The contamination of filter feeder mussel *Perna viridis* Linnaeus, 1758 (Bivalvia: Mytilidae) by organophosphate pesticide at Brebes marine waters Central Java, Indonesia

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Abstract. In the last five years, organophosphate pesticides have been introduced in red onions fields in Brebes. This will broadly impact to the ecosystem, including the filter feeder marine organisms such as green mussel *P. viridis*. This article presents the occurrence level of organophosphate pesticides residues in marine waters and green mussel which has collected on Brebes marine areas. Some selected organophosphate pesticides compounds (Chlorpyrifos, Profenofos, Diazinon, Fenitrothion, Malation and Methidathion) of contaminant have been determined. The samples were analyzed by using gas chromatography and followed by using the method of Standard Method Examination. The results showed that average of six residues (Chlorpyrifos, Profenofos, Diazinon, Fenitrothion, Malation and Methidathion) in marine water were undetected (<0.0004 ppm). The merely concentration of PPOs detected was Chlorpyrifos 0.31 µg/L detected in green mussel. The concentration of organophosphate pesticides in these areas might contribute by the usage of organophosphate pesticide from red onion fields.

1. Introduction

Organophosphate pesticides (OPP) are a group of highly toxic chemicals that are widely used in agriculture including paddies and red onion [1]. In fact, these type of pesticides is very effective to control various types of insects [2]. However, the use of these pesticides cause many problems throughout the world due to the effects of contamination carried by rivers flow through into the marine system [3]. This is the major reason why the OPP residues have been found in animal and fish products [4]. Moreover, the pesticides have polluted the overall ecosystem by its toxic substances [5]. OPP residues have considered to reach the marine environment, especially in river flow areas around Brebes. Since the application of paddies and red onion being massive, the contamination become stronger.

During the last decades OPPs become more popular compared to organochlorine pesticides. Organochlorine pesticides are persistent and damaging the environment [6,7]. On the other hand, OPPs break down more rapidly, have a milder impact and are safer and less persistent. These most popular pesticides and their usage is still growing, mainly because of the low cost, reliability, wide spectrum of applications, multi-pest control capability and lack of pest resistance [8,9,10]. The widespread application of the OPPs has been suspected as a potential risk to human health [11]. Basically the OPPs residues on marine aquatic environmental such as fresh water and marine waters will give seriously affected on environment and organism such as bivalves. Some organophosphate pesticide such as...
Chlorpyrifos, Fenitrothion and Profenofos has contaminate bivalves (Anadara inaequivalvis, P. viridis and Atrina pectinata) in Surabaya and Semarang [12].

P. viridis is a filter feeder bivalve commonly found in Java seas. It is reported that this invasive species was found in Brebes coastal areas. At present P. viridis were consumed as a food source and their cultivation is evaluated to control and manage the ecosystem. Based on facts above, the present study OPPs determined the OPPs in soft tissues from specimens of P. viridis collected in the coastal waters of the Brebes. Secondly, to assess the contamination status in this ecosystem. So, therefore, the aim of the research was to investigate the contamination of Organophosphate pesticide residue on P. viridis on Brebes marine waters

2. Methodology

2.1 Samples collection
Samples were collected on Brebes coastal waters. Water and green mussel samples were collected in bottles and polyethylene plastic bags from different areas of Brebes coastal waters. Then, plastic bags were labelled properly and sealed tightly. All the samples were brought to the laboratory to analyze the Organophosphate pesticide.

2.2 Organophosphate extraction
The methods of mussels tissue extraction was conducted to the protocol for determination of organophosphate pesticides published by the DFG (1982). The mussels were thawed, shucked and excess water was drained off and discarded. Pooled tissue (whole mussels and organs) of 2 to 10 mussels was ground with anhydrous sodium sulphate (6 times of mussel wet weight and were then homogenized with a mortar with a mortar until homogeneous. The homogenate was extracted under reflux three times for 10 min with 50-ml petroleum benzene. The petroleum benzene extracts were evaporated in vacuo to dryness and the residue. No further clean-up steps were necessary [13]. Recoveries of 55 6 6% and 86 6 6% (n 5 3) were found for thionet and disulfoton, respectively. From the subacute demeton-S-methyl experiment, seven water samples (10 ml) were taken at different timepoints within the first 3 d, and once daily on days 11 to 13. These samples were extracted three times with 10 ml of petroleum benzene. Petroleum benzene extracts were evaporated in vacuo to dryness. The residue was taken up in 1-ml n-hexane. Recovery was 53 6 1% (n 5 2).

2.3 Sample analysis
The analysis of the tissue green mussels and water samples was carried out with a Carlo Erba HRGC gas Chromatograph (GC) equipped with a split/ splitless injector and a nitrogen phosphorus detector (NPD). Separation was performed on 20 m 3 0.30 mm glass capillary column coated with 0.15 µm OV-31-OH (polysiloxane containing 83% methyl and 17% 3-cyanopropyl). The carrier gas was hydrogen at a pressure of 40 kPa (linear velocity 0.5 ms21). The temperature of the detector was 280°C. One microliter of the sample dissolved in n-hexane was injected at a split ratio of 1:10. The temperature was held at 200°C (isothermal analysis). For quantification, reference compounds dissolved in n-hexane were employed. The NPD signals were recorded and integrated with a Spectra-Physics SP 4290 integrator. Concentrations of the respective organophosphates were determined by integrating the area of the organophosphate specific peak and using a standard curve. The detection limit was at 0.5 ng.

3. Results and Discussion

Brebes coastal waters is rich in marine product such as fishes, bivalves, molluscs and crustacean. On the other hand, Brebes coastal land have been cultivated with massive red onion and paddies field well as brackish-waters fish pounds. The wastes and disposal of agriculture and fisheries will be discarded and finally flow to marine waters and accumulate in marine organisms. Unfortunately, after organochlorine pesticide banded, many farmers turn to organophosphate pesticide. The concentration of organophosphate pesticides in green mussels from Brebes were analysed and shown in Table 1 and Figure 1.
Generally, result of these research indicate the concentration of OPPs in green mussel and Brebes marine waters was low detected. The residues concentration of Chlorpyrifos was 0.31 µg/L. The concentration of Profenofos, Diazinon, Fenitrothion, Malation were low detected, respectively. Figure 1 show that only chlorpyrifos was found on mussel *P. viridis* and low detected on marine waters. This is proven, that green mussel absorb and accumulate organophosphate pesticides from the marine waters.

Green mussels are known as an efficient absorbance and accumulate anthropogenic contaminants from their surroundings. Compare to fish and other vertebrates, mussels have a limited biotransformation capacity of pollutants. Even though the concentration of pesticide in environment was very limited, this pesticide is still found in green mussel’s tissue (Chlorpyrifos 0.31 µg/L). A filter feeder mussel *P viridis* collected from Demak marine waters has contaminated Chlorpyrifos (0.221 µg/L). Different from other area, samples collected from Surabaya was detected. Overall, the sediments and marine waters in those area were detected [12]. Reported that OPPs contamination on *P viridis* surrounding Semarang and Demak were Fenitrothion 0.413 µg/L and Chlorpyrifos 0.22 µg/L. OPPs was rapidly degrade in the water column and sediment compare to persistent pesticide, OCPs [14]. It was indicated that existence of organophosphate pesticides in environment and organisms are few. Mussels are being a models in pollutant bioconcentration/ bioaccumulation. Bioconcentration is the process in which chemical substances are absorbed by receptor organisms solely through uptake over respiratory and dermal surfaces, i.e. exposure via diet is not included. Bioaccumulation similar to bioconcentration but includes chemical exposure and uptake from the die. Although pesticides enable to control of the quantity and quality of farm products and food, and help to limit diseases in humans transmitted by insects and rodents, they are regarded to be the most mobility and long term effects on living organisms [15].

Table 1. The concentration of organophosphate pesticides residues in *P viridis* and marine waters

| Samples       | Chlorpyrifos | Profenofos | Diazinon | Fenitrothion | Malation | Methidathion |
|---------------|--------------|------------|----------|--------------|----------|--------------|
| P viridis     | 0.31         | < LoD      | < LoD    | < LoD        | < LoD    | < LoD        |
| Marine waters | < LoD        | < LoD      | < LoD    | < LoD        | < LoD    | < LoD        |

LoD = Low Detected

Figure 1. The concentration of Chlorpyrifos organophosphate pesticides residues in *P. viridis* and marine waters
Until now, there is not any data available in Indonesia informing the contamination of organophosphate pesticides on marine organism especially on green mussel. So, therefore, this research is very important due to the information on the status of organophosphate pesticides residues on marine environmental such as marine water and green mussel. This research indicates that organophosphate pesticides (Chlorpyrifos) on filter feeder mussel in Brebes areas have been detected.

4. Conclusion

The organophosphate pesticides of Chlorpyrifos has contaminated filter feeder mussel P. viridis in Brebes marine waters. The other OPPs (Profenofos, Diazinon, Fenitrothion, Malation and Methidathion) has low detected both in mussel and marine waters. The concentration of chlorpyrifos in P. viridis was 0.31 µg/L.

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