A PRELIMINARY STUDY ON THE RESPONSE OF AMPHIPOD *GRANDIDIERELLA* SP. TO CONTAMINATED SEDIMENT OF JAKARTA BAY

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ABSTRACT

A preliminary study on the response of amphipod exposed to contaminated sediments of Jakarta Bay was carried out in October 2009. The objective of the study was to determine the mortality rate of amphipod in response to exposure of contaminated sediments. Seven sediment samples were taken from the bay, *i.e*, reference site (St. A), and six-contaminated sites (St. B3, B5, C3, C5, D3, and D5). Amphipod (*Grandidierella* sp.) was collected from reference site. The study showed that average of mortality rate of amphipod ranged between 50.0 and 76.3% at contaminated sediments and 38.3% at reference sediments. There was significant difference on mortality rate of amphipod exposed to sediments of reference site and that of contaminated sites. The mortality of *Grandidierella* sp. was not strongly correlated with the concentration of Pb and Cu in contaminated sediments. The critical mortality factor of amphipod in this study might be due to variation in grain size of sediments and short period of acclimatization.

Keywords: Contaminated sediments, Amphipod, *Grandidierella* sp., Jakarta Bay

INTRODUCTION

The Jakarta bay bound by the capes of Tanjung Pasir to the west and Tanjung Karawang to the east is mainly influenced by land-based activities. With rapid development of the Jakarta Metropolitan Area (JMA), Jabodetabek (Jakarta-Bogor-Depok-Tangerang-Bekasi), in the last 20 years, the coastal waters has increasingly affected by both human and natural impacts, which include natural ecosystem transformation, non-sustainable practices on resource exploitation, and pollution. There are about 50 industries ranging from transportations, dockyards, and dairy products to recreation industries on the area. The bay is also exposed to high pollution load transported from up-land region by several rivers i.e., Angke, Bekasi, Cakung, Cidurian, Ciliwung, Cikarang, Cimancuri, Ciranjang, Cisadane, Citarum, Karawang Krukut and Sunter (Arifin, 2004).

Sediments can be used as an indicator of pollution due to its role as a sink of various contaminants. Once contaminants have been present in sediments, they will be potentially absorbed by benthic organisms/benthos that transfer the metals from sediments to food chain levels. Metal bioavailability to benthic organanisms depends on various factors, such as geochemical of sediments (Wang *et al*., 2002), animal behaviour, and exposure duration (Arifin and Bendell-Young, 2000).

Estuarine amphipods have been extensively used worldwide for an evaluation of contaminated sediment, due to their sensitivity to contaminants and changes in the benthic environment (Nipper and Roper, 1995). Amphipods are also important source of food for higher aquatic life, serving as diet to larger crustaceans and birds. Bioassays of these organisms were essential for the development of sediment quality guidelines (Canadian Council of Minister of the Environment, 1995; Long *et al*., 1995). Hence, the objective of this study is

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to determine how amphipod, *Grandidierella* sp., response to contaminated sediments of Jakarta Bay.

MATERIALS AND METHODS

Animal Collection and Maintenance

Grandidierella sp. was obtained from Muara Kebo estuary, Tangerang (Station A, Figure 1). The amphipod was identified as *Grandidierella* sp. first by Drs Indra Aswandy of RCO-LIPI, then Prof. Rahim A. of the University Kebangsaan Malaysia. The upper layer of sediment from the low intertidal zone was collected using a stainless-steel spoon.

Amphipods were transferred to a plastic container containing a 2- to 3-cm layer of sediment and seawater. Ice packs were used to prevent from over heating during transferring samples to the laboratorium. Subsequently, in the laboratory, amphipods were sieved out of the sediment and sorted by sizes. Animals were transferred to 30 L glass aquaria containing filtered seawater 5 μm pores size of the amphipod collection site. Amphipods were kept in the laboratory before tested.

Sediment Collection and Treatments

The sites were located in areas suspected to be highly contaminated, as well as corresponding to reference site. Tested sediments were collected on the same date as the amphipod collection at seven sites in Jakarta Bay in October 2009 (Figure1) using a 0.05 m^2 McIntyre grab.

Sediment from the organism collection site was used as control and referred as station A. Sites B3, C3, and D3, received wastes from Ancol estuary near a commercial recreation place and public harbour, whereas B5, C5, and D5 received domestic wastes from Sunter estuary. The upper 5 cm of sediments were scooped from the three grabs, composited and stored in 1 L bottle and kept inside coolers with ice until arrival at the laboratory. Both control and tested sediments were kept refrigerated at 4°C up to 8 week, prior to experiment running.

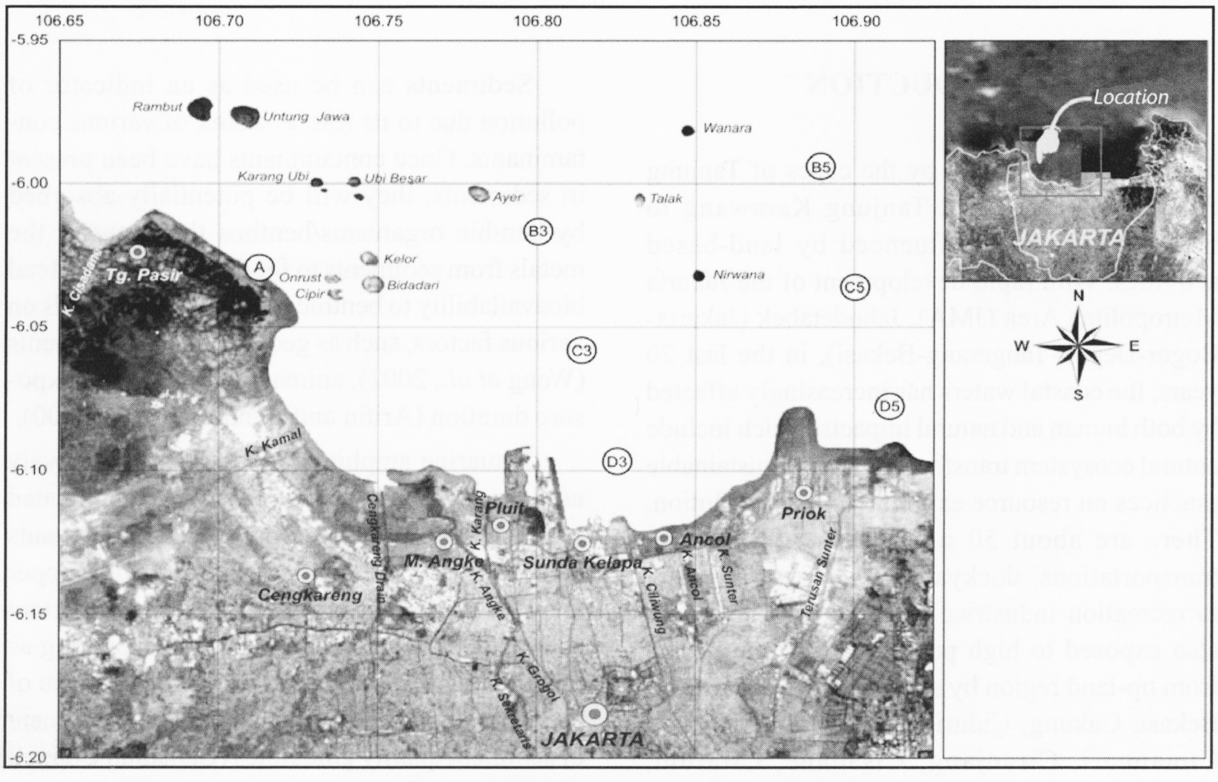

Figure 1. Sediment sampling sites in Jakarta Bay. There were seven stations which had been investigated for sediment contamination.

Analysis of Sediment Samples

Sediment analysis were subsampled for heavy metal Pb and Cu in reducible and aquaregia phases, total organic matter (TOM), and grain size. Heavy metal analysis was conducted using simultaneous method described by Bendell-Young *et al.*, (1992) in Thomas and Bendell-Young (1998). TOM of sediments was determined using by practicing Standard Method (1992). Grain size was analyzed following Wentworth (1922).

Reference toxicant

Before starting sediment experiments, the toxic effects of Cd on *Grandidierella* sp. were examined at several concentration levels with different orders of magnitude so called range finding test (Asean Canada Cooperative Programme on Marine Science-II (AC-CPMS II, 1995)).

The purpose of the range finding test was to determine the upper and lower limits of concentration to be applied in the definitive lethal toxicity test (AC-CPMS II, 1995). Five concentrations were tested in order to determine the concentration range in which the effects of cadmium appeared. The highest concentration completely caused mortality (or at least at 50%) and the lowest concentration resulted in no effect compared to the control (Gatidou and Thomaidis, 2007). In our study, the concentrations of range finding test varied from 0, 0.01, 0.1, 1, and 10 mg L^{-1} Cd. Result of the range finding test demonstrated that the lowest and highest concentration to cause lethality was 0.1 and 1 mg L^{-1} Cd, respectively.

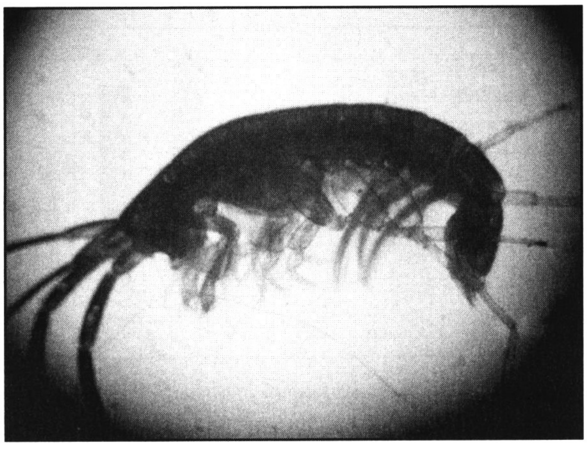

Figure 2. *Grandidierella* sp. as a sediment tested organims

Hence, the nominal concentrations were 0, 0.18, 0.32, 0.56, 1.0 and 1.8 mg L^{-1} Cd. All tests were performed at 96 hour of exposure within triplicate experiment.

Sediment Toxicity Test

The whole sediment toxicity test methodology followed the procedures described in American Society for Testing and Materials (ASTM, 2006). Field-collected organisms were exposed to the whole sediments after five days acclimation at laboratory conditions. One-litre glass beakers with lids were used as test chambers. Approximately two cm of sediment (175 ml sediment) and 775 ml of filtered seawater (30 ppt) were added to each beaker. Test sediments were not sieved, but macrofaunas were removed by visual inspection. Sediments were homogenized before added to the beakers. Filtered seawater was added carefully to avoid sediment resuspension.

The exposure chambers were prepared one day before amphipod addition and placed in constant temperature places. Mild aeration was added. Non-gravid amphipod of approximately the same size were randomly selected and then transferred to the test beakers. Four replicates per treatment and 20 animals per test beaker were used in the experiments. No food was supplied during the experiments.

Water quality (salinity, DO, and pH) was measured at daily basis. Dead amphipods were observed and removed daily, and the number of amphipods on the sediment surface was recorded. The number of alive amphipod observed on the sediments or in the water was used to calculate the mortality rates. At test termination, sediments were sieved through a 500-mm mesh screen and alive amphipods were counted. Test endpoints included amphipod mortality after 10 days during exposure. Missing amphipods were assumed as dead and being decomposed.

Mortality was the ecotoxicological endpoint considered in water-only 96 h acute bioassay and 10 days whole sediment test. Water and sediment toxicity test were always used a negative control (ASTM, 2006) for statistical comparison with test treatments. The amphipod mortality at contaminated sites were analyzed using one-way analysis of variance (ANOVA) to compare the amphipod mortality in each site to that in reference site (Gulley *et al.*, 1990).

RESULTS

Sediment Characteristics

Physical characteristic of sediments in research sites was presented in Table 1. Contaminated sites were dominated with silt ranging from 41.9% to 63.3%, while reference site was dominated with sand (46.0%). High total organic matter (TOM) at sites D5 and D3 (closer to shore) indicated richness in organic material.

Chemical characteristic of sediments was represented by reducible fraction of Pb and Cu in sediment was showed in Figure 3 and 4. Reducible fraction of Pb ranged from 0.43 (St. A) to 15.36 mg kg^{-1} (St. D5). The range of reducible Cu was from 0.28 (St. A) to 0.74 mg kg^{-1} (St. C5). The trend of both total Pb and Cu tended to decreased from near-shore to off-shore (St. D > St. C > St. B) which was similar to the trend of total metals (reducible + residual metal). Based on the Pb and Cu speciation in sediments, it was shown that in the reference site (St A), both Pb and Cu were in the form of residual which indicated potentially not biologically available to biota.

Sediment Toxicity Test

A water only 96-h acute bioassay using cadmium as reference toxic was conducted to assess the sensitivity of the test organism. Toxic effect of cadmium on mortality of amphipod was presented in Figure 5. Mean mortality of amphipod in negative control was 35%. Mortality in each concentration ranged from 35-100%. Since mortality in negative control experiment was higher than 10%, LC_{50}-96 h could not be calculated.

Table 1. Total organic matter (%) content and grain size distribution of sediments

Parameter	Station						
	A	B3	C3	D3	B5	C5	D5
TOM (%)	7.0	7,6	11,5	9,6	11,6	9.0	**14,5**
Grain size							
% pebbles	2,9	0.0	0.0	0.0	0.0	0.0	0.0
% granules	1,9	0.0	0.0	0.0	0.0	0.0	0.0
% sand	**46.0**	2,9	1,9	18,8	0,1	6,7	6,8
% silt	30,1	**53,9**	**63,3**	**41,9**	**53,9**	**53,4**	**57,7**
% clay	19,0	43,2	34,8	35,7	45,3	39,9	35,5

Note: Sands, silt, and clay were classified according to Wentworth (1922). Bold is the highest value.

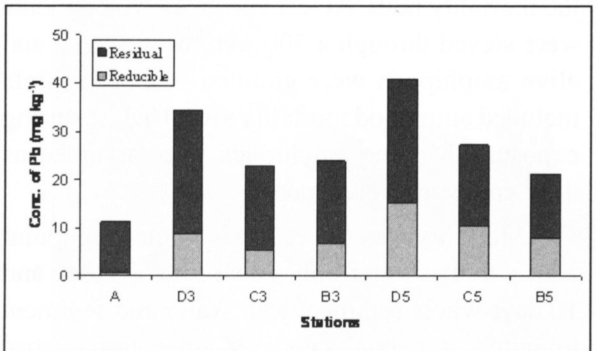

Figure 3. Concentration of Pb (*reducible fraction*) in mg kg^{-1} dry weight of sediment in October 2009. A is reference sediment, D = 5 km; C = 10 km; B= 15 km from shore.

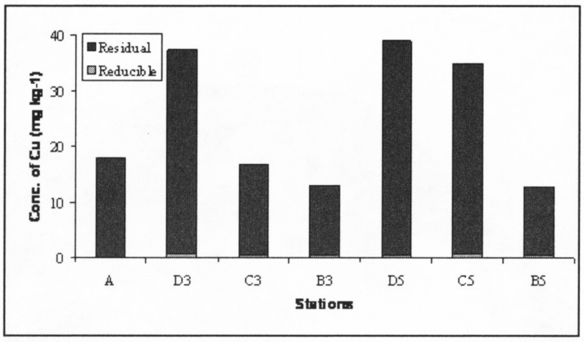

Figure 4. Concentration of Cu (*reducible fraction*) in mg kg^{-1} dry weight of sediment in October 2009. A is reference sediment, D = 5 km; C = 10 km; B= 15 km from shore.

An average of amphipod mortality in a static test with sediments ranged from 50% in D5 to 76.25% in B5. Highest mortality rate in contaminated sites was detected in St. B which was the farthest site from the shore, while the lower mortality was detected at nearest to shore (St. D). The lowest amphipod mortality was 38.6% in the reference site, St. A (Figure 6).

DISCUSSION

In order to determine how *Grandidierella* sp. responsed to contaminated sediments of Jakarta Bay, water only 96 h acute bioassay and 10 days whole sediment test were conducted. This was the first effort to investigate the *Grandidierella* sp. as the test organism for sediment bioassay in Indonesia. Because of high mortality in negative control experiment after 96 hour in both range finding test and definitive test, LC_{50} 96 h values could not be calculated. There were several reasons why the high mortality occurred *i.e.*, water quality of the media, health status of biota, and other technical factors. From the water quality of media, it seemed that there was no potential effect on amphipod, as our experiment was carried out under conditions as follows: dissolved oxygen (DO) from 7.08 to 8.60 mg L^{-1}, water temperature from 25.3 to 27.9°C, salinity 30 ppt, and pH from 8.01 to 8.48. Possible explanation for high mortality of *Grandidierella* sp. was because of a short period of acclimation (5 days period). Hence, to provide further study, we used available collected mortality data based on 48 hour period of exposure, the test exhibited that the LC_{50}- 48 h value was 0.64 mg L^{-1} Cd.

Based on ANOVA, 10 days whole sediment test showed only site B3, B5, and C5 having a significance difference mean percent mortality compared to the reference site, St. A($p = 0.05$). The mortality of *Grandidierella* sp. with an average of 38.75% in control sediment was relatively high compared to expected minimum average mortality of 10% under control conditions (ASTM, 2006). The site closed to the estuarine (5 km from coast; sites D3 and D5) had significantly lower mortality than that of further stations (15 km from coast; sites B3 and B5). Water quality in the 10 days whole sediment test chambers remained within acceptable levels throughout all tests, with salinity (32 ppt), pH (7.76-8.62), dissolved oxygen (4.2-6.13 mg $L^{-1)}$, and water temperature (25.3-31°C).

Concentration of Pb and Cu in sediment showed that the closer the site to estuarine, the higher the reducible concentration of Pb and Cd was. Suedel and Rodgers (1994) reported that the sensitivity of amphipods to natural sediment features in acute tests varies considerably among species and habitats. The marine amphipod *Rhepoxynius abronius* is most strongly affected by sediment particle size although no sediment property stood out as the best predictor of survival (DeWitt *et al.*, 1988). To see if there was a correlation between average mortality of amphipod and metal concentration, a simple linear regression analysis was carried out. The analysis showed that both reducible and total concentration of Pb and Cu did not have significant correlation to an average mortality of amphipod (Figure 7). It showed that the mortality rate of amphipod did not caused by Pb and Cu concentration. This

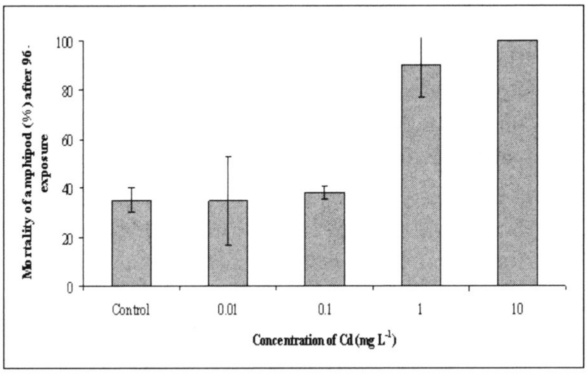

Figure 5. Mean Mortality of Amphipod *Grandidierella* sp. in Range Finding Test of cadmium (n = 3, bar = standard deviation)

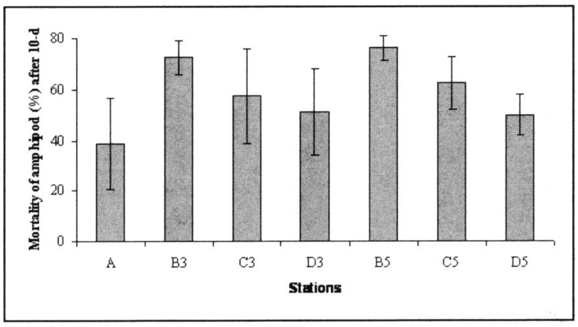

Figure 6. Mean Mortality of Amphipod, *Grandidierella* sp. in sediment toxicity Test (n = 4 replicates, bar = standard deviation)

might be due to difference of sediment features in their original habitat (the reference site) compared to the condition in each contaminated sites. All of sediment features in contaminated sites were dominated with silt whereas in reference site (St A) consisted of sand. In the present study, mortality rate of *Grandidierella* sp. seemed to be affected by grain size of sediment rather than Pb and Cu concentration.

Identification of the relationship between mortality and natural environmental variables were a priority in the development of further toxicological assay, and should be conducted before responses can be described to contaminant effects (Costa *et al*., 1998). Among the non-contaminant variables, sediment features, temperature, salinity, and organism density were suspected to be critical in amphipod, *Gammarus locusta* sediment toxicity testing (Costa *et al*., 1998). In our research, the difference type of grain size of sediment and period of acclimation were suspected as a critical factor in mortality of amphipod.

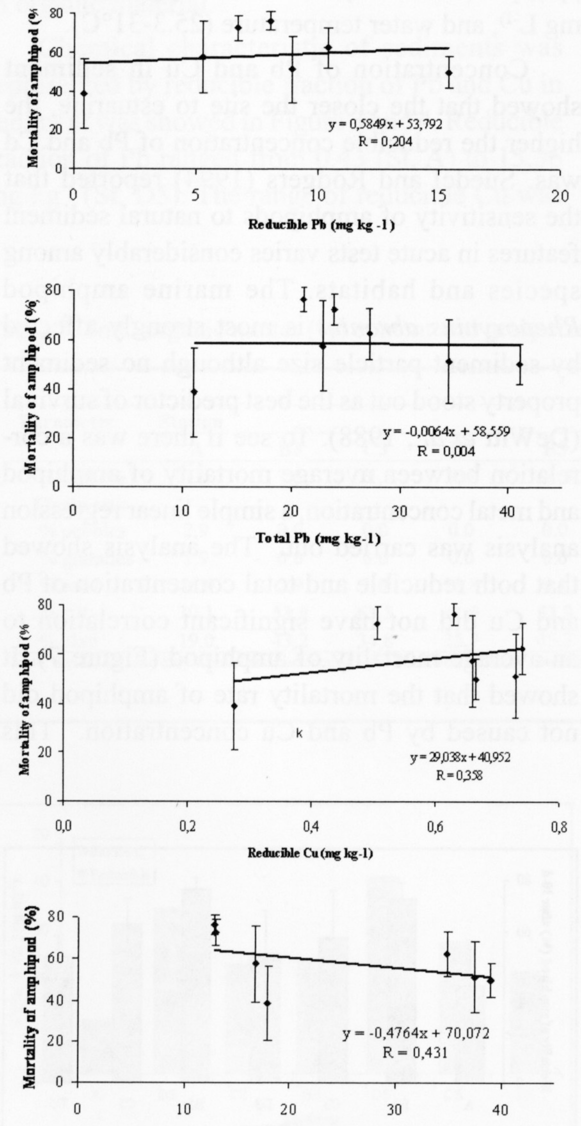

Figure 7. Linear regression of average mortality of *amphipod* and heavy metal (Pb and Cu) concentrations. Bar – standard deviation.

CONCLUSION

The research revealed that mortality of *Grandidierella* sp. In Jakarta Bay did not strongly correlated with concentration of Pb and Cu in contaminated sediments. The critical factor mortality of amphipod in this study might be due to variation on grain size of sediments and short period of acclimatization. Hence, we recommended further study on the area of acclimation period and behaviour of *Grandidierella* sp. under different environmental variables (sulphide, grain size, and food preferences).

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