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# Toxicity of Drilling Waste and Its Impact on Gill Structure of Post Larvae of Tiger Prawn (*Penaeus monodon*)

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**Abstract:** The study to evaluate the toxicity of used drilling muds (drilling wastes) and their impact on gill structure of the post larvae of tiger prawn *Penaeus monodon* has been done. The results showed that the 96 h LC50 of used drilling muds ranged from 30740 and 78271 ppm SPP. All toxicity values considered to be above the standard of Indonesian government (LC50≤30000 ppm SPP). Histological observation showed that only haemocytic congestion in the gill lacunae of post larvae was recorded. The gill lacunae exposed to high concentration of drilling waste (62500 ppm SPP) were reduced or absent.

**Key words:** Drilling wastes . toxicity . histology . gills . *Penaeus monodon*

## INTRODUCTION

Drilling muds usually to be used during drilling oil and gas well which serve several important functions: cool and clean the bit, maintain pressure balance between the geological formation and the borehole, lubricate the bit, reduce friction in the borehole, seal permeable formations, stabilize the borehole, carry cuttings to the surface for disposal and etc [1]. Two types of muds that are normally used in drilling operation are water based muds (WBMs) and oil based muds (OBMs). WBMs are by far the most commonly used muds, both onshore and offshore. WBMs are widely used in shallow wells and often in shallower portions of deeper wells, but are not effective in deeper well. The uses of WBMs generate 7000 to 13000 bbl of waste per well. Depending on the depth and diameter of the well, about 1400 to 2800 bbl of that amount are drill cuttings [2]. WBMs use water as their base fluid and do not contain any oil. WBMs are very economical and easy to dispose of because they can be fully biodegraded and considered as very low toxicity. In many countries, both WBMs and cuttings are discharged on site to the ocean.

During the past 30 years, OBMs have been developed and refined to overcome the limitation of WBMs applications. OBMs have been the mud of choice for a range of special situations, including high temperatures, hydratable shales, high angle and extended-reach well, high density mud and drilling trough to salt. Wells drilled with OBMs normally produced lower waste volume than those drill with WBMs because very little slumping or caving in of the walls of the hole occurs. Also, the muds is reconditioned and reused rather than discharged at the end of the well. Only the drill cuttings will be disposed to the ocean. The average volume of OBM waste is estimated at 2000 to 8000 bbl per well [2]. The base fluids of OBM are normally either diesel or mineral oil, even though nowadays many other types of low toxicity oil are developed. Because they contain oil, OBMs waste cannot be discharged on site under the regulation of many countries [3].

The Indonesian Ministry of Mining and Energy has set a guideline for discharging of drilling wastes (used drilling muds). The government of Indonesian (GOI) guideline for acceptability of drilling wastes, both WBMs and OBMs must exhibit 96 h LC50≤30000 ppm SPP (Suspended Particulate Phase) to shrimp. LC50 is a standard test to determine the concentration of the substance which will prove lethal to 50% of a test population of the marine organism in 96 h [4].

The exploration of oil and gas reservoirs in the Java Sea has been increased during the last ten years. Many petroleum companies, both national and international have authorities to develop oil and gas fields in this region. Some of oil and gas fields are located near coastal zone where number of shrimp ponds found. Drilling waste from exploration activity can pose a significant impact to the post larvae of tiger prawn (*Penaeus monodon Fab.*) lived in this area through acute lesion of gills.

For most aquatic animals, the gills are major sites through which waterborne pollutants can enter the body and gills are often affected by such substances [5, 6]. Since drilling waste can create a significant impact to

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the post larvae *P. monodon*; the objectives of this study were to evaluate the toxicity of used drilling muds and to observe any significant histological changes in the gill structures of the post larvae of tiger prawn.

## MATERIALS AND METHODS

**Animals:** Post larvae (PL15) of tiger prawn (*Penaeus monodon*) were obtained from a shrimp hatchery located in Pasuruan, East Java, Indonesia. After transport to the laboratory post larvae were kept for 2 days before the experiments at a salinity of 30% 0 at 27±1°C, with a 12 h light and 12 h dark light cycle. They were fed with *Artemia* (brine shrimp) nauplii during preacclimatory period.

**Acute toxicity of drilling wastes:** Acute toxicity biassays were conducted with post larvae *P. monodon* during 96 h. The tests were conducted in 1000 ml plastic boxes.

Used drilling muds (drilling wastes) to be tested were collected from active field systems. Samples were stored in uncontaminated polyethylene containers and immediately shipped and continuously maintained at 0-4 ? C until the time of testing. We tested drilling wastes from five exploration wells namely UP-North, UP-West, Sidayu-2, Hayam Wuruk-1 and WHP-A (Fig. 1).

A subsample of mud was mixed with filtered test seawater in a volumetric flask mud-to-water ratio of 1 to 9. This mud-water slurry mixed with magnetic stirrers for 5 minutes. Then, allow the slurry to settle for 1 hour. At the end of the settling period, carefully decant the Suspended Particulate Phase (SPP) into an appropriate container. The decanted solution is defined to be 100% SPP (equal to 1000000 ppm SPP). Any other concentration of SPP refers to a percentage of SPP that is obtained by volumetrically mixing 100 percent SPP with seawater. SPP samples to be used in toxicity tests shall be mixed for 5 minutes and must not be preserved or stored. Test solutions were 0, 3900, 7800, 15600, 31250, 62500, 125000 and 250000 ppm SPP. Thirty animals per concentration were used, divided into three groups of ten animals per tank. Test media were aerated and kept at 27±1°C. During exposure, post larvae were fed *Artemia* nauplii ad libitum [7]. Regular observations were made and dead individuals were removed 3, 6, 12, 24, 48, 72 and 96 h after beginning of the tests. The criteria for death were total lack of movement, immobility of the heart and lack of response after repeated touches with a probe [8]. Median lethal concentrations (LC50) and 95% confidence intervals were calculated with a computer program based on the probit analysis [9]. LC50's were calculated at 96 h.

**Effect of drilling wastes on histological structure of gills:** Animals were exposed over a 96 h period in 0 (control), 15600, 31250 and 62500 ppm SPP of UP-North drilling wastes. In order to determine the effect of drilling wastes on gills, we collected gills from control and drilling waste-exposed post larvae. Three to four prawns were chosen randomly for each exposure

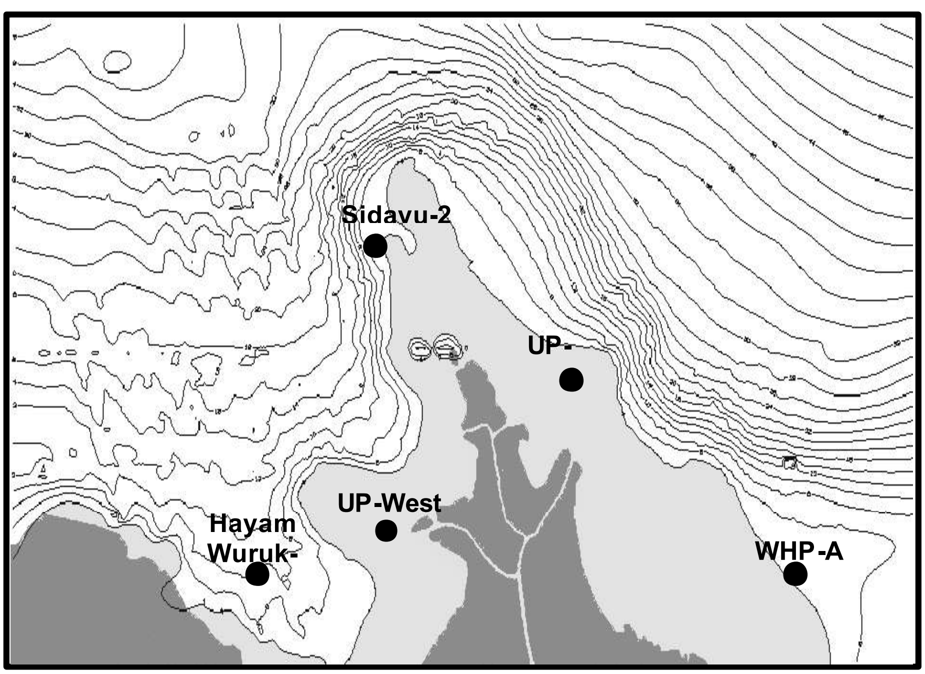


Fig. 1: Location of drilling platform

condition. For histological purposes, tissues were fixed in 10% neutral buffered formalin. Paraffin embedded tissues were cut at 6 µm thick and stained with hematoxylin-eosin. Samples were selected to histological study on the basis of showing the structural damage. The effect of drilling wastes on the histological structure of gill filament was assessed by using Mann-Whitney test. The results were considered to be statistically significant if $P<0.05$ [10].

## RESULTS AND DISCUSSION

Mortality of *P. monodon* in different concentration of drilling wastes is presented in Fig. 2. Variability in percentage mortality among treatments (drilling wastes) was high. In general, mortality increased with increasing drilling waste concentrations. Drilling wastes from UP North, UP West and Hayam Wuruk-1 caused 100% mortality of post larvae in concentration of 125000 ppm SPP. By contrast, 100% mortality of drilling wastes from Sidayu-2 and WHP-A was reached at concentration of 250000 ppm SPP.

The 96 h LC50 of drilling wastes for post larvae *P. monodon* ranged from 30740 and 78271 ppm SPP (Fig. 3). The lowest LC50 recorded for drilling waste of UP-North and the highest LC50 noted for Sidayu-2. All toxicity values can be considered above to the GOI limit for drilling waste (LC50 = 30000 ppm SPP). Only the LC50 value of UP North is close to GOI limit. According to the GOI standard, however, all used drilling muds can be discharged directly to the ocean. The results demonstrated also that drilling wastes from UP-North, UP-West and Hayam Wuruk-1 were more toxic than those from Sidayu-2 and WHP-A. Drilling muds are complex mixtures which contain many types of additives and chemicals. The barium in barite, a sparingly soluble mineral used to increase drilling fluid density, dominates the heavy metal content of wastes from drilling process. Other trace metals leached from the rocks are possible present in used drilling muds [3]. It's possible that drilling wastes from UP North, UP West and Hayam Wuruk-1 contain more toxic substances than those from Sidayu-2 and WHP-A.

Our results showed that the effects of used drilling muds from active platforms to post larvae *P. monodon* were varied. A comparison of LC50 data between post larvae *P. monodon* and other crustaceans indicates that the 96 h LC50 values for the eleven drilling muds tested to larvae of the grass shrimp *Palaemonetes intermedius* was also varied from 142 to >100000 ppm SPP [11]. The 96 h LC50 of water base drilling fluids to *Mysidopsis bahia* was equal to 27000 ppm SPP and to *Mysidopsis juniae* was 29100 ppm SPP [12]. The 96 h LC50 of water base drilling fluids commonly use in petroleum perforation and extraction in Campeche Sound of the Gulf of Mexico were about 475000 to 700000 ppm SPP [13]. Other research showed that survival of crab larvae *Callinectes sapidus* decreased as concentration of drilling fluids increased from 5000 ppm SPP to 50000 ppm SPP and no larvae reached the first crab stage in 100000 ppm SPP [14]. Exposure to 25000 and 250000 ppm SPP of used drilling fluids during 96 h reduced the growth and reproduction of *Daphnia magna* [15].

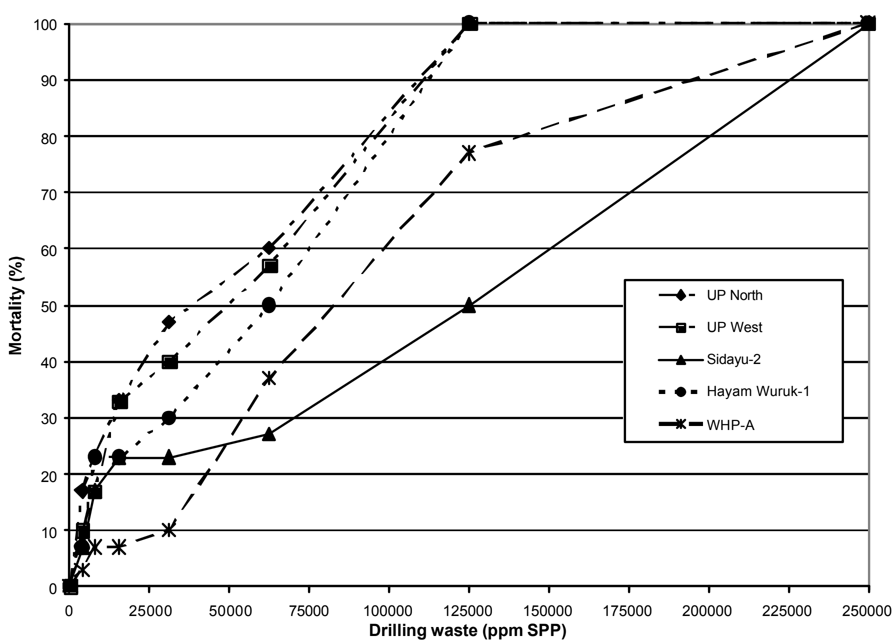


Fig. 2: Mortality (%) of post larvae *P. monodon* exposed to different concentrations of drilling wastes for 96 h

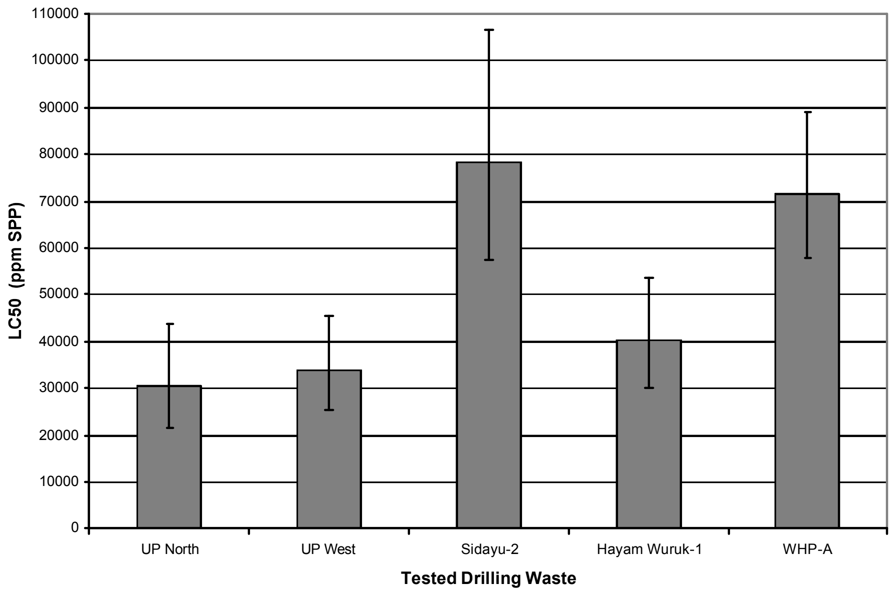


Fig. 3: The 96 h LC50 values of drilling waste with 95% confidence interval in post larvae (PL15) *Penaeus monodon*

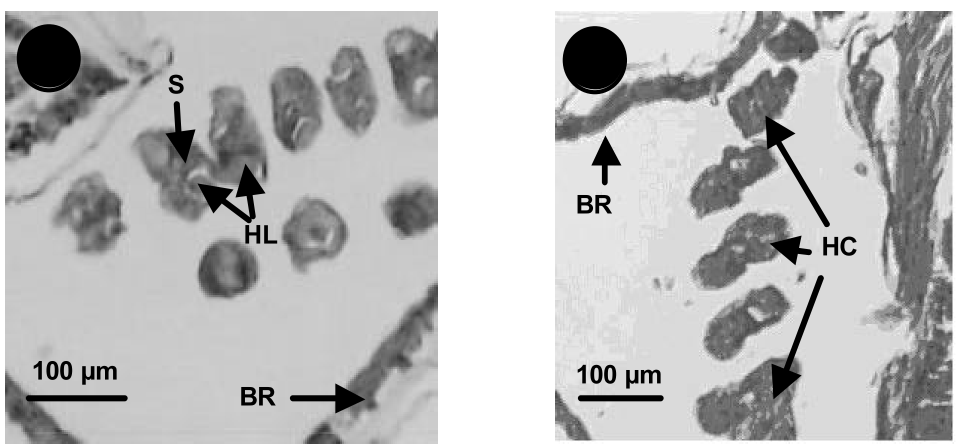


Fig. 4: Gill filaments of post larvae *Penaeus monodon*. A: cross-section of gill cavity of control PL15; B: cross-section of PL15 gill after 96 h exposure to 62500 ppm SPP of drilling waste. (BR: branchiostegite, HC: haemocytic congestions in the lacunae, HL: hemolymphatic lacuna, S: septum)

In control PL15 *P. monodon*, the gills are about 1.0-1.25 mm long. The morphology of gill filaments is similar to other species of penaeid shrimps [16]. The small hemolympathic lacunae located at the tip of filaments. The septum that divides the lacunae of the gill filament is present (Fig. 4A). The ultrastructural observations demonstrated that the filament of penaeid post larvae containing few nuclei and very few organelles was not differentiated [16].

In the histological study, only slight changes in gill filaments were observed after exposure to different concentration of drilling waste. Examination for organ damage showed only haemocytic congestion in the gill lacunae of post larvae exposed for 96 h to drilling wastes. In control animals, the haemocytic congestion was very low (8% ) and it was slightly higher in post larvae exposed to 15600 ppm SPP (24%). There was, however, no statistically significant difference between treated (15600 ppm SPP) and control post larvae. Compared to the controls, the haemocytic congestions increased significantly by 650 and 1125% in prawns exposed to 31250 and 62500 ppm SPP respectively (Table 1). In exposed gills, we observed that the lacunae which the hemolymph passes were reduced or absent (Fig. 4B).

Table 1: Structural changes in gills of post larvae *Penaeus monodon* after exposed to different concentration of drilling waste

| Treatment (ppm SPP of drilling waste) | Haemocytic congestion (%) |
|---|---|
| 0 (control) | 8 |
| 15600 | 24 |
| 31250 | 52* |
| 62500 | 90* |

Note: * = Significant difference with the controls (P<0.05); Number of filament observed for each condition: 100

The severity of lesions to gill filament was directly proportional to the concentration of drilling waste. In the gills, the haemocytic congestions observed can be considered responses to tissue damage caused by the pollutant. Haemocytic congestion can account for another possible role for gills during intoxication, namely a route of elimination for toxic substances, as explained by Lowe [17]. Haemocytic congestions gave rise to acute respiratory distress. This coupled with reduction in surface area of the respiratory barrier of gill filament and the inhibition of enzyme mitochondrial transport systems led to the inevitable death of aquatic animals [18].

On the basis of the present study, we concluded that the alterations of gill filaments seemed to be responsible for the mortality of post larvae *P. monodon*.

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