Journal of Coastal Development
Vol. 1, No. 3, June 1998: 201-206

ISSN : 1410-5217

# PRELIMINARY STUDY ON THE EFFECTS OF 2,4-D HERBICIDE FORMULATIONS ON REEF BUILDING CORALS

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## ABSTRACT

*The increasing use of herbicides in agriculture sectors have received great attention with respect to their potential toxic effect on reef-building corals of Indonesia. One chlorinated compound, 2,4-dichloro phenoxy acetat (2,4-D), has widely been used as a herbicide and has become a substantial environmental pollutant. Controlled tolerance experiments testing 2,4-D was performed on Porites sp. and Galaxea sp in Marine Station, Teluk Awur, Jepara. The effects of 2,4-D on coral mortality and zooxanthellae expulsion were investigated. The results showed that short duration (48 h) laboratory test demonstrated dramatic effects on sloughing and death of coral. The median lethal concentration ($LC_{50-48}$) was determined to be 23.20 ppm on Porites sp and 10.26 ppm on Galaxea sp. There were no significant differences between Porites sp and Galaxea sp on the toxic response of 2,4-D compounds.*

## I. INTRODUCTION

Many urban, industrial, and agricultural developments in coastal areas, highly productive coastal wetlands and estuaries are often poorly planned and regulated. They have introduced new organic pollutants which were used as refrigerants, fire retardants, paints, solvents, herbicides and pesticides (Scheneider, 1979). Most of this compounds are recalcitrant to biodegradation, and their entry into the sea, might be, poses many challenges to the existing coral reefs and even to live in this vicinity in general.

The use of herbicides in Indonesia began in the 1960's when the government launched plantation rehabilitation programme. Among eight highly toxic pesticides commercially available in the market, for examples, Lindane, Endrin, Dieldrin, 2,4-D was the most heavily used on plantations, crops, livestock production and fisheries (Djamin, 1983).

There is a lack of information available on pesticide effects to the coral reefs. Glynn *et al* (1983) reported that herbicides can have a deleterious effect on corals, at relatively low concentrations and for short term. But, the coral mortality that occurred in eastern Pasific can not be attributed to herbicide alone because of the other confounding factors (sea warming).

Several studies have demonstrated that many organic pollutants are strongly bound to sediments, particularly to the organic matter fraction and are highly refractive, resulting in a long sediment residence time (years) after deposition (Elder *et al*, 1979). Thus, contaminants associated with sediments are potentially available to benthic organism and coral reefs even after these compounds are no longer produced or introduced into the aquatic environment.

The aims of this study were to obtain $LC_{50}$ and to estimate the toxic response of *Porites sp* and *Galaxea sp.* on dissolved 2,4-D.

## II. MATERIALS AND METHODS

### 2.1. Sample Collection

The reef-building coral *Porites sp* and *Galaxea sp.* served as the test species in this experiment. Specimens were collected from the reef flat,Teluk Awur, Jepara, from a depth 1-3 m and placed in 15 l aquarium at the Marine Station. The corals were maintained in running sea water for 48 h acclimation prior to use in tolerance test after being cut them off in 2x2 cm in size.

### 2.2. Tolerance Test

A master standard of pure 2,4-D was made up in 90 % etanol equivalent to 1 gr/lt. Test solutions were prepared by appropriate dilution of master standard with filtered sea water. An initial range-finding experiment was set up to assess the possible toxicity of the 2,4-D to *Porites sp.* and *Galaxea sp.* Dilutions of the 2,4-D were prepared to give final concentrations of 2,4-D of 1000, 100, 10, 1, and 0.1 ppm.

Five small colonies (2x2 cm) of *Porites sp* and *Galaxea sp* specimens were exposed to 10 l of each of the solutions for 48 h. All test solutions and controls were aerated througout the experimental period. Based on the results obtained in the initial–finding experimental, a second series of exposures at different range concentrations were conducted. Coral mortality was investigated visually on percentage bleaching and quantification of loss of zooxanthellae which was attempted using a chlorophyll extraction procedure.

Variance components were estimated with the SPS procedure Nested Design, with 2,4-D concentrations nested within coral species. Two replicates were made for each concentration, and the median lethal concentrations were determined through probit analysis.

## III. RESULTS

### 3.1. 2,4-D Tolerance Test

Result of range-finding tolerance of *Porites sp* and *Galaxea sp.* to 2,4-D concentrations was 10 to 100 ppm as lower and upper limit. The condition of the test corals after 48 h exposure is given in Table 1.

Table 1. Results of range to 2,4-D after 48 h-finding tolerance of *Porites sp* and *Galaxea sp*

| 2,4-D Concentration (ppm) | Condition of coral genera | |
|---|---|---|
| | *Porites sp* | *Galaxea sp* |
| 0 | normal | normal |
| 0.1 | normal | normal |
| 1.0 | Mucus, slightly turbid | Polyps withdrawn |
| 10 | Stressed, water very turbid | Mucus, water turbid |
| 100 | Dead , loss of tissues | Dead, loss of tissues |
| 1000 | Dead; bleached | Dead; bleached |

### 3.2. 48 hour LC50 assay

Based on the results obtained in Table 1 above, a second series of exposure of corals in concentrations of 13.89, 19.30, 26.83, 37.27, 51.79, 71.97 and 100 ppm 2,4-D were conducted. The corals were inspected regularly every 15', 30', 1h, 2h, 4h, 8h, 16h, 24h, 48h and their condition recorded. The experimental data and the estimates of median lethal concentration ($LC_{50}$) values on *Porites sp* and *Galaxea sp* are shown in Table 2 and 3.

Table 2. The experimental data and the estimates LC50 values on *Porites sp.* for 48 h.

| 2,4-D (ppm) | Individual mortality/hour | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 15' | | 30' | | 1 | | 2 | | 4 | | 8 | | 16 | | 24 | | 48 | |
| | I | II | I | II | I | II | I | II | I | II | I | II | I | II | I | II | I | II |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13.89 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 19.30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 2 |
| 26.83 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 2 | 3 | 4 |
| 37.27 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 2 | 3 | 4 | 4 |
| 51.79 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 3 | 3 | 5 | 4 | 5 | 5 |
| 71.97 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 2 | 3 | 4 | 4 | 5 | 4 | 5 | 5 | 5 | 5 |
| 100 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 2 | 3 | 4 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| LC-50 (ppm) | | | | | | | 115.20 | | 71.98 | | 56.93 | | 46.51 | | 30.89 | | 23.20 | |

Table 3. The experimental data and the estimates LC50 values on *Galaxea sp*. for 48 h.

| 2,4-D (ppm) | Individual mortality/hour | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 15' | | 30' | | 1 | | 2 | | 4 | | 8 | | 16 | | 24 | | 48 | |
| | I | II | I | II | I | II | I | II | I | II | I | II | I | II | I | II | I | II |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13.89 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19.30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 2 | 3 | 4 | 4 | 4 |
| 26.83 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 3 | 2 | 3 | 3 | 4 | 4 | 5 |
| 37.27 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 3 | 3 | 4 | 4 | 5 | 4 | 4 | 5 | 5 |
| 51.79 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 2 | 3 | 4 | 4 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 71.97 | 0 | 0 | 0 | 0 | 1 | 0 | 4 | 3 | 4 | 4 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| 100 | 0 | 0 | 0 | 0 | 1 | 1 | 4 | 4 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| LC-50 (ppm) | | | | | | | 57.91 | | 33.75 | | 30.00 | | 23.15 | | 13.63 | | 10.26 | |

### 3.3. Loss of Zooxanthellae

The experimental results of loss of zooxanthellae as quantitative estimate of the to toxic response of *Porites sp*. and *Galaxea sp*. to dissolved 2,4-D are shown in Table 4. Statistically, there were no significant differences between *Porites sp* and *Galaxea sp* on quantity of zooxanthellae. However, 2,4-D treatments influenced significantly on the loss of zooxanthellae.

Table 4. Mean of zooxanthellae ($10^6$) on the effects of 2,4-D treatmens (ppm) on *Porites sp* and *Galaxea sp* after 48 h

| 2,4-D concentrations (ppm) | Coral genera | | | |
|---|---|---|---|---|
| | *Porites sp.* | | *Galaxea sp.* | |
| 0.00 | 3.599 | a | 3.155 | a |
| 13.89 | 3.677 | a | 2.389 | a |
| 19.30 | 2.806 | ab | 1.094 | b |
| 26.83 | 2.436 | b | 0.872 | b |
| 37.27 | 1.143 | c | 0.627 | b |
| 51.79 | 0.585 | c | 0.535 | b |
| 71.97 | 0.547 | c | 0.527 | b |
| 100.00 | 0.494 | c | 0.489 | b |

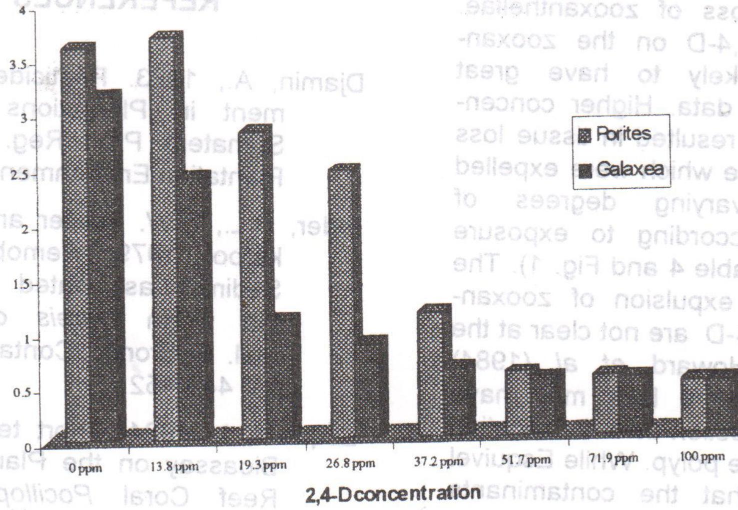


Figure 1. The effects of 2,4-D treatments on the loss of zooxanthellae ($10^6$)

## IV. DISCUSSION

Laboratory tolerance test (Table 1) demonstrated stressed effects on corals both *Porites sp* and *Galaxea sp*. at a concentration of 10 ppm 2,4-D. Coral tissue sloughing and death occurred after exposure to the 2,4-D at a concentration 100 ppm. This result was unexpected because previous studies in Hawaii have shown that herbicide can have a deleterious effect on corals, at relatively low concentrations (0.02 ppm) and for brief exposures, the coral mortality that occurred in Panama and elsewhere in the eastern Pacific (Glynn, 1983). Since this laboratory exposure was of short duration, not exceeding 48 h, it is possible that longer exposure at lower concentrations would also cause morbidity and death in corals.

The 48 h LC50 of 23.20 ppm for *Porites sp* and 10.26 ppm for *Galaxea sp* were obtained (Table 2 and 3). No similar data are avaiable for other experiments with which it may be compared. These 2,4-D concentrations are approximately 100 to 200 times greater than that affected at field specimens in Panama and Hawaii. Visually, there was a gradation of bleaching proportional to the concentration of 2,4-D. The higher the concentration of 2,4-D, the greater degree of bleaching. Most corals were dead after 48 h exposed on high concentrations. This trend supported by the loss of zooxanthellae where an analysis of variance indicated highly significant differences among treatments.

There were no significant difference between *Porites sp* and *Galaxea sp* in toxic response to 2,4-D by estimating the loss of zooxanthellae. The effect of 2,4-D on the zooxanthellae seem likely to have great variability in the data. Higher concentrations of 2,4-D resulted in tissue loss and zooxanthellae which were expelled demonstrated varying degrees of pigment loss according to exposure concentration (Table 4 and Fig. 1). The mechanisms of expulsion of zooxanthellae due to 2,4-D are not clear at the present time. Howard *et al* (1984) stated that pigment loss may have occurred after ejection as a detoxication mechanism of the polyp. While Esquivel (1984) noted that the contaminants penetrate the tissue where they act intracellularly on metabolic processes or they could exert their effect by absorbing onto the surface membrane of the organism and interfere with such vital processes as respiratory exchange or osmoregulation. It might be reasoned that these mechanisms also could be possible explanation for the effect of 2,4-D on coral mortality.

## V. CONCLUSION

This study has established that 2,4-D herbicides can have a harmful effect on corals. Since the high level of 2,4-D concentrations(10-100 ppm) were obtained in this study, the result of $LC_{50}$ experiment (acute lethal) must be viewed with concern. Therefore, for further research it is necessary to use lower concentrations of 2,4-D over a prolonged period of time.

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