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Effect of Tributyltin pollutant toxicity of sea alga cells, Kappaphycus alvarezii

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Abstract

The sublethal effect of TBT on the morphology of algae cells occurs at concentrations of 2-10ppm, starting with a fading color change followed by bleaching in the tallus. Histopathologically, the concentration of 2-4ppm algae cell structure is still intact as in control, where the cortex and medulla consist of a series of round cells. At a 6ppm TBT concentration, the boundary between the cortex and medulla cells is almost non-existent and the cells become very thin, while the higher concentrations (8 and 10ppm) of the cortex and medulla cells are completely damaged.

Keywords: Tributyltin; Toxicity; Seaweed (Kappaphycus alvarezii).

1. Introduction

The rapid development of science and technology provides opportunities in the use of natural resources to meet human needs. Coastal and marine areas have potential, both in the form of renewable and non-renewable resources, so that the coastal and marine areas are the areas with their developed activities.

Even though the activity of utilizing marine resources is very beneficial for the economy, but without realizing it can bring adverse impacts on the environment. This adverse impact can be in the form of environmental damage caused by pollution. Pollution is an innate impact that always follows every development activity. According to Law No. 23 of 1997, environmental pollution is the entry or inclusion of living things, substances, energy and/or other components into the environment

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by human activities so that the quality drops to a certain level that causes the environment to unable to function in accordance with its designation.

Pollution is caused by the entry of pollutants in the water, in the form of waste, pesticides, oil, heavy metals or other chemicals. Pollutants can cause a decrease in population in living things, affect metabolism and reproduction, changes in behavior and can cause a decrease in environmental quality quickly so that it cannot function, as it should (Rand and Petrocelli, 1985).

The sea, which covers around two-thirds of Indonesia's territory, contains a variety of natural resources. Biological life sustainability awareness of diversity in the sea, raised by the understanding that the increase in waste disposal due to rampant industrial activities, transportation around the coastal areas, expansion of residential areas and reclamation threaten the productivity and biodiversity of the sea.

Red algae has an important economic value because it contains iota carrageenan, served as a salad, and as a source of nutrition, algae contain carbohydrate, protein, less fat, and ash (sodium, potassium, phosphorus, sodium, iron, iodine). There are also vitamins such as A, B1, B2, B6, B12, C, and beta-carotene. This makes red algae one of the dominant species cultivated in Arakan Village Beach, Tatapaan District in South Minahasa Regency.

The existence of algal populations in waters is disrupted due to environmental pressure, because of the limitations of algae to survive in certain environments, such as contamination of anthropogenic waste containing toxic compounds for algae including TBT (tributyltin). This is because that TBT has been used extensively and uncontrollably as an anti-fouling material mixed with paint raw material. TBT is a substance that is very toxic in aquatic environments and to high concentrations can cause death to organisms (Bryan *et al*., 1986; Gibbs *et al*., 1987; Ohji *et al*., 2002).

The entry of TBT in the waters can affect the presence of marine organisms including algae. However, the extent of the influence of TBT has not been widely applied. Red algae Kappaphycus alvarezii is applied to control water pollution, where cell changes that occur in algae can provide information regarding the alleged contamination of organotin compounds in waters. Based on this, a study was conducted with the aim of analyzing the effects caused by tributyltin through histopathological techniques on algae cells Kappaphycus alvarezii.

2. Research methodology

The algae test, Kappaphycus alvarezii that was used in this study was taken from the algae cultivation area in the Coastal Waters of Arakan Village, Tatapaan District, and South Minahasa Regency. Algae samples were taken to the laboratory to be adapted to laboratory conditions for 3 days before testing. Maintenance was carried out at a temperature of 29-30 ºC, pH 7-8 and salinity 35 ‰. The culture technique of the test organism is a static system (water does not flow).

Before conducting the test, a preliminary test was carried out to determine the range of chronic concentrations, which will be used in further research. The concentrations used in the preliminary test were 0.01ppm, 0.1ppm, 1ppm, 10ppm plus control. The test organism was maintained in a jar container with a volume of 250 ml water and a predetermined concentration, and each jar was put into 5 pieces of algae. From the results of this preliminary test, five chronic concentrations were chosen namely 2ppm, 4ppm, 6ppm, 8ppm, and 10ppm for further testing.

Testing the effects of TBT was done by maintaining the Kappaphycus alvarezii algae in a 300 ml jar with a volume of water containing a TBT test solution per treatment. Each container is inserted 10 pieces of algae measuring about 4-5cm and given aeration. The test was carried out for 15 days and at the end of the test TBT toxicity checks were carried out on algal cells, while observations of algal morphology were carried out every day.

To find out the impact of TBT compounds on the structure of Kappaphycus alvarezii algae, microscopic examination was performed with histopathological procedures. Samples were taken in each jar for each treatment, and then the samples were fixed in 4% formalin. After fixation, the sample was cut as thin as possible using a razor blade. The incision was placed on a glass preparation and stained with aniline blue, then covered with a cover glass that was observed under a microscope. The descriptive and histopathological changes of algal cells were compared with the controls, based on the preparation of cell structure (Doty, 1985). The part was visualized using a microscopic camera.

3. Results and Discussion

Based on the observation of morphological forms of the algae, Kappaphycus alvarezii, which is the control, exhibits morphological character as in the natural habitat. Where the thallus and its color are brown, it was used as a reference or comparison to the TBT-treated algae. The algae exposed to TBT concentrations of 2ppm and 4ppm did not undergo any changes in their shape and color. Morphological changes occurred in the second weekend (day 15), where the color of the thallus began to fade, but the tallus did not change.

At the concentration of TBT 6ppm, the color of tallus was faded, but at the end, the thallusnya did not experience bleaching. Then, at the end of bleaching on the day 8th, thallus showed and followed by a color change that faded in comparison with the control, however, the shape of the thallus did not change.

Morphological changes in algae exposed by TBT concentration of 8 ppm, the color of thallus looked fading and the bleaching that occurred at the tip of the thallus, which was getting bigger and soft. At a concentration of 10 ppm, the process of changing the color of thallus occurred quickly, namely starting on day 3, then on day 4, the color of thallus changed and bleaching of the thallus was more and more, almost all have turned white, then the thallus became soft on day 12.

Based on the results obtained from TBT testing on Kappaphycus alvarezii algae tested at different concentrations ranging from 2ppm, 4ppm, 6ppm, 8ppm and 10ppm, it has an impact on the morphological shape of the thallus and algal cells. Tributyltin compounds contained in water were transferred from water bodies into the body of organisms through the process of absorption of these organisms. The process of absorbing TBT that has high toxicity by the Kappaphycus alvarezii algae can be carried out in all parts of the thallus, so that the contaminated algae undergo changes in morphological conditions. This shows that sublethal TBT has an influence on algae. Loban and Wayne (1981) stated that morphological changes in the thallus that become soft and change in color to white can affect changes in cortical cells and medulla, which can affect the metabolic processes and algal growth. Rompas (1997) states that toxic effects can occur due to pollutants in the form of chemicals classified as foreign material that can affect tissue activity and even deadly bias. Furthermore, Bryan (1976) in Connell and Miller (1995) suggested that the effects of sublethal a pollutant in living things cause changes in morphology, histology, physiology (growth, development, the ability of swimming, breathing, circulation), biochemical (the state of blood chemistry, the activity of enzyme , endocrinology), behavior / neurophysiology and propagation.

The results of cross section Kappaphycus alvarezii algae cells not treated with TBT (control) at the end of test showed that the medulla and cortex were intact. The cortex was still in regular shape consisting of 1-3 series cells and medulla cells were still in full shape. In Figure 1A, the medulla and cortex are clearly visible. The results of this identification can

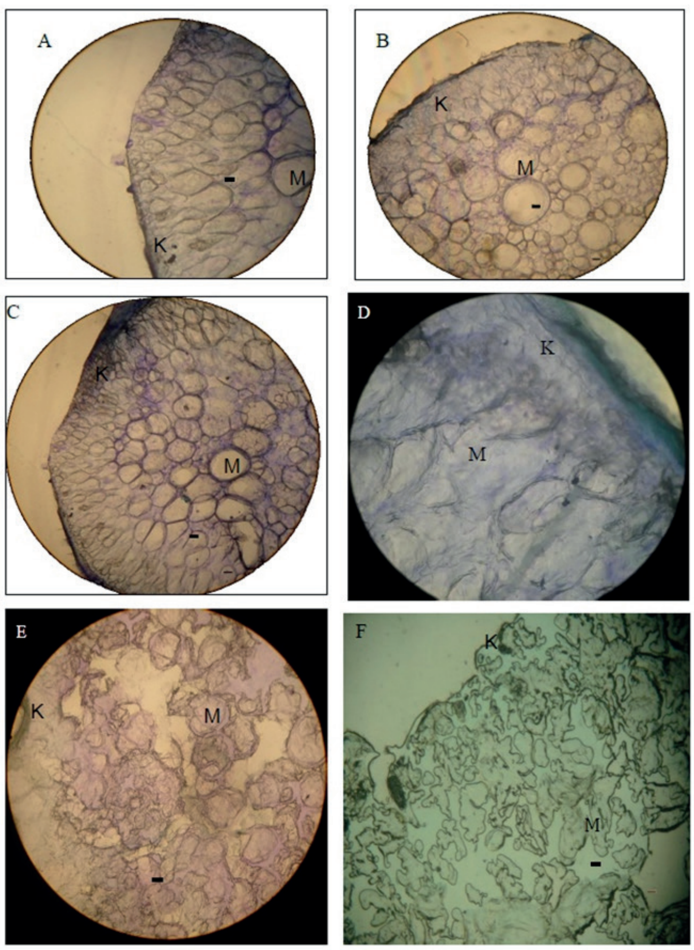

Figure 1. Forms of algal cells, Kappaphycus alvarezii before and after treatment with tributyltin compounds

(A = Normal cell shape (control), B = Concentration 2 ppm, C = Concentration 4 ppm, D = Concentration 6 ppm, E = concentration 8 ppm, F = Concentration 10 ppm, M = medulla, K = Cortex) compared against algae that were contaminated with TBT.

The alga exposed with TBT of 2ppm, showed that the medulla and cortex were still intact, as the cells in the control still looked perfect. The same result was observed in algae cells that had been treated with TBT of 4ppm, showing intact medulla and the cortex was arranged on a series of cells as in control (Figure 1B & C).

After algae cells were treated with 6-ppm TBT concentration, it turned out the shape of the cortex fringe, where the boundary between the cortex and its medulla was almost non-existent. Then, the cortex was already very thin and broken, this also occurs in parts of the medulla that were already incomplete and the cells almost merged with the cortex. Figure 1D shows the changes occur at a concentration of 6ppm TBT. The same change also happens in the higher concentration of test when the concentration is 8ppm, in comparison with the control. It appears that the shape of the medulla is irregular but not damaged because there are parts of the medulla that can still be observed according to their shape, whereas cortical cells appear to be out of shape (Figure 1E).

Figure 1F shows the shape of cortex and medulla cells that were given in TBT concentration of 10ppm, in which the arrangement of cells had been totally damaged. The boundary between the cortex and medulla is gone because the cells have fused. So, the greater the concentration of TBT given, the greater the level of damage caused and the faster the time required to cause the damage.

Observations on algae Kappaphycus alvarezii cells prove that TBT absorbed into the algal thalus accumulates in cortical and medulla cells causing changes in shape in concentrations of 6ppm, 8ppm, and 10ppm. The repeated introduction of TBT even in small amounts will increase its concentration in the cortex, causing changes in cortical cells. The cortex is the tissue that surrounds the medulla and contains filaments at the ends. According to Atmadja *et al*. (1996), the middle tissue of Kappaphycus alvarezii consists of colored filaments surrounded by large cells and covered by cortical layers. A filament is a type of thallus consisting of one or more lines connecting cells, with or without a layer of gelatin and adhesive. If pollutants have damaged the cortex, it will be easier to continue to enter all algal cells. The transition of cells from the cortex to the medulla can cause pollutants to damage the medulla cells. Changes that occur in cortical cells are visible at each concentration of the test with different levels of damage where the results of observations indicate there has been a change in the shape of the cortical cell structure.

Changes in the cortex can also cause 'pit connection' damage. Loban and Wayne (1981) stated that the connection of one cell to another cell is due to a 'pit connection'. Damage to the 'pit connection' will affect the supply of oxygen and food to all algal cells. Damage to the cortex will cause pollutants more quickly enter the cell, thus accelerating damage to the medulla cells. This change is starting from a concentration of 6ppm, 8ppm and 10ppm (Figure 1 D, E, and F). The cortex is part of the algae, which contains chlorophyll pigments, to carry out photosynthesis. Chlorophyll is a pigment because it absorbs light, like electromagnetic radiation in the visible spectrum. With the presence of pigments, algae can synthesize organic materials by absorbing light as an energy source (Tokida *et al*., 1975; Kimbal, 1998). Changes that occur in the cortex are thought to be caused by loss of pigment in algal cells, where the presence of tin (Sn) in the TBT test solution is accumulative which allows a buildup in the cortex, thereby inhibiting the process of photosynthesis, which results in algae losing the ability to grow.

According to Connel and Miller (1995), the element tin is a substance that can be accumulated biologically in the food chain (bio-accumulative), can be somewhere and not undergo a process of degradation (persistent) so that it has the potential as a poison (toxic) for organisms. Furthermore, Bowman (1982) states that organotin compounds are toxic because they are easier to enter the body's tissues through their soluble organic elements, especially when bound to lipids or fats.

This research proves that the compound tributhyltin has a toxic power for aquatic organisms. So with the increasing number of human activities, more and more pollutants are produced, especially pollutants produced through the release of ship paint material that is antifouling and contains TBT. This pollutant will be very dangerous if it pollutes the waters, because it will be very toxic and easily accumulate in the body of organisms, so that it can poison the entire food chain in the ecosystem and the way of accumulation of other organisms that prey (bio-magnification) (Dobson and Cabridenc, 1990; Rumengan *et al*., 2008).

Most of these pollutants will reach water areas such as rivers, lakes and oceans. These pollutants have toxic effects on marine organisms through a rapid deterioration in environmental quality, can affect tissue and can even be deadly which ultimately reduces the biota population. Uncontrolled use can have major effects that have long-term impacts on marine life.

Algae are aquatic organisms that depend on environmental quality conditions. Considering that algae are aquatic organisms that are very beneficial for human needs, the condition of the waters where algae live must be considered so that they are not easily polluted with pollutants, especially organotin compounds, such as TBT.

Conclusion

TBT contamination in Kappaphycus alvarezii cell algae can cause physical morphological changes, which are characterized by the fading of thallus color and bleaching at the end of the thallus (bleaching) and changes in the shape of cell. At TBT concentrations ranging from 6ppm to 10ppm, because of cell changes in the cortex and medulla that are increasingly apparent, it can result in cell damage and algal death. Damage caused by TBT is influenced by the amount of concentration given, where the greater the concentration, the greater the damage caused by a relatively faster time. Based on the results obtained, it is suggested that efforts should be made to control the use of TBT as an anti-fouling material in Indonesia given the adverse effects are very dangerous if contaminated in aquatic organisms, especially algae.

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