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Short Note

Effects of triphenyltin exposure on the red alga *Eucheuma denticulatum*

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Abstract—Toxic effects of triphenyltin (TPT) on the marine alga *Eucheuma denticulatum* obtained from Nain Island, North Sulawesi, Indonesia have been examined in laboratory condition. The algal samples were first acclimated in laboratory prepared seawater for three days. The algae were then divided into 12 culture chambers for treatments with different concentrations of TPT between 5–30 $\mu g\,l^{-1}$ with 5 $\mu g\,l^{-1}$ interval, and in another container for control. After two-week experimentation, some samples of algae were obtained from each chamber for histopathological examination. The aniline blue stained tissue of alga *E. denticulatum* showed that at all TPT concentration tested, the cortex had morphological changes, even almost disappeared at higher concentration (>20 $\mu g\,l^{-1}$). The medullary cells of alga exposed to 5–15 $\mu g\,l^{-1}$ have been decreasing in size compared to those in control. Cell walls of some medullary cells have damaged at 20 $\mu g\,l^{-1}$ and totally lysed in all cells of alga exposed to 25 and 30 $\mu g\,l^{-1}$. The pattern of zonatedly dividing tetrasporangium had not occurred anymore in alga exposed to TPT even at the lowest tested concentration (5 $\mu g\,l^{-1}$) and had totally damaged in higher concentrations, such as that of 20 $\mu g\,l^{-1}$. The tetraspores in tetrasporangium seem to be fused or shrinkage, no more dividing process occurred as in control. Therefore, reproductive cells of alga is more sensitive than somatic cells, indicating that no further development of alga will occur in aquatic environment contaminated even with unlethal TPT levels.

Key words: triphenyltin, red alga, medullary cells

Introduction

Triphenyltin as a member of organotin compounds (OTs) has been used as biocides in antifouling paints applied to vessels to prevent the attachment of organism (Harino et al. 1998). Widespread contamination by organotin and their toxic effects on aquatic organisms have been major concerns and have received considerable attention during the last decade. Indonesia is located between two continents (Asia and Australia) and two oceanic waters (Pacific and Indian Ocean), could possibly serve as emission sources for the surrounding waters. Therefore understanding of organotin effects may help increase knowledge to contribute for developing a monitoring program of organotin contamination in coastal waters.

Widespread occurrence of organotin compounds has been reported, such as those of Harino et al. (1998, 2003). They concluded that triphenyltin (TPT) and tributyltin (TBT) give harmful effects on aquatic organisms when they released into the environment even at ultra trace levels. Even though usage of TPT is less common compare to TBT but Harino et al. (1998) has detected high concentrations of TPT in many biological samples in Otsuchi Bay, Northeast Japan, with many fishing boats around. However, there is no report on its biological effects on alga.

Various toxic effect of pollutant have been identified such as reducing biodiversity, bearing negative impacts on the metabolism and reproduction efficiencies, even changing the behavior and structure and form of an ecosystem, and rapidly degrading environmental quality, therefore influence on the whole aquatic resources (Rand and Petrodelli 1985). Such effects of organotin have been reported by Ohji et al. (2002a, b) for the caprellid amphipod, and Horiguchi et al. (1997) for a mollusk; indicated a significant effect on intersex ration or imposex. So most biological effects of organotin were based largely on the use of aquatic animals. This is marked contrast to the aquatic environment, where micro and macro alga constitute the most basic ecological rank, i.e. primary producers.

The marine alga *Eucheuma* has an important role in aquatic system as a primary producer in coastal water system, and as a control agent of heavy metal pollution (Trono 1999). This alga is economically valuable because of its high carrageenan content (Bold and Wynne 1985) and its usage as freshly prepared vegetable salad (Trono 1999). It becomes an important export commodity in Indonesia. *E. denticulatum* is a dominant species cultured in Nain Island, a small Island next to the famous Bunaken Island in North Sulawesi. More information is necessary to contribute a deep insight that alga may indicate the quality of water in some ways.

In North Sulawesi, where laboratory facilities for measuring chemicals in water are still limited, using alga as a biological indicator of water quality to assess the level of contamination will be more realistic. This study was performed in order to know the toxic effects of TPT exposure to the alga in laboratory condition. Morphological change of the alga exposed to TPT may be useful information for developing an assessment of organotin contamination in coastal waters.

Materials and Methods

The test organisms, *E. denticulatum*, were obtained from a seaweed culture area in Nain Island, next to the famous Bunaken Island, North Sulawesi. Samples were acclimated in autoclaved seawater for three days in aquaria. The algae were then divided into 13 groups for six treatments and one control, so that there were two replicates for each treatment. The treatments were different concentration of TPT between 5 to 30 $\mu g\, l^{-1}$ with 5 $\mu g\, l^{-1}$ intervals. Each culture chamber was inoculated with 50 gram of alga, slightly aerated, closely tied and placed under illumination.

After two-week exposure of TPT, algal sample was taken from each culture chamber and immersed in 4% formalin for an hour. After this fixation, algal tissue were finely cut, and stained with an aniline blue solution Microscopic examination was performed under a compound microscope to record the histopathological change of cell structure and reproduction organ.

Results and Discussion

The most distinctive trait of this alga is the manner in which cortex and medulla composed in the tissue. The usual distinction between small cortical cells with many chloroplasts and larger medullary cells with fewer or no chloroplast is discernible as commonly occur in most larger forms of Rhodophyta (Bold and Wynne 1985). As shown in Fig. 1, normally an algal tissue as in control (0 $\mu g\, l^{-1}$) has cortex, which composed of outer and inner smaller cells, and medulla that is very compact and composed of larger cells. These medullary cells are initiated from the inner cortex. The cell structure changed after two-week exposure to TPT at different levels (5–30 $\mu g\, l^{-1}$).

It can be seen that, the cortex had morphological changes, even almost disappeared at higher concentration (>20 $\mu g\, l^{-1}$), and its influence on the transition of cells from cortex into medulla was clear. The medullary cells of alga exposed to 5–15 $\mu g\, l^{-1}$ had been decreasing in size compared to those in control. Cell walls of some medullary cells had damaged at 20 $\mu g\, l^{-1}$ and totally lysed in the treatments with 25 and 30 $\mu g\, l^{-1}$. Normally among outer and inner cells there is such a kind of a pit connection, which enables oxygen and nutrient supply into all inner cells (Loban and Wynne 1981). The change of cortical cell structure could eventually damage the pit connection, and therefore, the inner cells have no longer ability to grow. So the outer cortical cells were first influenced, then the inner cells, indicating there was a TPT penetration from surface tissue.

Bold and Wynne (1985) describe that Euchema commonly has a typical diplobiontic life history, in which male and female plants are different in some ways. Male plants have spermatangial sori, the spermatia being cut off the tips of elongate cells. Female plants produce the three-celled, inwardly directed carpogonial branches with reflexed trichogynes. Following fertilization, a single connecting filament emenates from each carpogonium and fuses with a remote auxiliary cell, from which the carposporophyte develops. The conspicuous fusion cell is surrounded by a candelabrum-shaped cluster of gonimoblast cells bearing terminal carpospores, and the entire carposporophyte is surrounded by a dense pericap of vegetative cells. Tetrasporic plants bear zonately divided tetrasporongia in the outer cortex. As shown in Fig. 2, normally a tetrasporangium (in 0 $\mu g\, l^{-1}$) constitutes the site of meiosis, the four haploid nuclei being incorporated into four tetraspores. This pattern of zonatedly dividing tetrasporangium has not been occurred anymore in the alga exposed to TPT even at the lowest tested concentration (5 $\mu g\, l^{-1}$) and had totally destroyed in higher concentration such as that of 20 $\mu g\, l^{-1}$. As medullary cells has decreased in size at 5 $\mu g\, l^{-1}$, the tetraspores in tetrasporangium seems to be fused or shrinkage, no more dividing process as in control. Normally the spores in tetrasporangia will get a mature stage and develop into a new organism. At higher concentration the tetraspores have totally damaged where tetrasporangium lysed, no more further development afterwards.

A similar phenomenon has been reported by Loban and Wynne (1981) who observed the reproductive cells of alga contaminated with hydrogen sulfide (H_2S). The spores developed unnormally, and no regeneration occurred afterwards. As for TPT, toxic effects on the alga may associate with the factor-inducing meiosis in primary reproductive cells even at very low concentration. It is clear that reproductive cells are

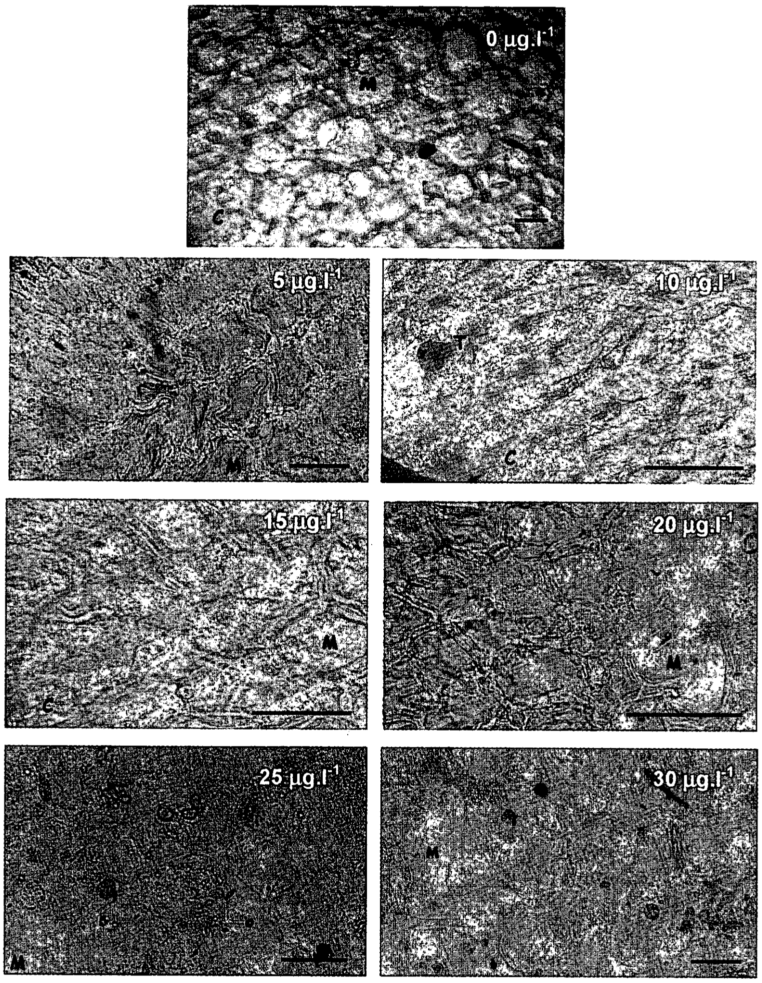

Fig. 1. Portion of transection of *E. denticulatum* showing medulla (M) and cortex (C) of control, and those exposed to 5, 10, 15, 20, 25 and 30 $\mu g\,l^{-1}$ of triphenyltin. Bar shows 100 μm.

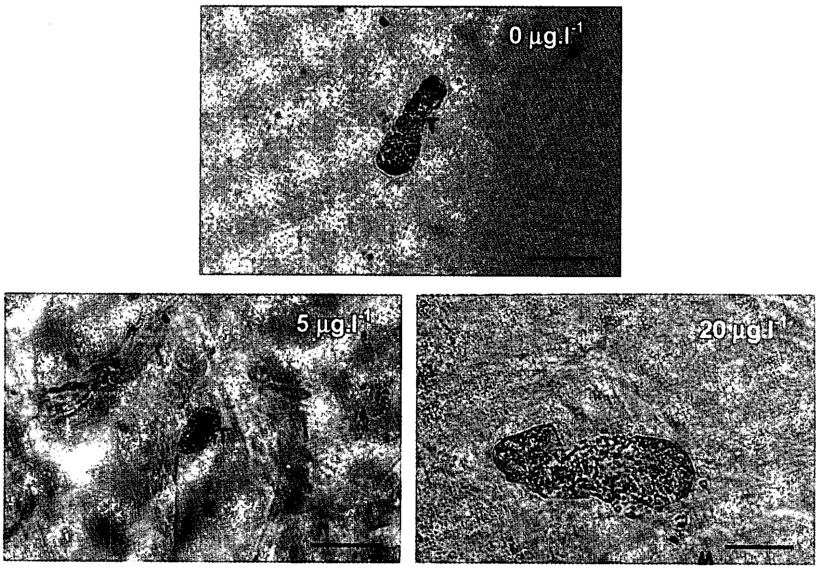

Fig. 2. Portion of transection of *E. denticulatum* showing the zonatedly divided tetrasporangium (T) in control, and those exposed to 5 and 20 $\mu g\,l^{-1}$. Bar shows 100 μm.

more sensitive than somatic cells, which implies that TPT inhibits the totipotency of plant. Even the unlethal levels may have negative impacts on cell division, so the alga could no longer reproduce.

The findings in this study is of course still very limited data, so further studies are necessary for developing a monitoring program to assess any water contaminated with organotin, or any other pollutant. Assessment of algal sensitivity to TPT contamination in nature could then understood from the morphological change of alga exposed to it. Even though TPT is less common than TBT in scientific communications, but as dominant compound among the PTs (Harino et al. 1998) its harmful effects is negligible. Attempts to prove that TPT can incorporate in many aquatic organisms and environment should be made, and its presence in all environmental compartments remain to study, especially in North Sulawesi that has a big plan regarding the AFTA, where some container harbors and fishing landings are being constructed. As Harino et al. (1998) found that the levels of TPT at station closed to coastal area with many shipyards around were the highest one, which decreased in stations located further from the coast, presumably the TPT and other organotins discharged from the shipyard. The vertical distribution of TPT levels from the intertidal zone has been also reported, in which the concentrations in mussels decreased with increasing depth. Although TPT levels of their finding was still too low (about $0.009\,\mu g\,l^{-1}$), but this could somehow occur in coastal water in North Sulawesi especially in harbor area.

As Whitton (1978) suggested long time ago that any photosynthetic plants may indicate the quality of waters in a wide variety of ways. It remains for future study that their distribution in time or space may be used to detect important features of the water including phenyltin contamination. Morphological change of this alga in nature may be useful to assess known organotin and any other pollutant as well. This could be a useful tool in a monitoring program.

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