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The Preliminary Investigation of Organophosphate Pesticide Residues on Green Mussel *Perna viridis* (bivalvia: *Mytilidae*, *linnaeus*, 1758) at Demak Coastal Waters Central Java Indonesia

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The preliminary investigation of organophosphate pesticide residues on green mussel *Perna viridis* (bivalvia: *Mytilidae*, *linnaeus*, 1758) at Demak coastal waters central Java Indonesia

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Abstract. The organophosphate pesticides pollution in the Demak coastal waters and their impact on green mussel were estimated. The paper presents the occurrence level of organophosphate pesticides residues in marine waters and green mussel which were collected from marine coastal areas. Six selected organophosphate pesticides compounds (Chlorpyrifos, Profenofos, Diazinon, Fenitrothion, Malation and Methidathion) of contaminant have been determined in the marine water and green mussel of Demak coastal waters. The samples were then analyzed by using gas chromatography and followed by using the method of Standard Method Examination. The result showed that the average concentration of organophosphate pesticides Chlorpyrifos, Profenofos, Diazinon, Fenitrothion, Malation and Methidathion in green mussel were 0.22 μ g/L, below detected, below detected, 0.413 μ g/L, below detected, below detected, respectively. But all of organophosphate pesticides residues in marine waters were below detected. The highest concentration of organophosphate pesticide in green mussel showed on Fenitrothion (0.413 μ g/L) and than Chlorpyrifos (0.22 μ g/L). The concentration of organophosphate pesticides in these areas might contribute four paddy and red onion agriculture.

1. Introduction

Organophosphate (OP) pesticides are a group of highly toxic agricultural chemicals that are widely used to control a wide range of insect pests [1]. The organophosphate and carbamate pesticides methyl-parathion and carbaryl have a common action mechanism: they inhibit acetylcholinesterase enzyme by blocking the transmission of nerve impulses [2]. In agricultural areas worldwide, there is an increasing concern about watershed contamination due to the widespread use of pesticides [3]. The worldwide growth of the aquaculture industry has increased concerns about the effects of aquaculture on the marine environment [4]. The blue mussel, *Mytilus edulis*, is widespread in littoral and shallow temperate waters, forming large beds in both open waters and brackish estuaries where it may be vulnerable to the effects of aquaculture runoff [5]. Its wide distribution, sessile filter-feeding lifestyle, low rates of metabolic transformation and ability to accumulate pollutants have led to its use as a sentinel species in many monitoring programmes and research protocols [4].

Organophosphorus pesticides (OPPs) have been extensively used throughout the world for crop protection and orchard treatment due to their high efficiency of pest control and low cost [6] [7]. These so called new generation insecticides are gradually replacing organochlorine pesticides (OCPs), because they are readily degradable in the environment and have brought significant economic benefits during the last five decades [8] [9] [10]. However, it has been reported that OPPs have a moderate persistence (for weeks) and can accumulate in the environment for a long time due to their frequent application in intensively cultivated areas [11] [12]. Qiu et al [13], recently found that OPPs were less accumulative and persistent than OCPs in roots, and their accumulation and persistence in leaves and stems were similar. Generally, OPPs are used for various vegetables, grain crops, and fruits to improve quality and yield. The residues of OPPs in the environment can be toxic to humans, microorganisms, and other organisms [14] [15] [16]. For instance, many kinds of OPPs, especially parathion, phorate, and dimethoate, may cause health effects, including neurological damage,



endocrine disruption, and genotoxic effects [17] [18] [19]. Recent studies reported that organophosphorus insecticide intoxication is a worldwide health problem with around three million poisonings every year and contributes to the largest proportion to human acute toxicity [20] [21]. Therefore, there is an increasing human health concern regarding OPP residues in the environment and food [22] [23]. Agricultural activities are the major sources of OPP pollution in the environment [20]. Continuous and excessive use of OPPs has led to the pollution of water and sediment in different regions of the world [20] [12]. The contamination of grains, vegetables, and fruits with OPPs was also studied [24] [25] [26]. However, the knowledge of the pollution status of OPPs in marine bivalve is limited. Marine waters could receive OPPs from both direct inputs and atmospheric deposition [27]. Marine environmental may also be polluted by agricultural irrigation waters with OPPs [28]. On the contrary, OPPs in soil could be discharged to surface water and leached to groundwater and finally damped in the marine [29]. Emission from soil is also an important source for air and marine pollution [30]. Many studies suggested that the residues of absorbed pesticides in marine waters could enter marine organism via food chain or direct exposure. Therefore, the study on organophosphate pesticides in marine organism which is the green mussel from Demak coastal areas is very important to be conducted to discover how far the contamination level of those materials in the green mussel.

2. Research Methods

Samples collection: Sampling points were located on coastal waters of Demak. Figure 1 shows the sampling sites, the samples of green mussels were collected in polyethylene plastic bags bottles from different areas along the coastal. The samples were collected in 3 kg plastic bags. Then, plastic bags were labelled properly and sealed tightly. All the samples were brought to the laboratory for the Organophosphate pesticide to be analysed.

Organophosphate Extraction: The tissue of green mussels extraction followed the protocol for determination of organophosphate pesticides [31]. The mussels were thawed, shucked and the mussel water was drained off and discarded. Pooled tissue (whole mussels or organs) of 2 to 10 mussels was ground with six times its wet weight of anhydrous sodium sulphate 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 was taken up in *n*-hexane (1 ml or 100 μ l). No further clean-up steps were necessary as shown [31]. Recoveries of 55.6% and 86.6% ($n = 3$) were found for thiometon 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 53.61% ($n = 2$).

Sample analysis: The analysis of the tissue and water samples was carried out with a Carlo Erba HRGC gas chromatograph (GC) equipped with a split/ split less injector and a nitrogen phosphorus detector (NPD). Separation was performed on 20 m \times 0.30 mm glass capillary column coated with 0.15 μ m 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 ms⁻¹). The temperature of the injector and 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

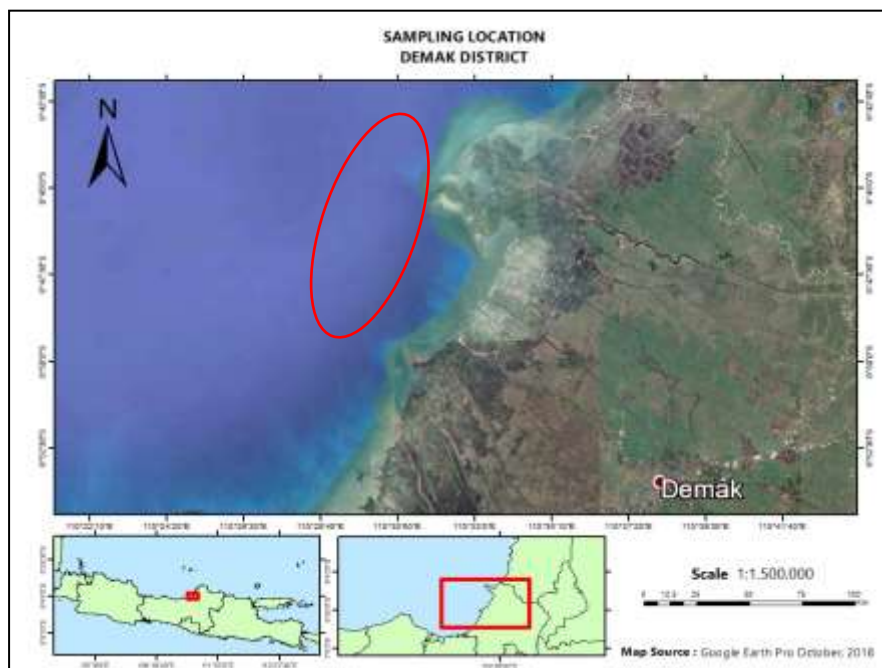


Figure 1. The sampling areas at the Demak Coastal waters

3. Results and Discussion

Demak coastal waters is very productive of marine resources such as bivalves, crustacean and fishes. On the other side Demak coastal land have used to agriculture, fish pound and human settlement. The wastes of their activity finally on marine environment and accumulate on marine organisms. Unfortunately, after banded using organochlorine pesticide, many farmers change to Organophosphate pesticide as like Chlorpyrifos, Profenofos, Diazinon, Fenitrothion, Malation and Methidathion. The concentration of Organophosphate pesticides in green mussels from the coastal area of Demak was analysed. The concentration of Organophosphate pesticides is shown in Table 1 and Figure 2.

Table 1. The concentration of organophosphate pesticides residues in green mussel

	Concentration of organophosphate (μ g/L)					
	Chlorpyrifos	Profenofos	Diazinon	Fenitrothion	Malathion	Methidathion
Means	0.22	nd	nd	0.413	nd	nd
SD	0.03			0.034		

nd = not detected

The recent research result by taking the mussels samples in Demak marine waters was found the concentrations of Chlorpyrifos, Profenofos, Diazinon, Fenitrothion, Malation and Methidathion residues concentration from the highest to the lowest as followed Fenitrothion (0.413μ g/L), Chlorpyrifos (0.22μ g/L), while Profenofos, Diazinon, Malation and Methidathion showed the number of not detected.

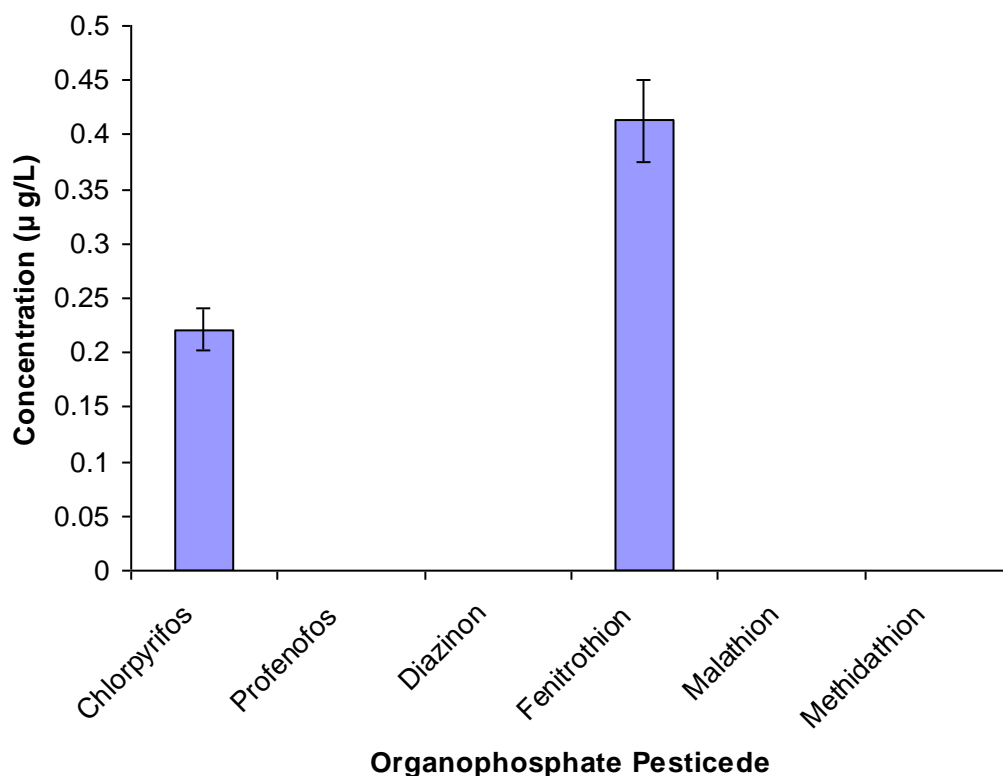


Figure 2. Mean \pm SD of occurrence of organophosphate pesticides residue levels (μ g/L) of green mussel *P. viridis* from Demak coastal waters

Marine mussels like as green mussel are known to efficiently absorb and accumulate anthropogenic contaminants from their surroundings and they have a limited biotransformation capacity for pollutants in comparison to for example fish and other vertebrates. Even the environment of green mussel life was very limit of concentration of pesticide but it is still found in tissue of green mussel (Fenitrothion 0.413 μ g/L and Chlorpyrifos 0.22 μ g/L). Mussels are therefore suitable as animal 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; whereas bioaccumulation is the same (as bioconcentration) but includes also chemical exposure and uptake from the [32]. Although pesticides enable 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 as among the most dangerous environmental contaminants because of their tendency to bioaccumulate, and their mobility and long term effects on living organisms [33]. Even in Indonesia no data was informed the contamination of organophosphate pesticides on marine organism. So, this research was very important to inform the organophosphate pesticides have been found in marine organism such as green mussel *P. Viridis*.

4. Conclusion

Some kinds of organophosphate pesticides have been contaminating green mussel *P. Viridis* in the Demak coastal waters. The OPPs including Chlorpyrifos, Profenofos, Diazinon, Fenitrothion, Malation and Methidathion has been contaminated the samples of green mussel. That was the high concentration of pesticide showed on Fenitrothion 0.413 μ g/L and Chlorpyrifos 0.22 μ g/L and, Profenofos, Diazinon, Malation and Methidathion was not detected

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