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Presence and Toxicity of 2,4-D Herbicide in Coral *Galaxea fascicularis* of Java Coast, Indonesia

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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-dichlorophenoxyacetat (2,4-D), has been widely used as a herbicide and has become a substantial environmental pollutant. Contamination of 2,4-D was assessed in stressed or dead coral tissues of *Galaxea fascicularis* from the coastal waters of Java Sea. Controlled tolerance experiment testing 2,4-D was performed on this coral. The effects of 2,4-D on coral mortality were investigated. Comparison of the residue levels in coral tissues showed that the highest presence of 2,4-D concentrations was detected in a sample collected from Jepara coastal waters. While small amounts of a contaminant 2,4-D can still lead to detectable in West Java and East Java coastal waters. Contamination of 2,4-D was not found in coral samples collected from Karimunjawa islands. The toxicity of 2,4-D on corals showed that short duration (48 h) laboratory test demonstrated dramatic effects on sloughing and death of coral. The $LC_{50\text{-}96}$ was determined to be 18.82 mg L^{-1} 2,4-D. These results demonstrated the possibility that 2,4-D herbicide could act and lead to coral mortality in the Java coastal waters.

Key words: Dichlorophenoxyacetic acid, *Galaxea fascicularis*., Java Coast, median lethal concentration (LC_{50})

INTRODUCTION

Indonesia is the world's largest archipelagic country with approximately 17,508 islands containing valuable coastal and marine resources such as coral reefs. About 85.707 km^2 or 14 percent of total corals in the world extending all the way in the Indonesian sea (Tomascik *et al*., 1997). The Java Sea is Indonesia's second largest and most polluted among Indonesia’s seas. It is 162,662 square nautical miles and is bounded by Sumatra to the west, Borneo to the north and Java to the south (Fig. 1). Java Island, with 2,885 km long shoreline, has a dominantly humid tropical climate with an annual mean

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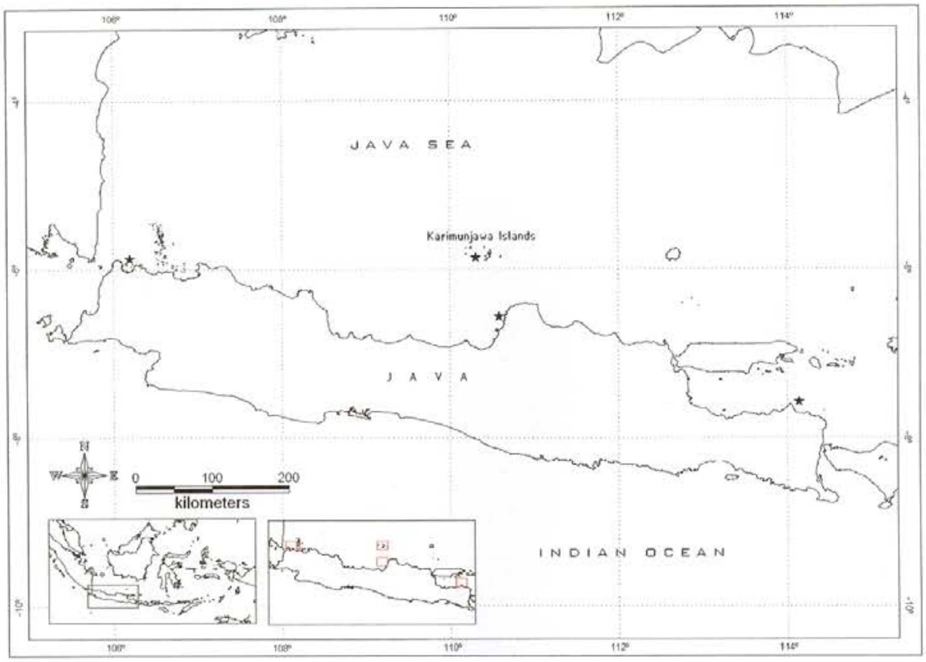

Fig. 1: Sampling sites of Java Island, Indonesia

precipitation slightly in excess of 2,000 mm (Anonymous, 2006). The reefs near Java coastal areas are under serious stress which subject to intense agricultural activities (brackishwater and paddy crop). Moreover, many urban, industrial and agricultural developments in this coastal areas have introduced new organic pollutants. Most of this compounds are recalcitrant to biodegradation and their entry into the sea, might be, poses many challenges to the existing coral reefs.

The use of herbicides in Indonesia began in the 1960's when the government launched plantation rehabilitation programme. One chlorinated compounds, 2,4-Dichlorophenoxyacetate (2,4-D), has been widely used as herbicide represent the largest crop protection category for control of weeds, brush and other unwanted vegetation. It is covering 25% of the total pesticide sales in Indonesia (Budiman, 2006). The Indonesian Pesticide Committee (1999) listed 190 approved herbicide whose main ingredients are phenoxy acid. Java island is highly productive farming region in Indonesia. Four of the principal crops in north of Java coast were paddy, corn, tobacco and red onion which herbicide applied regularly. These crops need more frequent spraying and higher doses during rainy season. Since this toxic chemical is used each year in immense quantity and has become a substantial environmental pollutant, effective handling of its production wastes and the contaminated environment is required. This compound does not persist for long in the environment since it is susceptible to microbial degradation (Evan *et al*., 1971; Sandmann *et al*., 1988; Sinton *et al*., 1986; Bhat *et al*., 1994; Fulthrope *et al*., 1996). However, adverse conditions such as low soil moisture (Wadson *et al*., 2006), salinity (Bondarenko *et al*., 2004), low pH (Wang *et al*., 1994; Thompson *et al*., 1984) and low temperature (Nesbitt and Watson, 1980; Thompson *et al*., 1984) are known to promote its longevity. Numerous cases of 2,4-D herbicide residue have been detected in river water (Wang *et al*., 1994), wastewater (Hill *et al*., 1986), golf courses (Scott *et al*., 1998), artificial ponds (Birmingham and Colman, 1985), Pasific Ocean (Young, 2006) and in the pesticide treated soil (Grover *et al*., 1985). Very few of information available on pesticide effects to coral reefs that have been the subject of scientific study. Glynn *et al* (1984) reported that herbicides can have a deleterious effect on corals, at relatively low concentrations and for short term. In this study, we performed the detection of 2,4-D herbicide residues in the tissues of stressed and dead corals from different sampling sites with specific geographic regions and controlled toxicity test of 2,4-D herbicide on coral in Java coast. The coral *G. fascicularis* was selected for test species because it is present in the most plentiful coral that underwent bleaching in Jepara, Central Java Province. We hypothesize that the coral bleaching in several groups of reef *G. fascicularis* was promoted by the high intensity agriculture activities along seashore and therefore, bleaching of the coral reefs may actually be the 2,4-D herbicide toxicity.

MATERIALS AND METHODS

Sample Collection

Coral *G. fascicularis* is found on open reef bottoms and form morphologically similar colonies of individual, upright tubes (Veron, 1985). Sampling points were located along the North Coast of Java Sea, Indonesia flowing from the west to the east of Java, namely Cilegon, West Java Province (industrial coastal waters), Jepara, Central Java Province (high intensity agricultural activities), Situbondo, East Java Province (high density coastal settlement) and Karimunjawa Islands (pristine areas). Figure 1 shows the sampling sites. Specimens of the hard corals *G. fascicularis* were collected randomly by scuba diving on June, 2005 at depths of 2 to 3 m, broken away with chisel and hammer and placed in plastic bag submerged in sea water. Upon collection coral fragments were put into sterile plastic bags (Whirl-Pak, Nasco, USA) and immediately brought to our laboratory with dry-ice. In laboratory, corals were dried in room temperature, ground and sieved. Fraction of particles less than 2 mm size was used for chemical analysis.

The reef-building coral *G. fascicularis* served as the test species in toxicity experiment were collected from the reef flat and placed in aquarium at the Marine Station. The corals were maintained in running sea water for 48 h acclimation prior to use in toxicity test. All corals used in this experiment were obtained from the same colony.

Sample Analysis

Coral tissue extraction was treated using the methods previously reported by Glynn *et al*. (1984) and Greenberg *et al*. (1985) with slightly modification. Coral samples were broken into small pieces, grinded in ceramic mortar and transferred into pre-weighed 250 mL round-bottom flasks. Coral and flask were weighed and 15 mL ethyl ether was added. The tissue was shaked gently for 10 h. Extract were collected in 250 mL erlenmeyer flask containing 15 mL distilled water, 2 mL KOH solution and a small boiling stone. Then, the ether was evaporated in a steam bath for 60 min and solution was transferred to a 125 mL separatory funnel, extract twice with 20 mL diethyl ether and discard the ether layers. The aqueous layer was acidified with 2 mL cold 25% H_2SO_4 and extracted with 20 mL and then 10 mL of ethyl ether. The ether extracts were combined and dried over 1 g of acidified anhydrous sodium sulfate. Then the extracts were transferred to evaporator tube and concentrated in water bath after adding 0.5 mL benzene. The concentrated extracts were removed from the water bath and allowed to cool before adding 0.5 mL of 14% boron trifluoride-methanol esterification reagent. After concentrated in water bath, 4.5 mL of 5% Na_2SO_4 were added and shaked vigorously. The benzene layer was pipetted and placed on the florisil pipet column until all the benzene was removed. More benzene was added and the procedure was repeated until 5 mL of volume was obtained. The samples were then analyzed by gas chromatograph Model Hitachi 163 FPD (Flame Photometer Detector) and nitrogen high pure (HP) was used as the carrier gas. A 2 m glass column (3 mm ID) packed with 3% Silikon OV1 on 80-100 mesh Supelcon was used. Gasflow at 30 mL min^{-1}, column temperature at 160-230°C, detector temperature at 290°C and the injector temperature at 290°C were maintained.

2,4-D Toxicity Assay

DMA-6, a commercial herbicide, marketed by Pacific Chemical Indonesia Co, Ltd., was obtained from a local traditional market in Central Java. The manufacturer's ingredients was given as: Dimethylamine salt of 2,4-dichlorophenoxyacetic acid 865 g L^{-1}. An initial range-finding experiment was set up to assess the possible toxicity of the 2,4-D. Dilutions of the 2,4-D were prepared with filtered sea water to give final concentrations of 2,4-D of 100, 10, 1 and 0.1 mg L^{-1}. Five small colonies (4×4 cm) of *G. fascicularis* were placed in 10 L of each of the solutions for 48 h. All test solutions and control were aerated throughout the experimental period. Coral mortality was investigated visually on percentage bleaching.

96 h LC_{50}

Based on the results obtained in the range-finding experiment of 2,4-D tolerance, groups of ten small *G. fascicularis* colonies were exposed to 2,4-D concentrations in sea water of 6.25, 12.5, 25, 50 and 100 mg L^{-1} 2,4-D were conducted. The corals were inspected regularly and their condition recorded. All test solutions and control were also aerated throughout the experimental period. When polyp tissue could no longer be seen within the calices, the corals were considered dead.

RESULTS

2,4-D Herbicide used in Indonesia

There are more than 150 herbicides that were registered and permitted to be sold freely on the market today, (Indonesian Pesticide Commission, 1999). The most commonly used herbicides in Indonesia contains 2,4-D active compounds (Table 1).

Coral Tissue Analyses

2,4-D was detected in dead coral tissues in varying concentrations and frequencies of occurrence. None of the pesticide residues were detected in coral samples from Karimunjawa island waters (Table 2).

Table 1: Herbicides most commonly used in intensive agriculture in Indonesia

Trade name	Active compounds
Agroxone 4	Kalium MCPA
Ally 76	2,4-D Sodium
Rumpas	Phenoxaprop-p-ethyl
Fusilade	Fluazifop-p-butyl
Rumputox	2,4-D butyl esther
DMA-6	2,4-D dimethylamine
Hedonal	2,4-D dimethylamine
Indamin	2,4-D dimethylamine
Lindomin	2,4-D dimethylamine
Panadin	2,4-D dimethylamine
Rhodiamine	2,4-D dimethylamine
Shellamine	2,4-D dimethylamine
Weedrol	2,4-D dimethylamine
Glifomin	2,4-D dimethylamine
Ronstar	2,4-D iso oktil esther
Rilof	2,4-D IPE
Argold	2,4-D IBE
Rogue	2,4-D IBE
Satum	2,4-D IBE
Saber	2,4-D IBE
Tordon	2,4-D tri isopropanolamine
Bimastar	2,4-D isoprophylamine
Bumout	2,4-D isoprophylamine
Keris	2,4-D isoprophylamine

Table 2: Mean, range and percent frequency of occurrence of 2,4-D herbicide residue levels (μg kg^{-1}) in coral *G. fascicularis* from different sampling sites of Java Coast[a]

Parameters	Karimunjawa	Cilegon	Jepara	Situbondo
Mean±Sd[b]	nd	12.683±1.132	18.770±4.721	14.558±1.288
Minimum-Maximum	-	11.382-13.445	14.480-25.060	13.441-16.325
Positive (% frequency)[c]	0	60	100	80

[a]10 μg kg^{-1} = lowest working concentration (below which residues could not be quantified). [b]Mean was calculated for positive samples. [c]Total samples = 5. nd = non detected

Table 3: Results of range-finding toxicity of coral *G. fascicularis* to 2,4-D after 48 h

	Coral mortality (%)		
2,4-D concentration (mg L^{-1})	After 24 h	After 48 h	Coral conditions
Control	0	0	Normal
0.1	0	0	Normal, slightly turbid
1	0	0	Stressed, water turbid, partial bleached
10	0	20	Heavy turbid, dead, bleached
100	100	100	Dead, bleached

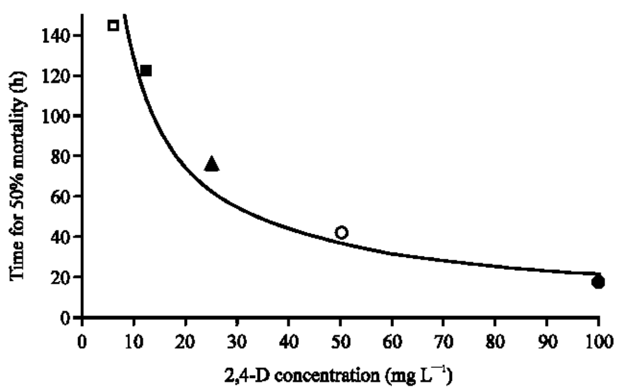

Fig. 2: Time for 50% mortality of *G. fascicularis* at various 2,4-D concentrations (□ = 6.25 mg L^{-1}; ■ = 12.5 mg L^{-1}; ▲ = 25 mg L^{-1}; ○ = 50 mg L^{-1} and ● = 100 mg L^{-1} 2,4-D)

2,4-D Toxicity Assay

In the range-finding experiment, coral *G. fascicularis* in 1.0 mg L^{-1} were producing mucus after 19 h of exposure. The process of sloughing off coral tissues showed to result in the release of polyps from calices. The coral condition after 48 h exposure is given in Table 3.

96 h LC_{50}

Fifty percent coral mortality figures were achieved for *G. fascicularis* colonies at 2,4-D concentrations of 6.25, 12.5, 25, 50 and 100 mg L^{-1} 2,4-D. Even after 168 h of exposure, no corals were dead in 6.25 mg L^{-1} 2,4-D. Figure 2 plots the time taken for 50% of colonies to die at each experimental 2,4-D concentration and results a 96 h LC_{50} of 18.82 mg L^{-1}.

DISCUSSION

One chlorinated compounds, 2,4-D, are among the most widely used pesticides. There are about 24 active compounds used as phenoxy herbicides (Table 1). Contamination of coastal waters by this compounds is of concern because of potential toxicity to marine organisms, especially to those at the coral ecosystem. Concentrations of 2,4-D herbicide were detected in 12 of the 20 coral *G. fascicularis* analyzed (Table 2). Phenoxy acid concentrations ≥ 15 μg kg^{-1} were detected in four coral specimens. Some corals sampled from Central Java coastal waters had relatively high concentrations of phenoxyacetic acids, whereas West Java and East Java coastal waters had lower amounts. No 2,4-D herbicide were detected in Karimunjawa Islands. Several bleached corals sampled in West Java and East Java waters were also 2,4-D herbicide free.

It is significant that relatively high 2,4-D concentrations, in the range 10-25 μg kg^{-1}, were found in coral tissues of Java Coast, especially in Jepara (Central Java) which an area of high intensity of agricultural activity. The presence of this chemical suggest that a large and constant source of this herbicide entering the reef region. Even this compound does not persist for long in the environment since it is susceptible to microbial degradation, however, the persistence of 2,4-D was much longer and further prolonged in seawater. Bondarenko *et al*. (2004) stated that microbial degradation contributed significantly to the dissipation of pesticides in freshwater, but was inhibited in seawater, leading to increased persistence. 2,4-D compounds was not found in a sample collected from Karimunjawa islands and, not surprisingly, this sampling site was assumed to be a pristine area.

The laboratory toxicity test demonstrated stressed effects on coral *G. fascicularis* at a concentration of 10 mg L^{-1} 2,4-D. Coral tissues sloughing and death happened after exposure to the DMA-6 herbicide at a concentration 100 mg L^{-1}. However, on the continuing investigation, the corals were also dead after observed in 131 h at a concentration 10 mg L^{-1} (data not shown). Based on this results, it is possible that longer exposure at lower concentrations would also cause coral mortality. Result of range-finding tolerance of *G. fascicularis* to 2,4-D concentrations was 10 to 100 mg L^{-1} as lower and upper limit.

The 96 h LC50 of 18.82 mg L^{-1} 2,4-D for *G. fascicularis* were obtained (Fig. 2). This value is still in the range of that reported for many other marine invertebrates (World Health Organization, 1989). No similar data are available for other experiments with which it may be compared. These 2,4-D concentrations are approximately 100 times greater than that of affected corals at field specimens in Java coastal waters.

The coral mortality in Java Coast could be attributed to 2,4-D herbicide factor. The presence of 2,4-D in stressed and dead coral in the fields and the demonstrated laboratory effects make the possibility that 2,4-D herbicide was addressed as one of the factors caused of coral mortality of Java Coast, Indonesia. Since the high level of 2,4-D concentrations(10-100 mg L^{-1}) were obtained in this study, the result of LC_{50} experiment (acute lethal) must be viewed with concern. Therefore, for further research is necessary to use lower concentrations of 2,4-D over a prolonged period of time.

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