# Identifikasi Kadar Timbal (Pb), Mercury (Hg), Klorofil-a, dan Morfologi Sel Rumput Laut *Kappaphycus alvarezii* di Bluto dan Saronggi Water, Sumenep, Madura, Jawa Timur

# Identification The Content of Lead (Pb), Mercury (Hg), Chlorophyll-a, and Cell Morphology of Seaweed *Kappaphycus alvarezii* in Bluto and Saronggi Water, Sumenep, Madura, East Java

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**Abstrak**

*Kappaphycus alvarezii* adalah salah satu komoditas ekspor Indonesia karena memiliki nilai ekonomi yang tinggi sebagai makanan dan industri. Salah satu daerah di Jawa Timur yang merupakan sentra budidaya rumput laut adalah Kabupaten Sumenep seperti Saronggi dan Bluto. Saat ini Kabupaten Sumenep merupakan kawasan untuk eksplorasi dan eksploitasi minyak dan gas. Jenis polutan yang dihasilkan dari kegiatan ini menyebabkan polusi di lingkungan perairan seperti timbal (Pb), merkuri (Hg) dan kadmium (Cd). Logam berat yang ditemukan di perairan dapat diserap dan terakumulasi dalam rumput laut thallus. Tujuan dari penelitian ini adalah untuk mengetahui kandungan logam berat Pb, Hg, Cd, klorofil-a, dan morfologi sel *E. cottonii* di Bluto dan Saronggi perairan. Penelitian ini adalah survei dan penelitian deskriptif. Hasil penelitian menunjukkan bahwa terdapat perbedaan dalam kandungan logam berat timbal dalam E. cottonii, air laut, dan sedimen di perairan Bluto dan perairan Saronggi. Perairan Bluto memiliki jumlah yang lebih rendah dari klorofil-a daripada Saronggi Waters. Ketebalan dinding sel *E. cottonii* di perairan Saronggi memiliki dinding sel yang lebih tipis dari perairan Bluto. Pengukuran kualitas air di Bluto dan Saronggi perairan melalui suhu, pH, salinitas, kecerahan dan DO parameter. Hasil pengukuran dari kedua perairan tidak ada perbedaan mencolok dan dalam kondisi optimal.

**Kata kunci**: *Kappaphycus alvarezii*, logam berat, klorofil-a, morfologi sel.

**Abstract**

*Kappaphycus alvarezii* is one of Indonesia's export commodities because it has high economic value as food and industry. One area in East Java which is the center of seaweed cultivation is Sumenep regency such as Saronggi and Bluto. Currently Sumenep Regency is an area for oil and gas exploration and exploitation. Types of pollutants resulting from these activities cause pollution in aquatic environments such as lead (Pb), mercury (Hg) and cadmium (Cd). The heavy metals found in the waters can be absorbed and accumulated in the seaweed thallus. The purpose of this research is to know the heavy metal content of Pb, Hg, Cd, chlorophyll-a, and cell morphology in E. cottonii in Bluto and Saronggi waters. This research is survey and descriptive research. The results showed that there are differences in heavy metal content of lead in *E. cottonii*, seawater, and sediments in Bluto waters and Saronggi waters. The waters of Bluto have a lower amount of chlorophyll-a than the Saronggi Waters. Cell wall thickness in *E. cottonii* in Saronggi Waters has thinner cell walls than Bluto waters. Measurement of water quality in Bluto and Saronggi waters through temperature, pH, salinity, brightness and DO parameters. The measurement results from both waters are not any striking difference and under optimal conditions.

**Keywords :** *Kappaphycus alvarezii,* heavy metal, chlorophyll-a, cell morphology.

## 1. Introduction

Seaweed is one of the mainstay export commodities to increase national income and foreign reserve. Seaweeds grown in Indonesia reach 555 species and four types have been known as export commodities, namely *Eucheuma* sp., *Sargassum* sp., *Gracilaria* sp., and *Gelidium* sp. (Alamsjah *et al.,* 2010). Among the many types of seaweed, the type of seaweed is the most widely used for cultivation in Indonesia is the type of *Eucheuma cottonii* or *Kappaphycus alvarezii* (Rozaki *et al*., 2013). This species is widely cultivated because its production technology is relatively cheap and easy and its post harvest handling is relatively easy and simple. In addition to industrial raw materials, this type of seaweed can also be processed into food that can be consumed directly (Wijayanto *et al.,* 2011).

One of the areas in East Java which is the center of seaweed cultivation is Sumenep Regency, Madura. In 2014, the cultivation area of *K. alvarezii* in Sumenep regency recorded 141,324 ha and the area that has gone through seaweed cultivation development is Saronggi, Bluto, Talango, Giligenting, Sapeken and Gapura sub-districts (Fatmawati and Wahyudi, 2015). However Sumenep Regency is an area for exploration and exploitation of oil and gas due to the potential of Sumenep. The potential of oil and gas in Sumenep Regency, East Java is believed to be great because a number of oil and gas companies are trying to explore the area (Bappeda Jatim, 2012).

One of the pollutants generated from these activities causes pollution in aquatic environments is heavy metals (Palar, 2012). According to Kementerian Negara Kependudukan dan Lingkungan Hidup (1990) *in* Sarjono (2009) that Pb, Hg, and Cd metals are heavy toxic. Heavy metals in the water will undergo the process of sedimentation and accumulate in the sediment, then accumulate in the body of marine biota existing in the waters through the process of gravity, bioconcentration and bioaccumulation by aquatic biota (Ma'rifah *et al*., 2016).

The mechanism of heavy metal absorption in seaweed in general that occurs is the membrane layer in algae consisting of lipid bilayer where the surface contains a layer that can bind the ions to be absorbed. The metal ions will enter the cell by penetration into the lipid layer (Andhini, 2011).

Seaweed growth and distribution is limited by several factors: temperature, salinity, substrate type, and brightness. The most influential factor on the growth of seaweed is the depth of water. The ups and downs of the water surface affect the light entering into the water column (Christon *et al*., 2012). Khan and Satam (2003) said that the best condition so that the seaweed can grow at best it when the depth between 30 cm to 60 cm so that the absorption of nutrients can still take place and the seaweed is not damaged by exposure to direct sunlight. These conditions can prevent seaweed from drought and optimize sunlight acquisition for photosynthesis.

Based on the above description, it is necessary to research the identification of lead (Pb), mercury (Hg), cadmium (Cd), chlorophyll-a and cell morphology of *Kappaphycus alvarezii* seaweed in Bluto and Saronggi waters, Sumenep, Madura, East Java. The information can be used as the basis of further research in the field of seaweed.

## 2. Materials and Methods

*Place and Time of Research*

This research was conducted in April 2017. The sampling location was conducted in Bluto and Saronggi Waters, Sumenep, Madura, East Java. The analysis of heavy metals was carried out at the Chemistry Laboratory of Universitas Negeri Surabaya. Analysis of chlorophyll-a content and cell morphology observation was done at the Laboratory of Faculty of Fisheries and Marine University Airlangga Surabaya.

*Tools and Materials*

The research equipments used for sampling are thermometer, refractometer, pH paper, DO test kit, 5 kgof plastic wrapping, and coolbox. Equipments used for heavy metal analysis are Atomic Absorption Spectrometry (AAS), beaker, aluminum foil,homogenizer, polypropylene bottle, porcelain cup, plastic funnel, desiccator, measurement cup, hot plate, roasted pumpkin,microwave, micropipette, oven, drop pipette, volumetric pipette, knive, freezer, plastic spoon, and analytic balance sheet. Equipments used for analysis of chlorophyll content are test tube, incubator, centrifuge and spectrophotometer. Equipments used for observation of cell morphology are microscope, glass object, glass cover, tweezer, and scapel.

The materials used in this research are *K. alvarezii* are seaweed, seawater, and sediment samples. The materials used for the analysis of heavy metals content in seaweed and seawater are HCl solution, concentrated $HNO_3$ solution, $H_2SO_4$, heavy metal standard solution and aquadest. The material used for chlorophyll test is 85% acetone.

*Procedures*

*Determination of Sampling Station*

Sampling stations for the analysis of heavy metal based on sources of pollutants which is the area near of oil and gas field. Geographical determination of each sampling station using Global Positioning System (GPS). In Bluto waters, the sampling station is located at 7° 12' 36.6"south latitude and 113° 77' 64.57" east latitude. While the station in Saronggi Waters located at 7° 12' 59.25" south latitude and 113° 89' 29.17" latitude east.

**Table 1**. The content of heavy metal (Pb, Hg, and Cd) on seaweed

| Heavy Metal | Heavy metal content (mg/L) | | Heavy metal standard (mg/L) |
|---|---|---|---|
| | Bluto | Saronggi | |
| Lead (Pb) | 0.008 | 0.013 | 0.05 |
| Mercury (Hg) | 0.1168 | 0.0716 | 0.0258 |
| Cadmium (Cd) | 0.3439 | 0.2406 | 0.0111 |


**Figure 1**. Map of research sites in Bluto waters, Sumenep (Google Map, 2017)

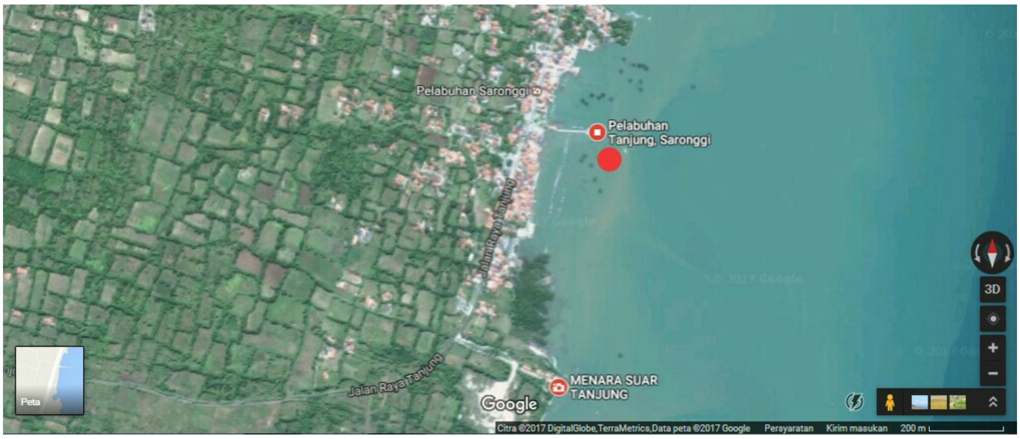


**Figure 2**. Map of research sites in Saronggi Waters, Sumenep (Google Map, 2017)

*Sampling*

Sampling is done directly in the waters of Bluto and Saronggi in the form of seaweed, sea water, and sediment. Water quality measurements are temperature, pH, salinity, dissolved oxygen (DO), and brightness. Samples taken are then stored in the coolbox to avoid damage to the sample. After that, the samples tested for heavy metal content, chlorophyll-a content and morphological observations of cells.

*Analysis of Heavy Lead Metal (Pb), Mercury (Hg), and Cadmium (Cd)*

Analysis of heavy metal content in seaweed and seawater using Atomic Absorbance Spectrophotometric (AAS). AAS is principled on the absorption of light by atoms. Atoms absorb the light at a certain wavelength, depending on the nature of the element. AAS encompasses the absorption of light by the neutral atoms of a metal element in its ground state. The absorbed rays are usually ultraviolet rays and visible light. The principle of AAS is essentially the same as the absorption of rays by molecules or ionic compound in solution (Skoog *et al*., 2000).

*Measurement of Chlorophyll-a*

Measurement process of chlorophyll-a on *Kappaphycus alvarezii* is using spectrophotometer. The procedure for measuring chlorophyll is by drying the seaweed samples. Chlorophyll pigment was obtained by tissue extraction of approximately 1 gram within 10 ml of 85% acetone in the test tube. The aqueous solution of acetone and seaweed was incubated at a temperature of 20-25 ° C for 24 hours. The chlorophyll extract is filtered using a buchner funnel. Then the extract is centrifuged at 5000 rpm for 5 minutes. The supernatant of the extract is used to measure chlorophyll. The aquadest is inserted in a cuvette as a blank and then placed on the spectrophotometer and then pressed the zero button to calibrate. The sample solution is inserted in the cuvette and placed on the spectrophotometer. Furthermore, by pressing the wavelength value of 664 nm and 647 nm on the spectrophotometer, the absorbance value will be known (Lobban *et al*., 1988).

The measurement of pigment extracts was carried out at wavelengths of 664 and 647 nm (Lobban *et al*., 1988). The formula for calculating chlorophyll-a is as follows:

$$\text{chlorophyll-a (mg/L)} = 11{,}93\ (\text{Abs } 664) - 1{,}93\ (\text{Abs } 647)$$

The known absorbance value is entered into the formula below:

$$\mu\text{mol chlorophyll in the extract} = \frac{\text{Chlorophyll in absorbance (nm)}}{\text{chlorophyll molecules weight } (\mu g)}$$

Chlorophyll-a molecule weight is 894 µg.

*Observation of Cell Morphology*

Observation of cell morphology is done by cross-cutting the seaweed thallus. After that, put a piece of thallus on top of glass object and closed with cover glass. The morphology of seaweed cells was observed using a microscope. Cell morphology observed was the difference in cell wall thickness.

**Table 2**. The heavy metal content of Pb, Hg, and Cd of seawater

| Heavy metal | Heavy metal content (mg/L) | | Heavy metal standard (mg/L) |
|---|---|---|---|
| | Bluto | Saronggi | |
| Lead (Pb) | 0.005 | 0.007 | 0.05 |
| Mercury (Hg) | 0.0731 | 0.1138 | 0.0258 |
| Cadmium (Cd) | ND | ND | 0.0111 |

Description: ND = No Detection

*Data analysis*

The data analyzed were informations about heavy metal content, chlorophyll-a and cell morphology at *K. alvarezii* in Bluto and Saronggi waters. This research is a survey research. The data obtained are then described descriptively.

## 3. Result and Discussion

*The content of heavy metal Pb, Hg, and Cd*

The Lead (Pb) content of *K. alvarezii* at both sites is still below the maximum limit. Based on BSN (2009), the maximum limit of Pb contamination on seaweed is 0.5 mg/L. While the contents of Hg and Cd in both locations has exceeded the threshold. Hg content of *K. alvarezii* seaweed on both locations exceeded the limit of 0.0258 mg/L (BSN, 2006). The Hg amount in sample of Bluto waters reached to 0.1168 mg/L and the numbers in sample of Saronggi waters reached to 0.0716 mg/L. The content of Cd in seaweed at both locations has exceeded the threshold. Based on BSN (2006), the maximum limit of heavy metal Cd contamination is 0.0111 mg/L. The farther the sampling location from the center of the oil and gas activities, the smaller the value of heavy metal content in the seaweed. When the location is closer to the center of the activity, heavy metals absorbed by the seaweed will be more numerous (Siaka et al., 2016). Seaweed binds heavy metal ions by means of ion exchange, where the ions in the seaweed thallus are replaced by heavy metal ions (Siswati *et al*., 2005).

Pb content of seawater in Bluto and Saronggi waters have Pb content below the threshold. Based on the Departemen Kesehatan (1990) that the threshold value of Pb content in water is 0.05 ppm. However, Pb content in Seawater of Saronggi Waters (0.007 mg/L) is higher than Bluto waters (0.005 mg/L) as the Pb content in seaweed in Saronggi Waters also higher than Bluto waters.

Hg content in seawater showed that Hg content in both waters exceeded the predetermined threshold of 0.0258 mg/L (BSN, 2006). Hg content in seawater in Bluto waters is 0.0731 mg/L while in Saronggi waters is 0.1138 mg/L.

The content of Cd in seawater in Bluto and Saronggi Waters is undetectable. Cd is only found in seaweed and sediment only. This indicates that the Cd is less soluble in water so that the Cd tend to settles on the sediment and absorbed by the seaweed. This proves that the ability of seaweed that can absorb heavy metals present in seawater can cause to small amounts of heavy metal content in the waters (Surahman, 2007 *in* Siaka *et al*., 2016).

**Table 3**. The heavy metal content of Pb, Hg, and Cd of sediments

| Heavy metal | Heavy metal content (mg/L) | | Heavy metal standard (mg/L) |
|---|---|---|---|
| | Bluto | Saronggi | |
| Lead (Pb) | 4.05 | 3.81 | 0.675 |
| Mercury (Hg) | 0.002 | 0.003 | 0.0258 |
| Cadmium (Cd) | 2.11 | 2.34 | 0.0111 |

**Table 4**. The content of Chlorophyll-a of *Kappaphycus alvarezii*

| Sample | µMol Chlorophyll | |
|---|---|---|
| | High Tide | Low Tide |
| Bluto | 0.0157 | 0.0159 |
| Saronggi | 0.0221 | 0.0294 |

The threshold value of Pb concentration of sediment based on NOAA (2004) is 0.675 ppm. Pb content of sediment in Bluto and Saronggi waters shows a value above the threshold. The content of heavy metal Hg on sediment does not exceed the threshold of 0.0258 (BSN, 2006). The water of Bluto is 0.002 mg/L and the waters of Saronggi is 0.003 mg/L. The content of Cd at both locations is exceeding the threshold. According to BSN (2006) that the maximum limit of Cd in sediment is 0.0111 mg/L. This indicates that many of the Cd accumulates and deposits in the sediments.

Heavy metals will accumulate in the water column and are carried away by currents that then sink to the bottom of the waters and will accumulate in the sediment. Flows and waves are the major force factors that determine the direction and distribution of sediments. Contamination of heavy metals in sediments will persist for long periods of time (Putri *et al*., 2014).

*Chlorophyll-a content*

The amount of chlorophyll-a on *K. alvarezii* at high tide and low didn't have much different. However, there is a difference in the amount of chlorophyll at both sites. The waters of Bluto have a lower amount of chlorophyll than the waters of Saronggi. Based on research by Wijaya (2012) that the content of chlorophyll-a on *K. alvarezii* when the normal condition is 0.0157 µg/ml.

Pigment concentrations in algae are closely related to light intensity. Similarly, the intensity of sunlight is very closely related to the water layer. The deeper the water layer, the intensity of light that penetrates the water layer is also decreasing (Rusdani, 2013).

The ups and downs of the water surface affect the light entering into the water column (Christon *et al*., 2012). Khan and Satam (2003) said that the best condition so that the seaweed can grow at best it when the depth between 30 cm to

60 cm so that the absorption of nutrients can still take place and the seaweed is not damaged by exposure to direct sunlight. These conditions can prevent seaweed from drought and optimize sunlight acquisition for photosynthesis.

Excessive concentrations of heavy metals will affect chloroplasts that cause degradation of the thylakoid membranes where the thylakoids are one part of the chloroplast receiving sunlight. Since chlorophyll is present in chloroplasts and leaf mesophyll cells, indirectly excessive heavy metal will affect the amount of chlorophyll-a in seaweed (Setiawati, 2009).

*Cell Morphology*

The membrane layer of *K. alvarezii* in the waters of Bluto has a thinner cell wall than the waters of Saronggi. However *K. alvarezii* cell wall in the waters of Bluto and Saronggi waters both experienced thinning of the cell wall. According Afriani (2012) that the normal cell wall *K. alvarezii* in normal circumstances have a thickness of 50 µm.

**Table 5**. Cell Wall thickness of *K. Alvarezii*

| **Sample** | **Cell Wall Thickness of *K. alvarezii*** | |
|---|---|---|
| | **High Tide** | **Low Tide** |
| Bluto |  Length of Cell Wall is 18.37 µm |  Length of Cell Wall is 18.36 µm |
| Saronggi |  Length of Cell Wall is 20.72 µm |  Length of Cell Wall is 21.01 µm |

**Table 6**. Water Quality Parameter Measurements

| Parameter | Perairan Bluto | Perairan Saronggi |
|---|---|---|
| Suhu (°C) | 29 | 30 |
| pH | 7 | 7 |
| Salinitas (ppt) | 27 | 28 |
| Kecerahan (m) | 2 | 3 |
| DO (mg/L) | 4 | 2 |

The accumulation of heavy metals occurs because the polysaccharides present in the cell wall bind the heavy metal ions and form complex compounds with organic substances contained in tallus (Lobban and Harrison, 1994). The algae consists of a lipid bilayer in which the surface contains a layer which can bind the ions to be absorbed. The metal ions will enter the cell by penetration into the lipid layer. The more heavy metals bound to the cell wall, the more heavy metals can enter the cells (Andhini, 2011).

*Water quality*

According to Parenrengi et al. (2007), *K. alvarezii* seaweed lives in tidal areas with water depths of 1-5 m at the lowest tide, requires sunlight for photosynthesis, requiring a pH range of 6-9 (optimum pH 7.5-8.0 ) and salinity between 27-34 ppt. The required nutrients are obtained from water and the temperature needed range from 27 to 30° C, and the brightness is 1.5 meters. Thus the water quality in both locations is in good condition.

## 4. Conclusions

The conclusion of this research is the content of Pb on *Kappaphycus alvarezii* in Bluto waters and Saronggi waters does not exceed the threshold while the content of Hg and Cd exceeds the predetermined threshold, the amount of chlorophyll-a on *K. alvarezii* in Bluto waters is lower than Saronggi Waters, The cell walls of *K. alvarezii* in the Waters of Saronggi have thinner cell walls than the Bluto waters.

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