY REDUCETING AGENT FOR HEAVY METALS Fe, Zn AND DYE PROCION RED, METHYL ORANGE, DIRECT GREEN AND NAPTHOL BLUE IN LIQUID WASTE MANAGEMENT IN THE SONGKET, BLONGKET, AND JUMPUTAN WEAVING INDUSTRY IN PALEMBANG

**NURHAYATI**

Master Student of Biology Sriwijaya University, JL. Raya Palembang-Prabumulih KM.32 Indralaya, OI, South Sumatra. Email: nurhayatisaje@gmail.com

**HILDA ZULKIFLI**

Supervisors of Master of Biology Sriwijaya University, JL. Raya Palembang-Prabumulih KM.32 Indralaya, OI, South Sumatra. Email: hilda.zulkifli@gmail.com

**RISFIDIAN MOHADI**

Supervisors of Master of Chemistry, Sriwijaya University, JL. Raya Palembang-Prabumulih KM.32 Indralaya, OI, South Sumatra. Email: risfidian.mohadi@unsri.ac.id

**Abstract**

This study aims to determine the growth of Spirulina sp. in culture media with the addition of heavy metals Fe and Zn with degradation cycles of Procion Red, Methyl Orange, Direct Green, and Napthol Blue. The method used is experimental by taking Spirulina sp. microalgae. Obtained from ponds in Palembang and Prabumulih, South Sumatra with laboratory-scale algae culture. The design in this study used a completely randomized design (CRD). The treatment used for each metal Fe, Zn and dyes Procion Red, Methyl Orange, Direct Green and Napthol Blue was 5 treatments with 3 replications, in 1 L medium containing Spirulina sp. with an individual average of 1×104 ind/L. Method validation for heavy metals Fe and Zn was calculated with AAS (Atomic Absor Btion Spectrophotometer) and dyes with Lambert Beer isotherm, SEM and FTIR tests. The results showed that the growth of Spirulina sp. in the control is greater than in the metal treatment with a span of 14 days. The average growth size of Spirulina in the Fe control was 652.62 ind/L, Zn was 636.3 ind/L and the decrease in Fe metal at 1, 3, 5, 7 ppm was 33%, 10%, 5.6%, 4 respectively. .28%. While the decrease in Zn metal at 1, 3, 5, 7 ppm was 15%, 8.67%, 4.2%, 4%. Furthermore, the growth of Spirulina sp. in the control dyes Procion red, Methyl Orange, Direct Green, Napthol Blue, namely 643.44 ind/L, 631.08 ind/L, 634.34 ind/L, 638.08 ind/L. Then the decrease in dyes in Procion Red at concentrations of 1, 3, 5, 7 ppm, namely 90.7%, 76.33%, 72%, 64.14%. Furthermore, the decrease in Methyl Orange 1, 3, 5, 7 ppm is 71.1%, 69.67%, 79.2%, 88.14%. In addition, the reduction of Direct Green dye at 1, 3, 5, 7 ppm was 36%, 28.33%, 48%, 81.42%. Furthermore, the decrease in Napthol Blue dye at 1, 3, 5, 7 ppm was 12.6%, 49%, 55.4%, 47.85%. FTIR test results of Spirulina sp. shows three sharp peaks which are in the absorption area 3259.65 cm-1 which is the OH group (Hydroxyl), Absorption which appears in the area 1640.76 cm-1 which is the aromatic C=C group, at wave number 1020.74 is the C-O group, 547.32 is a cluster of fingerprint regions.

**Keywords:** Spirulina sp., Heavy Metals Fe, Zn, Water pollution, Textile dyes Procion Red, Methyl Orange, Direct Green, Napthol Blue

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## 1. INTRODUCTION

Pollution of waters that exceed the threshold of environmental quality standards is a serious problem that has a negative impact on aquatic biota and humans in it because water is classified as an environmental component needed for survival (Firmansyah et al, 202: 1). Damage to the aquatic environment due to water disturbances in waters can occur due to the entry of pollutants into the aquatic environment which can cause a decrease in water quality. One of the pollutants that cause water pollution is heavy metals. The presence of heavy metals in waters can directly harm aquatic biota and indirectly affect human health. This is related to the properties of heavy metals which are very dangerous because they cannot be destroyed (non degradable) by living organisms and can accumulate into the body of living things and aquatic environments, especially settling on the bottom of the water to form complex compounds with organic and inorganic materials (Utami et al, 2018)

The capital city of Palembang, South Sumatra Province, is a city that produces songket woven textiles, jumputan which is not only in the category of large and medium scale handicrafts but also on a small scale and some even on a household scale such as songket weaving crafts in Palembang City. The manufacture of songket and jumputan fabrics requires a dying process that generally uses synthetic dyes. The use of synthetic dyes in the manufacture of songket and jumputan fabrics will have an impact on increasing the number of pollutants and waste they produce. After the dyeing process is complete, turbid and concentrated liquid waste will be produced which, in addition to containing dyes, also contains synthetic materials that are difficult to dissolve or decompose.

Dyestuffs are toxic to the human body, although there are certain dyes that are safe for humans, namely dyes for the food and pharmaceutical industries. In the dyeing process there are several dyes used. Detailed classification is usually based on the properties and uses of acid, base, direct, mordant, sulfur, vessel, dispersion and reactive dyes (Isminingsih, Djufri, and Rasjid, 1982). One of the synthetic dyes used in the dyeing process of jumputan cloth is a crane.

Generally, songket weaving crafts are carried out by craftsmen in densely populated village areas. If the craft disposes of liquid waste, the waste stream will go through the waters around the settlement leading to the Musi River. Conditions in the field show that most of the waste is not treated first before being discharged into the river. Even if there is treatment, it is just ordinary deposition, thus the environmental quality of river waters is feared to be reduced. Therefore, it is necessary to carry out further waste treatment so that this waste is safe for the Musi River aquatic environment as a polluting body.

One of the processes in songket weaving that produces liquid waste is the process of giving color or dyeing which in addition to requiring chemicals also requires water as a solvent medium. This causes the control of the environmental impact of textile crafts to generally focus on liquid waste because it provides the most extensive impact. Some of the substances needed in the coloring process are adsorbed by textile materials and will remain in the textile until the process is complete, while the rest is in solution and will be wasted with the water used for the

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wet process. The substances in the wastewater have the potential to cause environmental pollution problems (Effendi, 2000).

Heavy metal pollution tends to increase in line with the increasing industrialization process. Heavy metal pollution in the water, soil and air environment can pose a danger to health. Heavy metals enter the body tissues of living things through several ways, namely the respiratory tract, digestion, and penetration through the skin. Metal absorption through the respiratory tract is quite large, both in aquatic biota that enters through the respiratory system, and land biota that enters through dust in the air into the respiratory tract. Heavy metals can have disruptive effects on human health. Toxicity from heavy metals can block the work of enzymes that interfere with the body's metabolism, because allergies, are mutagens, teratogens, or carcinogens.

Iron is one of the chemical elements that can be found in almost every place on earth, in all geological layers and all water. In surface water it is rare to find Fe levels greater than 1 mg / L, but in groundwater these higher Fe levels can be felt and can stain fabrics and kitchen utensils. In drinking water Fe gives rise to taste, color (yellow). Deposits on pipe walls, bacterial growth and turbidity. Iron is a component of many enzymes that affect all important chemical reactions in the body. Iron is also a component of hemoglobin, which allows red blood cells to carry oxygen and deliver to body tissues (Naingolan, 2011). Iron in water comes from the ground itself in addition to other sources, including from the dissolution of iron pipes, iron water reservoirs or industrial waste deposits (Setiarto, 2020). At a pH of about 7.5 - 7.7 ferric ions undergo oxidation and bind to hydroxides to form $Fe(OH)_3$ which is insoluble and precipitates (precipitation) at the bottom of the waters, forming a reddish color on the bottom substrate. Therefore, iron is only found in waters that are in anaerobic (anoxic) conditions and acidic atmosphere (Cole, 1988 in Sembiring, 2010). Too much iron in the body can create reactive reactions that can easily bind to free radicals and cause oxidative stress that has the potential to damage cells, organs, and tissues in the body (Aldi et al, 2019). The high content of Fe metal will have an impact on human health, including causing poisoning, cancer, premature aging to sudden death (Atikah, 2021). Therefore, heavy metal pollution in the water, soil and air environment needs to be taken seriously considering the dangers posed to human health and to environmental equilibrium.

Zinc is a useful element in the human body, animals and plants. Such is its usefulness, Zn is found in water, plants and animals. According to the Ministry of Health, the maximum allowed maximum drinking water is 15 mg / L. The toxic effect of Zn on humans is at high concentrations between 300-360 ppm, which causes physical disorders such as severe diarrhea, stomach cramps and vomiting (Setiarto, 2020). A source of drinking water containing Zn 26.6 mg / L is not harmful to humans, but for drinking water with Zn levels of 30.8 mg / L has caused nausea and motion sickness. In terms of aesthetics, water containing Zn 30 mg / L will look like milk and when boiled, a layer like oil appears on the water substitution (Suprijanto and Agustina, 1988).

The highest concentration as the standard to be set must be below the limit of the concentration that can cause taste. In small amounts it is an important element for metabolism, because Zn deficiency can cause inhibition in child growth. In large quantities this element can cause bitter

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and astringent taste in drinking water (Sutrisno, 1991). The influx of Zn into rivers as a result of groundwater runoff is generally caused by rain. Hutagalung (1984) stated that the source of Zn metal in waters comes from geochemical materials carried or present in rivers, raw materials for oil, iron, paint and the remains of used cans. In the textile industry, zinc metal can function as an additional chemical in the final refinement process as well as for fiber preservation, especially antifungal (fungicide) and insecticide (Setiarto, 2020). Zinc metal is also part of the constituents of textile dyes, especially dyes from metal complexes and pigments.

Among the existing methods of removal of dyes, adsorption is considered the most convenient and economical method for the removal of dyes from aquatic systems. Adsorption is a phase transfer process that is widely used in practice to remove substances from the liquid phase (gas or liquid). Molecules or ions are set aside from aquatic solutions by adsorption onto the surface of solids (Worch, 2012). The main advantages of pollutant adsorption technology by biomass are its effectiveness in reducing pollutant concentrations to very low levels and the use of inexpensive biosorbent materials. The advantage of using agricultural waste materials as adsorbents is that they save disposal costs while reducing potential environmental problems. In fact, agricultural waste products are usually composed of lignocellulose materials, which contain mostly cellulose, hemicellulose and lignin, which are considered useful for adsorption.

One of the wetland aquatic microalgae used as a bioremidiation agent to reduce concentration is Spirulina sp. Thismicroalgae is a blue-green algae classified as Cyanobacteria, has a spiral, single-celled shape. Has a high protein content (Notonegoro et al., 2018). Metal ions will enter the cell wall of microalgae which there are variations of polysaccharides and proteins that have a number of active sides that are able to bind to metal ions (Purnamawati et al., 2015). According to Zulkifli et al (2016), Spirulina sp. It has a carbosyl functional group that is active in adsorbing heavy metals.

The ability of Spirulina sp. to reduce heavy metal concentrations is different for each heavy metal. This study focused on determining the potential of Spirulina sp. in combating heavy metals and reducing the concentration of heavy metals Fe and Zn by absorbing Red Procion, Methyl Orange, Green Direc and Blue Napthol. This research is carried out on a laboratory scale so that the environment does not become the main limiting factor. Based on the background above, several problem formulations are obtained as follows: How is the effect of various concentrations of heavy metals Fe and Zn, absorption of Red Procion, Methyl Orange, Green Direc, and Blue Napthol on the growth of Spirulina sp.? What is the ability of Spirulina sp. to absorb various concentrations of heavy metals Fe and Zn with the degredation cycle of Red Procion, Methyl Orange, Green Direk, and Blue Napthol? The purpose of the study was toanalyze the growth of Spirulina sp. in culture media with the addition of heavy metals Fe and Zn with the degredation cycle of Red Procion, Methyl Orange, Green Direk, and Blue Napthol. Testing the potential of Spirulina sp. in absorbing heavy metals Fe and Zn with the degredation cycle of Red Procion, Methyl Orange, Green Direk, and Blue Napthol. The benefits of this research are expected to be a solution in minimizing heavy metal pollution by reducing the concentration of heavy metals Fe, Zn and Red Procion, Methyl Orange, Green Direk, and Blue Napthol using Spirulina as a bioremidiation agent.

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## 2. RESEARCH METHODOLOGY

### 2.1 Time and Place

This research will be carried out from June 2022 to August 2022 at the Biology Education Laboratory, Faculty of Teacher Training and Education, Sriwijaya University Palembang for cultivation, the Integrated Research Laboratory of the Sriwijaya University Postgraduate Program for optical density (OD) measurement with UV-Vis Spectrophotometer, UPTD Environmental Laboratory of the Environment and Land Service of South Sumatra Province for Fe and Zn metal content test analysis. Use of microalgae Spirulina sp. was obtained from ponds in Palembang and Prabumulih, South Sumatra.

### 2.2 Tools and Materials

The tools used are aerators, aerator hoses, culture bottles, stirring rods, spatulas, fluorescent lamps, analytical scales, fume hoods, erlenmeyers, measuring cups and Sedgewick Raffter Counting Cell (SRCC). The materials used are aquades, aluminum foil, Fe and Zn heavy metal solutions, as well as chemical substances for the composition of making BG-11 medium including NaNO 3, K 2 HPO 4, $MgSO_{4.7H}$ 2O, CaCl 2.2H $_2$O, citric acid, ammonium ferric citrate green, EDTANa2, Na2CO 3, H 3 BO $_3$, MnCl $_{2.4H}$ $_2$O, $ZnSO_{4.}$ 5H2O, Na2MoO4.2H2O, FeSO, 4.5H2O, Co (NO3)2.6H2O (Strainer et al., 1971).

### 2.3 Experiment Design

The design in this study used Complete Randomized Design (RAL). The treatment used on each metal was 5 treatments with 3 repeats, in 1 L medium containing Spirulina sp. with an average of $1\times10^4$ ind/L. The determination of the initial number of individuals refers to the research of Yusuf, D. M. (2014), using an average of 10,000 ind/L Spirulina sp. was able to reduce the concentration of Fe in culture media by 89% at a concentration of 1 mg / L.

**Table 1: Research Design**

| Treatment | Concentration (ppm) | | Early Inoculum (Ind/L) and OD |
|---|---|---|---|
| | Fe | Zn | |
| 1 | Control | Control | $10^4$ Ind/L or equivalent to OD = 0.0015 |
| 2 | 1 | 1 | |
| 3 | 3 | 3 | |
| 4 | 5 | 5 | |
| 5 | 7 | 7 | |

The determination of concentration in this study refers to the research of Mohadi et al. (2020), using heavy metal concentrations of 1, 3, 5 ppm. At Cd 1 ppm, the final concentration was 0.53 ppm, at Cd 3 ppm and 5 ppm the final concentration was 2.75 ppm and 4.87 ppm. However, in this study added 1 different concentration, namely 7 ppm to determine the optimization of Spirulina sp. in absorbing heavy metals Fe, Zn, Procion Red, Methyl Orange, Direct Green, Napthol Blue with each treatment substance 0, 1, 3, 5, 7 ppm.

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## 3. RESEARCH METHODS

The research method used in this study was experimental. Experimental research is research carried out by manipulating the object of research and the existence of control (Nazir, 2005). This study used a quantitative approach. According to Sugiyono (2007) research data on quantitative approaches in the form of numbers and analysis using statistics.

## 4. RESULTS AND DISCUSSION

### 4.1. Effect of Heavy Metals on the Growth of Spirulina sp.

#### 4.1.1. Effect of Fe and Zn on Growth of Spirulina sp.

Growth observations were made using SRCC based on OD values, then converted into abundance of Spirulina sp. Growth of Spirulina sp. in a medium containing Fe metal variations was carried out for 14 days with an initial inoculum amount of $1x10^4$ ind/L or equivalent to an OD of 0.0015.

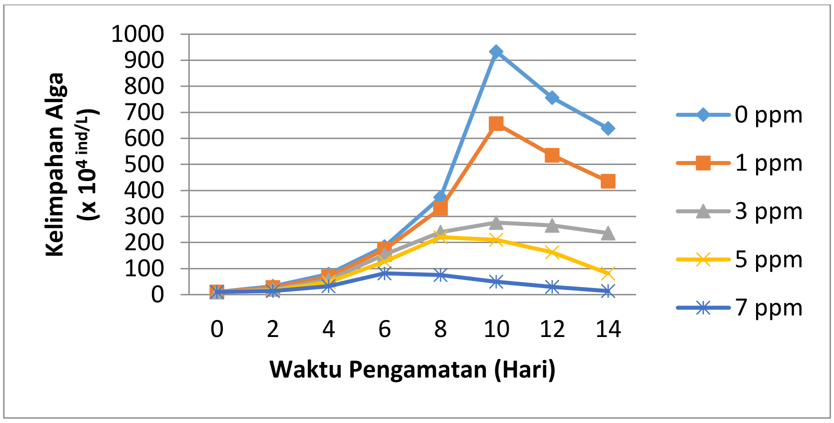


**Figure 1: Growth of Spirulina sp. on a medium containing variation heavy metals Fe**

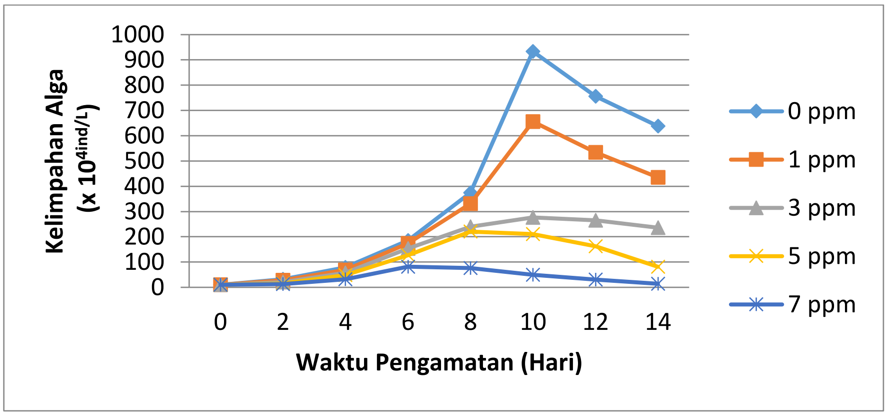


**Figure 2: Growth of Spirulina sp. on a medium containing variation heavy metals Zn**

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The growth phase of Spirulina sp. consists of 4 phases including the lag phase, exponential phase, stationary phase and death phase. Based on table 4.2. The lag phase was not seen in the control treatment, Zn 1 ppm, 3 ppm and 5 ppm were suspected to occur in a short time and the initial culture used was from the exponential phase. However, in the 7-pm treatment, the lag phase occurred until day 2, it was thought that the metal concentration was too high so that Spirulina sp. took a long time to adapt.

The exponential phase in the control treatment, (Fe &; Zn) 1 ppm and 3 ppm occurred until day 10, while in the Zn treatment 5 ppm and 7 ppm occurred days 4 to 6. The stationary phase of the entire treatment cannot be seen and is expected to occur in a short time. The death phase in the control treatment, (Fe &; Zn) 1 ppm and 3 ppm occurred on the 12th day, while in the 5 and 7 ppm treatment occurred on the 8th day marked by a decrease in the abundance of Spirulina sp. The phase that occurs in a short period of time is the lag phase in some treatments and the stationary phase in all treatments because the phase occurs in a short time and is estimated to be less than 24 hours while observations are carried out at intervals of 2 days. In addition, in the previous phase the nutrients used in the previous phase were very high so that the nutrients needed in the stationary phase were very few. This is in accordance with the statement of Meritasari et al. (2012), the stationary phase occurs in a short time so that the stationary phase does not appear at every treatment

Factors affecting the growth of Spirulina sp. that is, pH, in this study the pH in each treatment is between 6-7. In addition to pH, temperature, and light also have an important role in the growth of Spirulina sp. The ambient temperature is 22-27$^{o}$ C, DO 1-7.4 mg / L and light intensity 3000 lux. According to Santosa and Limantara (2007), light intensity affects the growth of Spirulina sp. If the light intensity is too high, photoinhibition will occur while if the light intensity is low, there will be a decrease in photosynthetic activity. The optimum light intensity for Spirulina sp. is 2000-3500 lux.

## 4.2 Heavy Metal Absorption Ability by Spirulina sp.

### 4.2.1. Absorption Ability of Fe and Zn Heavy Metals by Spirulina sp.

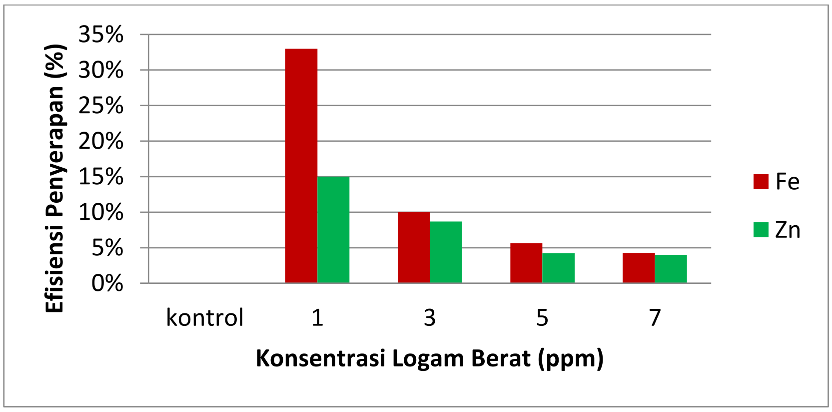


**Figure 3: Absorption efficiency of heavy metals Fe and Zn**

**Description:** Calculation of metal absorption efficiency (%) by AAS method

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Based on figure 4.23 it can be seen that the absorption efficiency of heavy metals Fe concentrations of 1 ppm, 3 ppm, 5 ppm and 7 ppm respectively is 33%, 10%, 5.6% and 4.28%. The absorption efficiency of heavy metals Zn concentrations of 1 ppm, 3 ppm, 5 ppm, 7 ppm respectively is 15%, 8.67%, 4.2% and 4%. This shows that absorption efficiency decreases with increasing concentration of heavy metals.

Based on the two-way factor anova test, results were obtained that had a real effect between metal concentrations on absorption efficiency and there was a real influence on the type of metal on absorption efficiency. Furthermore, an independent sample t-test to determine the difference in absorption in heavy metals Fe and Zn. At concentrations of 1 ppm, 3 ppm, 5 ppm, 7 ppm in Fe and Zn has a sig (2-tailed) value of <0.005, this shows that the absorption of Fe and Zn metals at various concentrations has differences.

The absorption of heavy metal Zn is lower than Fe, indicating that heavy metal Zn is more toxic than heavy metal Fe. This is thought to be because the heavy metal Zn is more toxic is a component in the medium used for the growth of Spirulina sp and the toxic power of Zn ions is stronger than Fe ions so that damage to the adsorption side of the cell wall due to Zn ions is greater, so Zn ions cannot be adsorbed more than Fe ions. According to Muyassaroh, et al (2018) Fe and Zn are essential heavy metals that function as micronutrients needed by Spirulina and can easily accumulate in the body of spirulina microalgae. However, because there is an increase in the concentration of Fe and Zn, it can experience greater inhibition of enzyme work than other metals.

### 4.3 Comparison of Spirulina sp. in Absorbing Red Procion Dyes, Methyl Orange, Green Direc and Blue Napthol

Microalgae Spirulina sp. have different absorption abilities in absorbing red procion dyes, methyl orange, green direk and blue napthol. A comparison of these capabilities can be seen in figure 4.24.

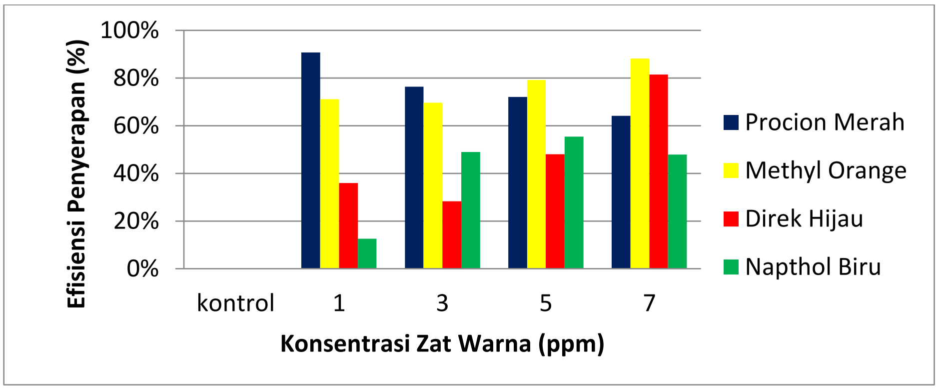


**Figure 4: Absorption efficiency of Procion Red, Methyl Orange, Direk Green, and Napthol Blue dyes**

**Description:** Efficient calculation of dye absorption calculated with Isotherm Lambert-Beer

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Based on figure 4.24, it can be seen that the absorption efficiency of Red Procion concentrations of 1 ppm, 3 ppm, 5 ppm and 7 ppm respectively is 90.7%, 76.33%, 72%, and 64.14%. Then for the absorption efficiency of Methyl Orange, the concentrations of 1 ppm, 3 ppm, 5 ppm, 7 ppm are 71.1%, 69.67%, 79.2%, and 88.14% respectively. Furthermore, for the absorption efficiency of Green Direc concentrations of 1 ppm, 3 ppm, 5 ppm, 7 ppm respectively, namely 36%, 28.33%, 48%, and 81.42%. Finally, for the absorption efficiency of Blue Napthol concentrations of 1 ppm, 3 ppm, 5 ppm, 7 ppm respectively, namely 12.6%, 49%, 55.4%, and 47.85%. From figure 4.23 can be seen the comparison of absorption of 4 types of dyes Procion Merha, Methyl Orange, Direk Hijau, and Napthol Blue.

Based on the two-way factor anova test, results were obtained that had a real effect between the concentrations of dyes on absorption efficiency and there was a real influence on the type of dye on absorption efficiency. Furthermore, an independent sample t-test to determine the difference in absorption of Procion Red, Methyl Orange, Direk Hijau, and Napthol Blue dyes. At concentrations of 1 ppm, 3 ppm, 5 ppm, 7 ppm in Red Procion, Methyl Orange, Green Direc, and Blue Napthol have a sig (2-tailed) value of <0.005, this shows that the absorption of Red Procion, Methyl Orange, Green Direk, and Blue Napthol at various concentrations has differences. The absorption of Green Direc and Blue Napthol dyes is lower than Red Procion and Methyl Orange, this shows that Green Direc and Blue Napthol are more toxic than Red Procion and Methyl Orange. In addition, the dye-treated spirulina medium showed a change in the color of spirulina which changed the color of the pigment. According to research Mohadi et al (2018) explained that Spirulina treated with Congo red showed pigment changes. This is thought to be due to toxic textile dyes. Toxic dyes can cause damage to the adsorption side of the Spirulina cell wall causing it cannot be adsorbed more so that there are changes in pigment and the abundance of Spirulina decreases.

Based on figure 4.23. It can be seen that Spirulina is more effective in absorbing the heavy metals Red Procion and Methyl Orange than Green Direc and Blue Napthol. This is suspected because the dyes of Direk Hjau and Napthol Biru are more toxic than Red Procion and Methyl Orange and the nature of Methyl Orange which is completely soluble in the algae culture medium as well as in the Red Procion compared to the Green Direc and Blue Napthol which are not completely soluble in the Spirulina medium and a precipitate is formed at the bottom of the spirulina culture medium. This is because the concentration of dyes absorbed by Spirulina has been maximized, so Spirulina limits itself to absorbing more dyes which has an impact on pigments and decreases the abundance of Spirulina.

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### 4.4 FTIR Test Results

### 4.4.1 Algae FTIR Test Results

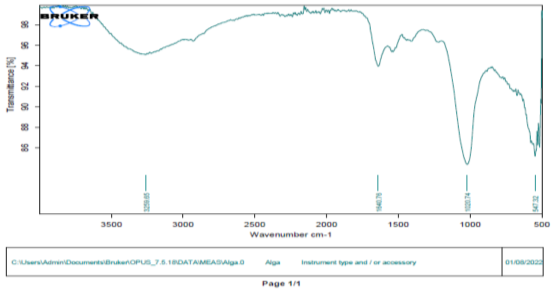

**Figure 5: FTIR Spectrum for Spirulina sp.**

FTIR Spectrum Spirulina sp. shows three sharp peaks located in absorption areas 3259.65 cm-1, 1640.76 cm-1, 1020.74 cm-1, 547, 32 $^{cm-1}$. The absorption that appears in the area 3259.65 is an OH (Hydroxyl) group. Michalak et al (2020) the shape of the wide band changes in the range of 2400-3800 $cm^{-1}$ the center of the band shifts towards a higher wavenumber, which is characteristic for undissociated carboxylate groups. This finding is consistent with results presented by other authors who examined metal ion biosorption by Spirulina sp. Ferreira et al. (2011) also underlined the role of carboxyl functional groups in the biosorption of Ni(II), Zn(II) and Pb(II) ions by the dry biomass of Spirulina platensis. The absorption that appears in the region 1640.76 cm-1 which is the aromatic group C = C. Michalak et al (2022) explained the wavenumber in the amide band (protein) 1657 cm-1I, the O-H bending vibration of the adsorbed water molecule – the extended vibration C=C. Absorption at wavenumber 1020.74 is a C-O group.

### 4.4.2 FTIR Test Results of Algae + Procion Red

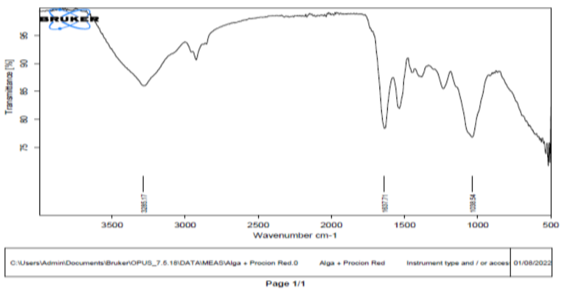

**Figure 6: FTIR Spectrum for Spirulina sp. Algae on culture medium containing Red Procion**

FTIR Spectrum Spirulina sp. shows three sharp peaks located in absorption areas of 3285.17 cm-1, 1637.71 cm-1, 1038.54 $^{cm-1}$. The absorption that appears in the area of 3285.17 $^{cm-1}$ is an OH (Hydroxyl) group. Celekli and Bozkurt (2011) explain that the peak at 3280 cm−1 can be associated with —OH and —NH groups. The absorption that appears

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at wavenumber 1637.71 $^{cm-1}$ is an aromatic group C=C. Celekli and Bozkurt also explain the existence of —C O stretches of aldehydes, ketones, and carboxylates can be confirmed by absorption peaks in the region 1645–1400 $cm^{-1}$. The absorption appears at wavenumber 1038.54 cm-1 $^{C-O}$.

#### 4.4.5 FTIR Test Results of Algae + Methyl Orange

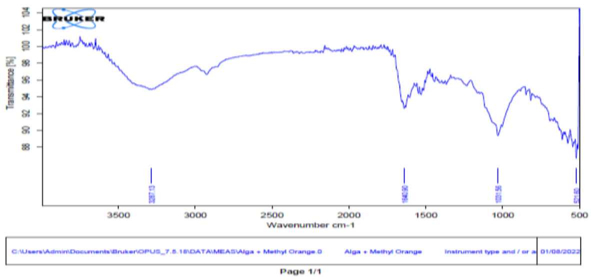


**Figure 7: FTIR Spectrum for Spirulina sp. Algae on culture medium containing Methyl Orange**

FTIR Spectrum Spirulina sp. shows three sharp peaks located in the absorption area of 3287.13 cm-1, 1640.90 cm-1, 1031.56 cm-1, and 521.6 $^{cm-1}$. The absorption that appears in the area of 3287.13 $^{cm-1}$ is the OH (Hydroxyl) group. Soni, R. A et al (2021) explained that IR spectra in the range of 3870-3550, 3300-2925 represent stretching hydroxyl (OH) and vibrational bending the presence of the amino acid carbohydrat. The absorption that appears in the area of 1640.90 $^{cm-1}$ is the aromatic group C = C. Michalak et al (2022) explained the wavenumber in the amide band (protein) 1657 cm-1I, the O-H flexural vibration of the adsorbed water molecule – the extended vibration C=C. Michalak et al (2020) wavenumbers at 1152, 1080, 1049, 932, 699 C-O and C-C extended vibrations, CH 2 shake vibrations, ring bending vibrations. The absorption that appears in the area of 1031.56 cm-1 is the C-O group. Michalak et al (2020) wavenumbers at 1152, 1080, 1049, 932, 699 C-O and C-C extended vibrations, CH 2 shake vibrations, ring bending vibrations. The absorption that appears in the 521.6 $^{cm-1}$ area is a cluster of fingerprint regions.

#### 4.4.6 Green Algae + Direct FTIR Test Results

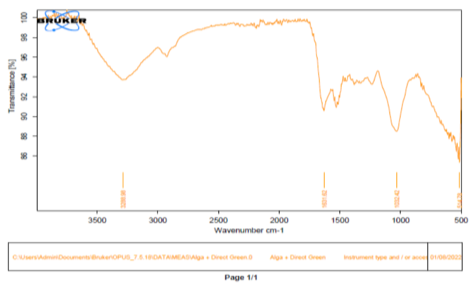


**Figure 8: FTIR Spectrum for Spirulina sp. Algae on culture medium containing Green Direct**

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FTIR Spectrum Spirulina sp. Shows four sharp peaks located in absorption areas of 3288.98 cm-1, 1631.62 cm-1, 1032.42 cm-1 and 514.78 $^{cm-1}$. The absorption that appears in the area of 3288.98 $^{cm-1}$ is the OH (Hydroxyl) group. The absorption that appears in the area of 1631.62 $^{cm-1}$ is the aromatic group C = C. Celekli and Bozkurt also explain thatthe existence of —C O stretches of aldehydes, ketones, and carboxylates can be confirmed by absorption peaks in the region 1645–1400 $cm^{-1}$. The absorption that appears in the area of 1032.42 cm-1 is the C-O group.

**4.4.7 FTIR Test Results of Algae + Napthol Blue**

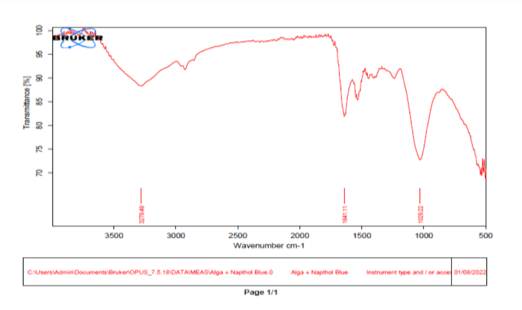

**Figure 9: FTIR Spectrum for Spirulina sp. Algae on culture medium containing Blue Napthol**

FTIR Spectrum Spirulina sp. shows three sharp peaks located in the absorption area of 3279.49 cm-1, 1641.11 cm-1, and 1029.22 $^{cm-1}$. The absorption that appears in the area of 3279.49 $^{cm-1}$ is the OH (Hydroxyl) group. Celekli and Bozkurt (2011) explain that the peak at 3280 $cm^{-1}$ cans be associated with —OH and —NH groups. The absorption that appears in the area of 1641.11 $^{cm-1}$ is the aromatic group C = C. Celekli and Bozkurt also explain thatthe existence of —C O stretches of aldehydes, ketones, and carboxylates can be confirmed by absorption peaks in the region 1645–1400 $cm^{-1}$. The absorption that appears in the area of 1029.22 cm-1 is the C-O group.

**4.5 SEM Test Results**

Spirulina sp. powder which has been characterized using Scanning Electron Microscope (SEM) analysis. The purpose of SEM analysis is to determine the surface morphology of Spirulina sp. samples. The results of SEM analysis can be seen in figure 4.22.

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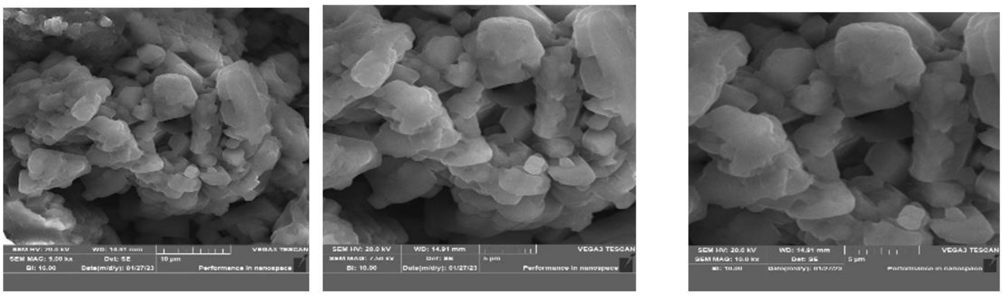

(a) (b) (c)

**Figure 10: Results of SEM analysis of Spirulina sp**

**Description:** (a) 5000X Magnification, (b) 7500X Magnification, and (c) 10000X Magnification

Based on the results of SEM analysis contained in figure 4.22 shows the size, morphological shape of the surface of Spirulina sp. powder. Which is rough, breaks, and produces a solid structure that crumbles because it has suffered significant damage after treatment.

## 5. CONCLUSION AND ADVICE

### 5.1 Conclusion

Based on the results of observations that have been made, the following conclusions are obtained:

Growth of Spirulina sp. in BG-11 culture media influenced by Fe and Zn heavy metal tests on a laboratory scale showed a decrease in Spirulina sp. Compared to Spirulina, the control was characterized by a decrease in the abundance of Spirulina sp. In addition, in the test of reducing heavy metal levels Fe and Zn concentrations of 1, 3, 5, 7 ppm using Spirulina sp. in the AAS method showed a decrease in Fe of 21% at 1 ppm, 9.3% at 3 ppm, 9.2% at 5 ppm, 3.8% at 7 ppm and in Zn showed a decrease of 66% at 1 ppm, 21% at 3 ppm, 20% at 5 ppm, 16% at 7 ppm.

Growth of Spirulina sp. in BG-11 culture media influenced by dye tests of Red Procion, Methyl Orange, Green Direk, and Blue Napthol on a laboratory scale showed a decrease in Spirulina sp. compared to Spirulina control characterized by a decrease in the abundance of Spirulina sp. Then in the concentration reduction test on each dye 1, 3, 5, 7 ppm using Lambert Beer isotherm showed a decrease in Red Procion concentrations of 1 ppm, 3 ppm, 5 ppm and 7 ppm respectively, namely 90.7%, 76.33%, 72%, and 64.14%. Then for the decrease in Methyl Orange concentrations of 1 ppm, 3 ppm, 5 ppm, 7 ppm respectively, namely 71.1%, 69.67%, 79.2%, and 88.14%. Furthermore, for the decrease in Green Direc concentrations of 1 ppm, 3 ppm, 5 ppm, 7 ppm respectively, namely 36%, 28.33%, 48%, and 81.42%. Finally, for the decrease in Blue Napthol concentrations of 1 ppm, 3 ppm, 5 ppm, 7 ppm respectively, namely 12.6%, 49%, 55.4%, and 47.85%.

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FTIR Spectrum Spirulina sp. shows three sharp peaks located in the absorption area of 3259.65 $^{cm-1}$ are OH (Hydroxyl) groups. The absorption that appears in the region 1640.76 $^{cm-1}$ which is the aromatic group C = C. The absorption that appears at wavenumber 547.32 is the fingerprint region cluster. FTIR Spectrum Spirulina sp. + Procion Red shows three sharp peaks in the absorption area of 3285.17 $^{cm-1}$ are OH (Hydroxyl) groups. The absorption that appears at wavenumber 1637.71 cm-1 is an aromatic group C = C. The absorption that appears at wavenumber 1038.54 $^{cm-1\ C-O}$. The FTIR spectrum of Spirulina sp. + Methyl Orange shows three sharp peaks in the absorption area of 3287.13 $^{cm-1}$ are OH (Hydroxyl) groups. The absorption that appears in the area of 1640.90 $^{cm-1}$ is the aromatic group C = C. The absorption that appears in the area of 1031.56 cm-1 is the C-O group. The FTIR spectrum of Spirulina sp. + Direct Green shows four sharp peaks located in the absorption area of 3288.98 $^{cm-1}$ are OH (Hydroxyl) groups. The absorption that appears in the area of 1631.62 $^{cm-1}$ is the aromatic group C = C. The absorption that appears in the area of 1032.42 cm-1 is the C-O group. The absorption that appears in the area of 514.78 $^{cm-1}$ is a cluster in the fingerprint area. The FTIR spectrum of Spirulina sp. + Napthol Blue shows three sharp peaks in the absorption area of 3279.49 $^{cm-1}$ are OH (Hydroxyl) groups. The absorption that appears in the area of 1641.11 $^{cm-1}$ is the aromatic group C = C. The absorption that appears in the area of 1029.22 cm-1 is the C-O group.

### 5.2 Suggestion

The next study should use a time interval of 1 day and XRF (X-Ray Fluorescence) analysis to determine maximum absorption of heavy metals.

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