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EARLY ASSESSMENT OF THE ORGANOCHLORINE PESTICIDES POLLUTION OF CORAL REEFS ECOSYSTEM ALONG JEPARA COASTAL WATERS, JAVA SEA

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Abstract

The contamination of seawater, sediments and coral tissues by organochlorine pesticides (OCPs) have presumed serious problems due to their persistence and toxicity. The research objectives were to determine the pesticide usage, occurrence, and toxicity of OCPs on Jepara coast. A questioner observation was used to inventory the types of pesticides used. Gas Chromatography equipped with Flame Photometric Detector (FID) detector was used to analyze selected pesticide concentrations of samples. The acute chronic exposure was used to investigate the toxicity effect of organochlorine on corals. The findings survey showed that farmers were most commonly using Gramoxone in the Jepara coastal regions, followed by Carbamate, Sevin, Converse, and Dupont. The farmers used no more OCPs.The pesticide analyses showed that the detected compounds in the coral samples were Lindane (γ-BHC) and Endrine with the range concentrations from <nd to 0.011 and from <nd to 0.15mg·g^{-1}, respectively. There were no organochlorine compounds determined in the water and sediment samples. The toxicity study showed that corals stressed and dead after 24h organochlorine exposure. The OCPs could kill corals in low concentration in the brief exposure. While small amounts of a contaminant organochlorines can still lead to detectable in Jepara coastal waters.

***Keywords**: Coral reefs; Pesticides; Organochlorine; Jepara; Java Sea*

Introduction

Jepara district, Central Java province, has a coastline of the 72km where the reefs distribute around Panjang island and close to the coast at water depths between 1 and 5m [1]. These reefs have experienced increasingly stressful conditions (Fig. 1) due to wood industries, high density of coastal settlements, high sedimentation and high intensity of agriculture activities [2]. The area along coastal waters are brackish waters and rice-paddy farming where pesticides largely use. Consequently, large-scale application of these toxic materials can contribute directly to the presence of those compounds in the coral reef ecosystems. Several studies have shown that pesticides were present and persisted in coastal areas and coral reef ecosystems [3].

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Fig. 1. Stressed corals on Jepara coastal waters

The use of organochlorine pesticides (OCPs) in Indonesia began when the government launched plantation rehabilitation programme in the 1970s [4]. To increase the quality and quantity of agricultural productivity, the use of pesticides is still crucial in paddy-rice fields [5-6]. However, their toxicity on living organisms and their presence in the environment pose serious issues [7-8]. Synthetic organochlorines such as DDT, Endrin, Lindane, were difficult to degrade biologically, chemically and physically [9]. Because OCPs have the toxic effects on living organisms, these pesticides banned [10]. Nevertheless, these pesticides are still used and sold illegally on the market [11]. Previous studies reported the occurrence of OCPs on sediments and living organisms from both marine and freshwater ecosystems. The results have continually shown the contamination of OCPs on these objects [12-13]. However, there were few studies addressed the impact of OCPs and their metabolites on corals. *P.W. Glynn et al.* [14] and *A. Sabdono et al.* [15] reported that the dichlorophenoxy acetate (2,4-D) were determined in coral tissues. Furthermore, *K.L. Markey et al*. [16] reported that organochlorines were detected on larval and adult corals. The objectives of the present study were to survey the pesticide usage in coastal regions, to determine the distribution of OCPs in coral tissues and to assess the toxicological implications of OCPs on corals.

Materials and Methods

Pesticide usage survey

A simple structured questionnaire observation was designed and used to survey pesticide usage in Awur Bay, Kartini beach, and Bandengan beach. No survey was done in Panjang island due to noninhabitant island. Data were collected by interview with peasants during farming activities in the fields. The interview was done directly on 47 respondents (10% of total 478 peasants in Jepara district).

OCP's Sample Analysis

Seawater, marine sediments and coral *P. lutea* colonies were collected from coastal waters of Awur Bay, Kartini beach, Panjang Island and Bandengan beach using Skin Diving (Fig. 2). Coral *P. lutea* was chosen as material study due to their persistence and abundance in this area. The coral specimens were broken away with chisel and hammer and placed in a plastic bag submerged in seawater. All samples were carried to Marine Station Laboratory of Diponegoro University and stored below 4 ^{o}C until analysis. The method used for the extraction of corals was treated using the methods previously reported by *P.W. Glynn et al.* [14]. Sediment sample extraction was conducted in the same manner as described by *S. Isworo et al.* [12]. After extractions, the samples were then analyzed by gas chromatograph Model Varian 450 GC in

Agrochemical Residual Laboratory, Environmental Agriculture Research Centre, Bogor, equipped with 1 nickel-63 electron capture detectors was employed, and nitrogen ultra high pure (UHP) was used as the carrier gas. A 2-meter glass column (3mm ID) packed with 1.5% OV110 and 2.5% OV210 on 80-100 mesh Supelcon was used. Gas flow at 30mL/min, column temperature at 160-250ºC, detector temperature at 270ºC and the inlet temperature of 270ºC were maintained.

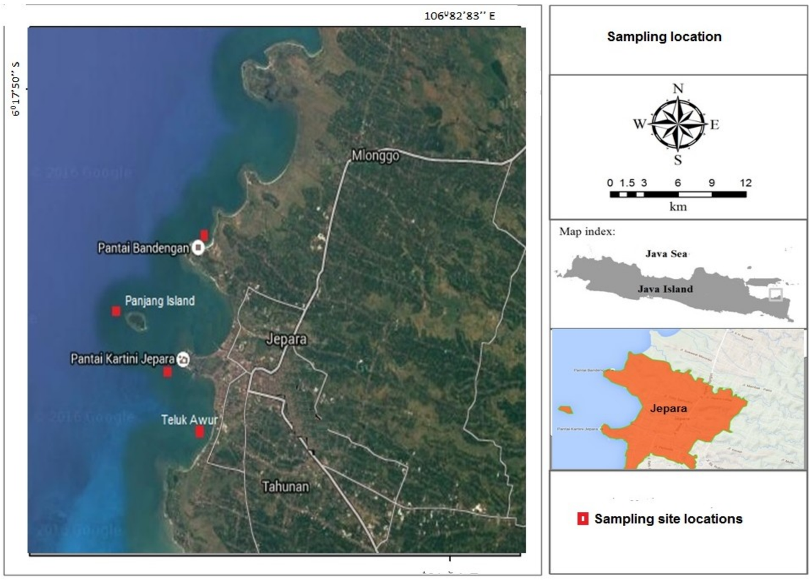

Fig. 2. Sampling site locations

Toxicity assay

Coral sampling

Coral *P. lutea* colonies were collected from 1- 2m depth on the flat reef at Awur Bay. Since then, all coral samples have been maintained and cultured at the Marine Station Laboratory of Diponegoro University in an isolated re-circulating seawater system before being subjected to toxicity experiment. Coral fragments were cut from a single colony of coral and allowed to recover from handling and observed for normal growth and performance. The healthy coral fragments only were used for experimentation. Coral fragments were then acclimated to the test conditions for one week before the start of the experiment.

Pesticide range-finding experiment

A commercial pesticide sold under the trade name of Gramoxone was purchased from a local market Jepara. The manufacturer's description of ingredients was given as paraquat dichlor. This type of pesticide was selected for toxicity studies because farmers widely use it. An initial range-finding experiment to assess the toxicity of paraquat dichlor at dilutions equivalent to 100.0, 10.0, 1.0 and 0.1mg·L^{-1} of the paraquat dichlor and control. Four small coral fragments (4x4cm) were used for each test concentration and control. Corals were placed in 10L of solution for 48h and continuously aerated throughout the experimental period. Coral mortality was investigated visually on bleaching. The qualitative characters such as color, polyp, bleached areas, mucous on coral fragments were also monitored throughout the bioassays. A colony was considered dead when it showed total bleaching and tissue loss accompanied by an increased turbidity in the water.

Estimation 96 hours LC_{50}

The 96 hour LC_{50} paraquat dichlor was conducted based on the results obtained in the range–finding experiment. The static of toxicity test was carried out without renewal of the test solution and for control (without pesticide, 0.0mg·L^{-1}) and five concentrations were used, each with three replicates.The ten small *P. lutea* fragments (4x4cm) exposed to pesticide concentrations in seawater of 15.85, 25.12, 39.81, 63.09 and 98.47mg·L^{-1} paraquat dichlor. The corals were regularly inspected after 15', 30', 1h, 2h, 4h, 8h, 16h, 24h, 48h, 72h and 96h and their condition recorded. All test solutions and control were also aerated throughout the experimental period. The physicochemical parameters (salinity, temperature, DO and pH) were also recorded during the test. However, these parameters were not presented in this paper.

Statistical analysis

The LC_{50} was calculated using the probit method [17]. The results were then graphed as regression curves with probit units against the logarithm base 10 of pesticide concentration using Excel 2016.

Results and Discussion

Pesticide Utilization

Geographically, the Jepara district location is on 6°35'32" (6°35'54) S and 110°40'15" (110°40'26'') E where is about 100km from the provincial city of Semarang. Farmers along the coastal area started to develop a vast region of brackish water ponds and paddy-rice fields since the 1970s [18]. The interviews were conducted in 3 coastal farm regions, Awur Bay (16 peasants), Kartini beach (9 peasants) and Bandengan beach (22 peasants). The survey showed that Jepara farmers used several different kinds of pesticides in their aquacultural practices for improving their farming yields. They commonly use more than 2 pesticide brands during growing and harvest seasons. Based on survey results, the active ingredients of pesticides commonly used by the farmers were Gramoxone, followed by Carbamate, Sevin, Converse, Dupont, Bulldog, Score and Regent (Fig. 3). It is not surprising that Gramoxone widely used in this region because Jepara small-holder farmers have been already trained and certified to use restricted/banned pesticides including paraquat dichlor. The farmers used no more organochlorine pesticide. Most of Jepara farmers use the Gramoxone pesticide in their farming due to labor-saving and cheap [19]. Moreover, *C. Wesseling et al.* [20] reported that paraquat herbicide was used widely in several developing countries such as Costa Rica, Trinidad, and Chili.

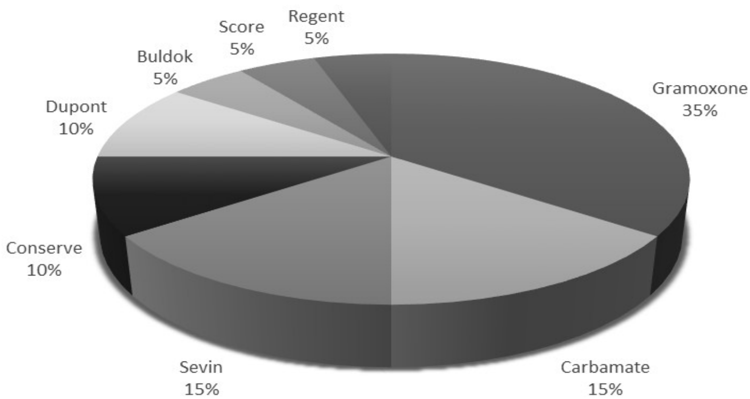

Fig. 3. Pesticide most commonly used in Jepara coastal waters

OCPs Detection on Corals

In the present study, a total of seven organochlorine compounds (Lindane, Aldrin, Heptachlor, Dieldrin, DDT, Endrin, and Endosulfan) were analyzed in seawater, sediments and coral tissues of four coastal regions (Table1). There was no organochlorine detected in water and sediment samples. Pesticides were detected in samples from south-west Jepara coastal waters, where two compounds OCPs were found. In Panjang island and Kartini beach, the presence of two organochlorines was detected, while in Awur Bay only one pesticide detected. No organochlorine was found on coral tissues from Bandengan. The important factors that determine the presence of organochlorines in these coastal regions might be the irrigation conditions as well as the amount of OCPs applications in the past. Pesticides applied to agricultural land in south-west regions may reach to coastal waters faster through of different rapid transport processes. The presences of two small rivers in this area could be the major causes of pesticide fate reached to coral ecosystems. Several previous studies on pesticide residues on the river, estuary, seawater, sediments and living organisms were reported [21-24]. In this study, small amounts of organochlorine detected on corals. *D. Wang et al*. [8] reported that HCH isomers, heptachlor, HCB, and DDT were detected in low concentrations on coral Porites. The persistent chlorinated pesticides such as DDT, BHC, Heptachlor, Aldrin, Dieldrin, and Endrin were prohibited for their application in agriculture and fish culture. However, DDT is still allowed to be used for domestic and health purposes in particular for fighting the malaria mosquito [25]. Dieldrin and Endrin were previously widely used, and in some places old stocks are still available, thus creating the possibility that these pesticides might still be found in the environment [26-27]. Then, it was not surprising that OCPs still detected in the environment.

There were no organochlorines detected in marine sediments in this study. The similar research reported by *S. Montenegro et al.* [28] showed that Aldrin, a-BHC, 0-BHC, 6-BHC, P-endosulfan and heptachlor epoxide were not detected in any of the samples analyzed. Singare [29] reported different results that organochlorine o,p′-, p,p′-DDT α,γ-BHC residues could be determined in low doses from marine sediments.

In our previous research [30], we reported that the OCPs residues detected in water and sediments of household wells of Java coastal urban areas. The OCPs residues might be degraded completely in sediments and released to water column affecting on terrestrial and marine organisms. *C. Chiu et al.* [31] reported that heptachlor in sediments was almost totally degraded in 12 days.

Table 1. Range of OCPs in sea water, sediment and coral samples

Location	Organochlorines (ppm):						
	Lindane	Aldrine	Heptachlor	Dieldrin	DDT	Endrin	Endosulfan
Awur Bay	nd-0.011	nd	nd	nd	nd	nd	nd
Panjang Island	nd-0.015	nd	nd	nd	nd	nd-0.011	nd
Kartini Beach	nd-0.011	nd	nd	nd	nd	nd-0.011	nd
Bandengan Beach	nd	nd	nd	nd	nd	nd	nd

Note: nd = no detection

Pesticide toxicity on corals

The result of range-finding organochlorine tolerance on corals was 10 to 100mg·L^{-1} as the lower and upper limit. The laboratory toxicity tests demonstrated deleterious effects on corals at paraquat dichlor concentrations. Coral tissue-sloughing and death occurred after exposure to the pesticide paraquat dichlor at a concentration of 100mg·L^{-1} for 24h (Table 2 and Fig. 4). When corals are stressed by pesticides, they expel the symbiotic algae (zooxanthellae) living in their tissues, causing them to turn completely white [32]. Coral bleaches are still alive. However, they are under more stress and are subject to mortality [33]. Based on the result of range finding, the acute toxicity exposure to find LC_{50-96} was conducted in the Marine Station Teluk Awur, Jepara. The results showed that the LC_{50-96} was 15.23mg·g^{-1}. These results were similar to those reported by Firman [34] who assayed the acute toxicity of Chlordane on coral *Porites divaricata* and *Montastraea faveolata*. The value of LC_{50-96} on *P. divaricata* and *M. faveolata* were 15.3mg·L^{-1} and 17.8mg·L^{-1}, respectively. *A. Sabdono et al.* [30] reported that the acute toxicity of 2,4-D on coral *Galaxea fascicularis* was 10.26mg·L^{-1}. The value of LC_{50-96} was various that influenced by the type of pesticides and coral species.

Table 2. Toxicity effect of Gramoxone on corals after 24 h exposure

Organochlorine(ppm)	Coral conditions
0	normal
0,1	turbid, draw tentacle into coralite
1,0	heavy turbid
10	partial bleached
100	bleached, sloughing off, dead

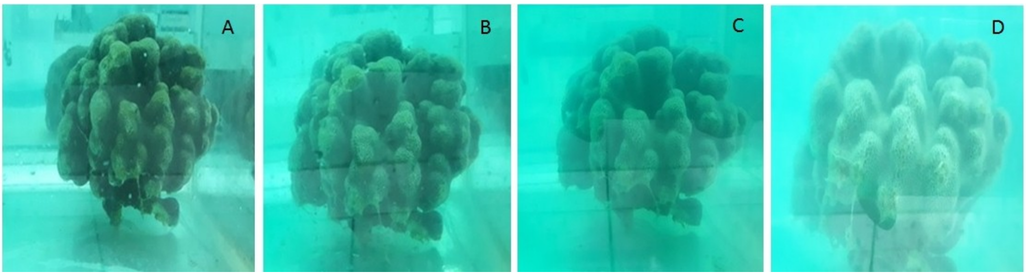

Fig. 4. Toxicity effect of Gramoxone on corals (A - 6h; B - 12h; C - 16h; D - 24h exposure)

Pesticides have been known be toxic to aquatic invertebrates, plants, and fish. Ecotoxicological studies of pesticide to fish have shown that these organisms were stress and mortality [35]. *J. Gao and J. Chi* [36] reported that algae were affected on morphologically and physiologically when exposed to the pesticide. Also, *J. Guo et al.* [37] stated that bacterial growth was inhibited in the presence of pesticide. It has been documented that the use of pesticides in brackish water, paddy field, plantation, and household can be toxic to aquatic organisms in various concentrations [38]. *V. Tonero and G. Hanke* [39] and *R.A. Relyea* [40] stated that marine organisms are negatively affected by the presence of active ingredients in pesticides, such as Roundup at concentrations ranging from 108 to 161mg·L^{-1}, respectively. The toxicity level may be more or less severe depending on the organism tested. In this study, the Gramoxone gave LC_{50} values of 15.23mg·L^{-1} against coral *Porites sp.* after 96h exposure (Fig. 5).

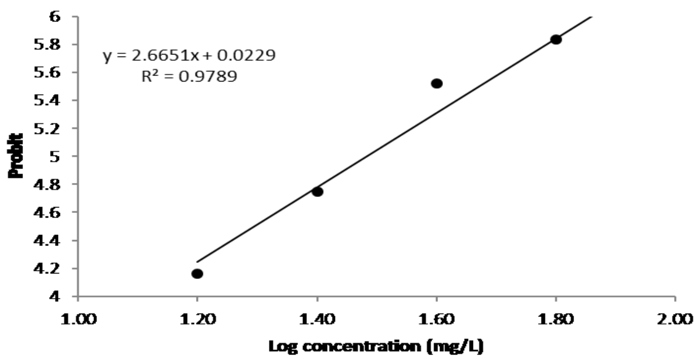

Fig. 5. Probit for the percent mortality of Porites sp. exposed to various concentrations of Gramoxone for 96 h under laboratory conditions

Several concentrations have been reported in the previous study for corals, revealing a range of sensitivities to different coral species and pesticides. *K.L. Markey et al.* [16] reported that the insecticides (chlorpyrifos, endosulfan, carbamate, and permethrin) caused polyp retraction and bleaching on coral *Acropora millepora* after 96h exposure to a concentration of 10μg·L^{-1}. However, this coral species was not affected by profenofos. *A. Negri et al.* [41] showed that coral **P. damicornis *were bleaching*** at 10μg·L^{-1} diuron after 96h exposure. While *R.J. Jones et al.* [42] reported that diuron was more toxic than that of atrazine on coral four species of coral *Acropora formosa, Montipora digitata, Porites cylindrica* and *Seriatopora hystrix*.

Conclusion

Jepara farmers used several different kinds of pesticides in their aquacultural practices for improving their farming yields. The findings survey showed that paraquat dichlor are most commonly being used. No more organochlorine pesticide was used by the farmers. Small amounts of contaminant organochlorines can still be detected in corals. The presence of OCPs in the coral tissue might be related to organochlorine use in the past and present illegally. These results suggest that the concentrations of the tested organochlorines found in corals were below the minimum levels and may not pose risks to corals. However, since the OCPs could kill corals in low concentration in brief exposures, further monitoring of this compound is recommended.

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