ASSESSING CONTAMINATION LEVEL OF JAKARTA BAY NEARSHORE SEDIMENTS USING GREEN MUSSEL (*PERNA VIRIDIS*) LARVAE

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ABSTRACT

Accumulation of heavy metals in the bottom sediment could pose serious environmental issues to marine ecosystems and as well as human health. Jakarta Bay is located adjacent to the capital city of Indonesia where the bay is notorious for its pollution problems. It is the aim of this study to analyze the toxicity of sediments from thirty one locations across Jakarta Bay using bivalves (i.e. green mussel *Perna viridis*) as a bioindicator. Specifically, we analyzed sediment toxicity using bivalve larva bioassay procedures and counted the amount of abnormal (non D-shaped) larvae. Our study finds that larval abnormality is prevalent after the larvae were being exposed to sediments taken proximate to the mainland (i.e. estuaries and within 5 km from the shoreline of Jakarta). These sites with high larval abnormality are located downstream from the Cengkareng Drain, Kamal River, Grogol River, Cakung River, North Jakarta integrated industrial area as well as along sea transportation lines. Combined, our findings highlight significant influence of anthropogenic activities from the mainland Jakarta to marine ecosystems in Jakarta Bay.

Keywords: sediments, toxicity, green mussel, larval abnormality, Jakarta Bay

INTRODUCTION

Jakarta Bay, located offshore from Indonesia's capital city, is heavily impacted by human activities where the bay acts as a disposal area for sewage, industrial and agricultural waste waters from the Jakarta metropolitan area. These wastes are mostly unprocessed and directly discharged to and mixed with the receiving waters via riverine pathways into the bay (Palupi *et al.*, 1995; Napitupulu 2009; Cordova *et al.*, 2011). Subsequently, toxic materials such as heavy metals have been accumulating in Jakarta Bay sediments (Williams *et al.*, 2000; Hosono *et al.*, 2001; Takarina, 2010; Sindern *et al.*, 2016). Throughout times, Hosono *et al.* (2001) reported that the accumulation of anthropogenic heavy metals (Zn, Cu, Pb) in Jakarta Bay sediments began in the 1920s, increased greatly from the 1970s until the end of the 1990s when the heavy metal concentrations especially Pb and Zn have since been declining. However, the concentration of other anthropogenic heavy metals tends to increase (Takarina, 2010; Sindern *et al.*, 2016), highlighting the complexity of environmental pressures in Jakarta Bay.

Accumulated pollutants in Jakarta Bay sediments could be a threat to marine organisms because sediments in the coastal area play an important role in providing habitats and food supply for various aquatic and benthic organisms. Pollutants accumulated in the sediment have the potential to be absorbed by benthic organisms (EPA, 1999; Rees *et al.*, 1999; Soliman *et al.*, 2015). In turn, increased heavy metals in the bottom sediment could be harmful to marine organism (Birch, 2013; Rumisha *et al.*, 2016). Therefore, it is important to understand the influence of heavy metals accumulated in the

bottom sediment on marine biota.

The analysis of sediment bioassay could provide a better understanding on the status and impacts of polluted sediments on biota. For instance, the sediment quality triad (SQT) as an integrated approach involving analyses on chemicals, sediment bioassay and benthic community structure has been used to provide sediment quality assessment (Chapman, 2000). Indeed, this line of research is currently needed to be applied more widely for marine pollution studies in Indonesia (Arifin *et al.*, 2012). A number of organisms have been used for sediment bioassay works such as bivalve (Phelps and Warner, 1990; Fathallah *et al.*, 2013), insect (Castro *et al.*, 2003; De Lange *et al.*, 2005), oligochaete (Reynoldson *et al.*, 1991), copepod (Hack *et al.*, 2008) and amphipod (Borgmann and Munawar, 1989; Bat and Raffaelli, 1998; Hindarti *et al.*, 2015a, b).

Given the lack of data on sediments toxicity to marine biota in Indonesia, it is the aim of this study to examine sediment toxicity from Jakarta Bay sediments on bivalve (green mussel *P. viridis*) larvae. Although still rarely used, bivalve is a sensitive and reliable biota for bioassay research (e.g. Fathallah *et al.*, 2013) with a well-established testing procedure (ASTM, 2006). Previous works also have shown that *P. viridis* can be used as a bioindicator of organic and inorganic pollutants (Phillips 1985; Boonyatumanond *et al.*, 2002; Liu and Kueh, 2005; Verlecar *et al.*, 2006, 2007). In Jakarta Bay, green mussel (*Perna viridis*) has been long cultivated for their economic benefits and has spawning time throughout the year (Kastoro, 1988), making it a potential biota for sediment bioassay study in the bay.

MATERIALS AND METHODS

Sediment sampling and heavy metal determination

Thirty one sediment samples were collected from Jakarta Bay using a Smith-McIntyre grab mud sampler in July 2015 (Figure 1). The sampling locations represent areas with increasing distances from the shoreline, which are estuary/river mouth (code M), 5 km (code D), 10 km (code C), 15 km (code B), and 20 km (code A) -away sites from the shoreline. Triplicate sampling were performed at each site. In the field, the sediment samples from each site were homogenized to obtain a composite sediment sample, immediately stored in 1L HDPE (Nalgene®) bottles, kept in a cooled ice box, and transported to the laboratory where they were stored in the dark at a 4 ± 2˚C temperature prior to toxicity testing. We determined the concentration of heavy metals (Cu, Pb, Cd, Ni, Zn, Hg) in the sediment samples using a Flame Atomic Absorption Spectrophotometer (Varian SpectrAA 20) following the US EPA 3050b method (USEPA, 1996). A reference material PACS-2 was used to ensure the accuracy of the data. The typical recovery ranges between 95-100% and with the percent difference for the reference material is <5%. The concentration of heavy metal is expressed in mg of metals per kg of sediment (dry weight).

Toxicity testing

The bivalve larvae bioassay procedures follow the methods outlined in ASTM (2006) and His *et al.*, (1999). Glass beakers were used as test chambers where 18 g of sediment sample (wet weight) was added into each chamber and filled with filtered seawater to a final volume of 900 ml. The contents of each chamber were vigorously stirred for 10 seconds to facilitate the release of toxicants from the pore water into the overlying water, which is the phase that developing embryos are primarily exposed to. After mixing, the contents were allowed to settle for 4 hours prior to toxicity testing.

The *P. viridis* green mussels (N=86, mean length: 65.48 ± 10.83 mm, mean weight: 14.13 ± 6.24 g) used in this research were taken from a cultivation in Muara Angke, Jakarta. The green mussels were cleaned from dirt and materials attached to their shells. Subsequently, spawning of the green mussels was conducted in a waterbath (Lauda®) witth seawater at a salinity of 32 pss. The green mussels were stimulated to spawn under temperatures started from 28˚C and then increased at a 2˚C increment every 10 minutes until reaching a maximum temperature of 36˚C. The spawn mussels were removed and put individually into a Pyrex® glass beaker containing filtered and sterilized seawater. The seawater filtration was done using a Whatman® cellulose nitrate filter papers (diameter 47 mm;

Figure 1. Sampling sites in Jakarta Bay.

pore size 0.45µm) and then the seawater was sterilized using an autoclave (ALP Co. Ltd; Model KT-30S).

Mature eggs and healthy sperms were selected to be used for the subsequent examination. The selected sperm cells were numerous and had active motions. Ova were filtered in sieve (diameter 0.25µm) in order to obtain clean and mature ones with round shape and granular surface. The sperm and ovum then put together in 500 ml glass beaker to allow fertilization while being stirred with a plunger to keep the homogeneity. The density of embryo solution that had been through fission phase after approximately 2 hours was calculated and adjusted to 300-400 embryo per ml.

Larvae counting was done using a Sedgwick rafter counting chamber (Wildlife Supply Company®, Model 1801-G20). 1 ml of embryo solution was inoculated to a 16 ml Pyrex® culture tube as a test tube, overlaid with 9 ml sediment sample and then sealed with parafilm. Five replicates of each sample, reference toxicant ($CdCl_2$) and control were prepared for counting analysis. The initial embryo density was counted at the beginning of the test by preserving the embryo using 1 mL of buffered formalin. Five test tubes filled with larvae were preserved with 1 ml of 50% buffered formalin to record the larvae density at the beginning of the test. The observation of embryo larval development was conducted after 2, 4, 8, 12, 16 and 24 hours by counting the number of larvae successfully evolving into the D-shaped development stage. The test was terminated when the percentage of D-shaped larvae reached ≥ 90% (ASTM, 2006; His *et al.*, 1999) by adding 1 ml of 50% buffered formalin into each test tube. The number of normal (D-shaped) and abnormal (not D-shaped) larvae were then counted.

Data analysis

The percentage of green mussel larval abnormality was estimated using the formula outlined in ASTM (2006) with statistical analysis of the larval abnormality data was calculated using RKWard 0.6.5 Software (Open Project, Free Software Foundation, Inc). To assess for any significant difference among sites, the non-parametric Kruskall-Wallis Test was conducted by classification of distances from shoreline, i.e. river mouth (code M), 5 km (code D), 10 km (code C), 15 km (code B), 20 km (code A) sites, and continued by the post-hoc Mann-Whitney-U-Test similar to conduced in Sedgwick (2012) and Nichols and Holmes (2007). P-value (<0.01) is used for testing significant difference between variables.

Table 1. Heavy metal concentrations (mg/kg dry weight) in Jakarta Bay sediments.

	Heavy Metal Concentration (mg/kg dry weight)					
	Cu	Pb	Cd	Ni	Zn	Hg
Estuary	55.36 ± 32.53	28.80 ± 16.73	0.28 ± 0.34	19.78 ± 8.96	441.91 ± 476.3	0.47 ± 0.19
5 km	46.46 ± 18.83	35.65 ± 12.71	0.18 ± 0.17	22.09 ± 2.46	228.52 ± 77.63	0.53 ± 0.17
10 km	34.15 ± 23.3	22.24 ± 4.9	0.16 ± 0.26	20.24 ± 2.61	152.21 ± 96.47	0.38 ± 0.05
15 km	15.13 ± 3.83	15.31 ± 6.47	0.11 ± 0.07	16.47 ± 3.76	82.73 ± 17.66	0.15 ± 0.05
20 km	14.79 ± 2.5	14.87 ± 4.78	0.22 ± 0.07	18.06 ± 3.08	82.05 ± 6.61	0.28 ± 0.36
TEL[1]	35.7	35	0.596	18	123	0.174
PEL[2]	197	91.3	3.53	36	315	0.486

[1]TEL (Threshold Effect Level) and [2]PEL (Probable Effect Level) of the Sediment Quality Guidelines (SQG) reported in Macdonald *et al.* (1996, 2000, 2004).

RESULTS

Heavy metals in Jakarta Bay sediments

Jakarta Bay sediment samples indicate that the accumulation of heavy metal tends to increase with proximity to the mainland Jakarta. Heavy metal concentrations in Jakarta Bay sediments is presented in Table 1. From the 31 sampling sites that represent five areas of increasing distances from the shoreline, the highest mean concentrations of Cu (copper) Cd (cadmium) and Zn (zinc) are found in river mouth sites; whereas the highest mean concentrations of Pb (lead), Ni (nickel) and Hg (mercury) are found at sites with 5 km distance from the shoreline.

In order to understand the impact of accumulated heavy metals in the bottom sediment to biota, the values of heavy metal concentration in the sediment samples are compared to the values of Threshold Effect Level (TEL) and Probable Effect Level (PEL) reported in the Sediment Quality Guidelines (SQG) of MacDonald *et al.* (1996, 2000, 2004). TEL is the level under detrimental effects that rarely cause death effects to aquatic biota; while PEL is the low concentration level in giving protection for aquatic biota. Heavy metal concentration that is under TEL would produce less than 10% harmful effects, while concentration that is higher than PEL would produce 50-70% harmful effects (Long *et al.*, 1998). Therefore Cu (at river mouth and 5 km sites), Pb (5 km sites), Ni (river mouth, 5 km, 10 km and 20 km sites), Zn (river mouth, 5 km and 10 km sites) Hg (river mouth, 5 km, 10 km and 20 km sites) concentrations pass the TEL value. And, the concentrations of Zn (river mouth sites) and Hg (river mouth and 5 km sites) pass the PEL value.

Green mussel larval abnormality

The percentage of larval abnormality is highest near the shoreline and decreases towards the sea. And statistically, the percentages of green mussel larval abnormality from the river mouth and 5 km sites are significantly different ($p<0.01$) compared to the control and other sites (Figure 2). The mean percentages of abnormal larvae in river mouth and 5 km sites are 50.61 ± 34.83% and 61.66 ± 36.52%, respectively.

Examining each sampling site (see Figure 1 for site names), the percentages of larval abnormality after being exposed to sediments taken from the river mouth sites vary widely (Figure 3). From the highest to the lowest, the percentages are: M4 station (100% larval abnormality) > M1 (91.20 ± 0.96%) > M7 86.30 ± 0.75%) > M9 (47.70 ± 9.79%) > M3 (30.77 ± 0.27%) > M2 (23.97 ± 1.30%) > M5 (13.16 ± 0.27%) > M8 (11.80 ± 0.27%). At the D (5 km) sites, high percentages (61.66 ± 36.52% larval abnormality) are found in three out of four sites, with site D3 being the only site with <10% larval abnormality. Within the 5 km sites, D4 site has 100% larval abnormality followed by D5 (80-85%) and D6 (55-65%) sites. Sites with 10-20 km distances (e.g. A, B, C) have <10% larval abnormality except in B1, B4, C2 and C4 sites (10-20%) and B6 (27-30%). Overall, there are six sites with larval abnormality above 60%, they are: D6 (60.22 ± 0.81%), D5 (81.38 ± 0.78%), M7

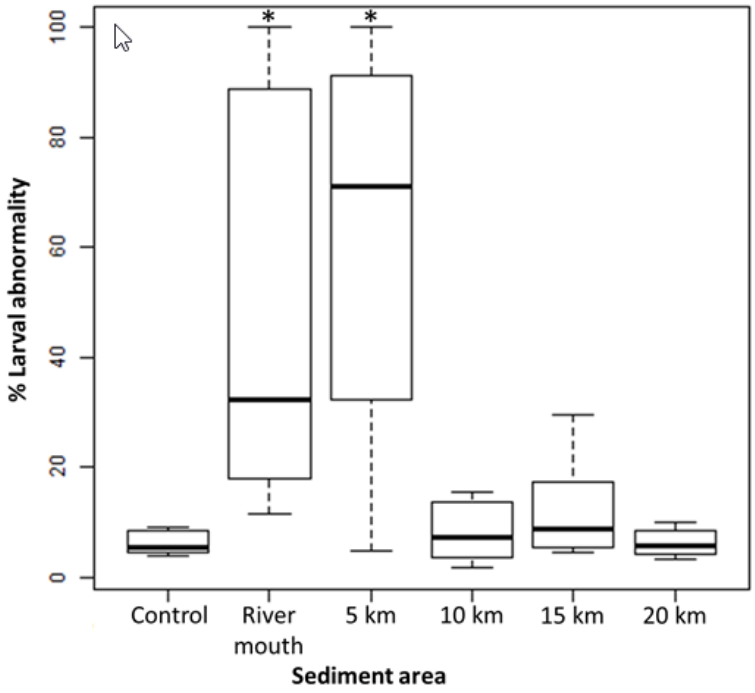

Figure 2. The percentage of abnormal (non-D shaped) *P. viridis* larvae after being exposed to Jakarta Bay sediments taken from increasing distances from the shoreline. Asterisk indicates sites whose values are statistically significantly different ($p < 0.01$) from the control site.

(86.30 ± 0.75%), M1 (91.20 ± 0.96%), and 100% larval abnormality in D4 and M4. In contrast, the controls have values 6.22 ± 2.19%.

The test on reference toxicant shows that the percentage of larval abnormality for the control experiment is 6.22 ± 2.30%. The percentage of larval abnormality increases to 21.38 ± 3.60% after being exposed to 0.56 mg/l $CdCl_2$. More than 90% larva abnormality was resulted from 1 mg/l $CdCl_2$ (94.60 ± 1.43%) and 1.8 mg/l (99.67 ± 0.33%), thus 100% larvae abnormality after being exposed to 3.2 and 5.6 mg/l $CdCl_2$. In order to compare the percentage of larval abnormality with heavy metal concentration in Jakarta Bay sediments, we apply a logarithmic transformation prior to determining the correlation coefficient. We find $R^2 > 0.90$ in D5, M7, M1, D4 and M4 sites and $R^2 > 0.70$ in D6 and M9 sites.

DISCUSSION

Higher heavy metal concentration in nearshore Jakarta Bay sediments reflect high anthropogenic impacts into the bay whose upstream waters are located in areas supporting a number of industries (e.g. transportation, pharmacy, paper, leather, chemicals and petrochemicals industry; BPLHD-DKI-Jakarta, 2015). Jakarta Bay also receives sediments derived from volcanic activity (Sindern *et al.*, 2016), however, the lack of wastewater management installation in the upstream industrial areas causes a significant amount of heavy metals to be discharged to the ecosystem of Jakarta Bay (Napitupulu, 2009).

Sites with high percentage of larval abnormality (i.e., M4, M1, M7, D4, D5, D6) correspond to areas of high anthropogenic pressures. These sites with >50% larval abnormality may be detrimental to biota as also suggested by the PEL value. A study by BPLHD-DKI Jakarta (2015) confirm that water quality at the mouth of the Cengkareng Drain and Kamal River (site M1), Grogol River (site M4), Cakung River (site M7) were in Class D or Class 4 on the Governor of Jakarta's decree No. 582/1995 on water quality standards (Pemerintah Provinsi DKI Jakarta, 1995). Furthermore, D4, D5 and D6 sites are located near sea transportation lines (Tanjung Priok Harbor) and the North Jakarta integrated industrial area. Napitupulu (2009) reported that only 5% of industries operating in integrated industrial area have waste water treatment plants, which again highlights the influence of untreated

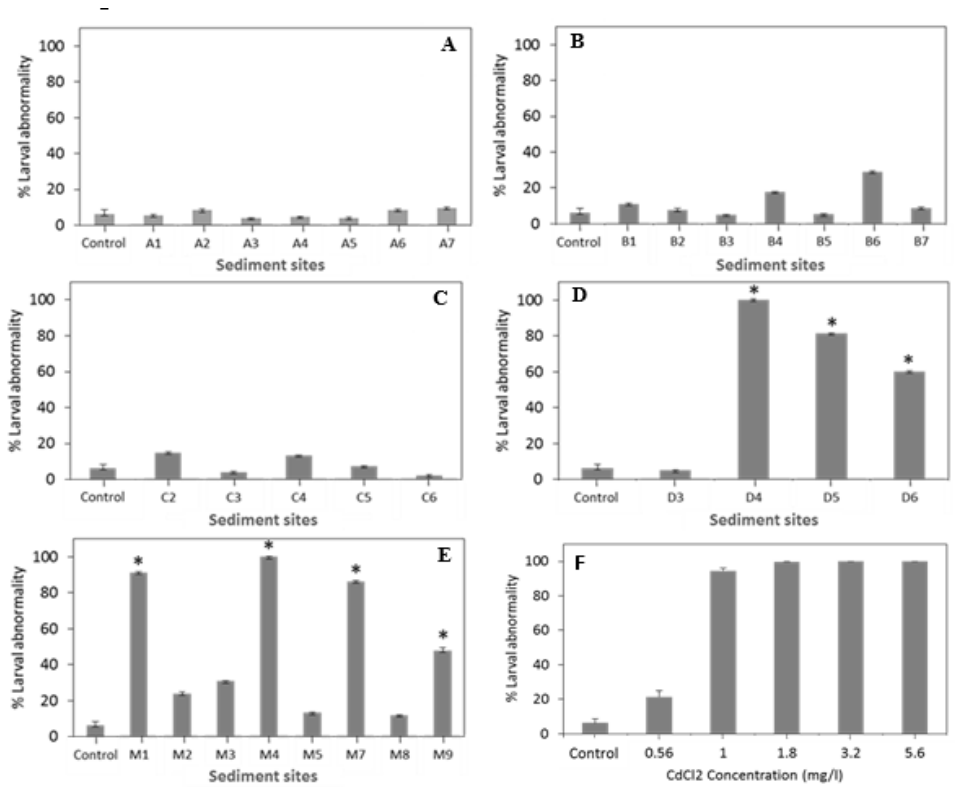

Figure 3. The percentage of abnormal (non-D shaped) *P. viridis* larvae after being exposed to Jakarta Bay sediments of increasing distances to the shoreline. (A) 20 km, (B) 15 km, (C) 10 km, (D) 5 km distances from the shoreline, (E) river mouth and (F) reference toxicant. Asterisk indicates sites whose values are statistically significantly different ($p < 0.01$) from the control site.

waste water to heavy metal accumulation in Jakarta Bay.

Our study shows a coherency between larval abnormality and heavy metal concentration in sediments (R^2>90%), in line with other works indicating that toxicant in sediments could cause direct effects to biota specifically during its embryo-larval development (e.g. Long *et al.*, 1998, Fathallah *et al.*, 2013). Heavy metals can impede the physiologic process of membrane and come in through transporter or endocytosis in cytosol, triggering the disturbance of homeostasis Ca^{2+} process and replacing natural ligand in cell organelle. These changes could trigger the forming of reactive oxygen species/reactive nitrogen species (ROS/RNS) that disturbs gene expression and process of antioxidant cell organelle (Varotto *et al.*, 2005, 2006, 2013). Also, heavy metals coming to the cell level could cause destruction and death of cell in necrosis or apoptosis, affecting DNA and triggering deregulations in the process of cell homeostasis (Varotto *et al.*, 2013). These physiological effects presumably disrupt the development of D-shaped green mussel larvae in this study.

CONCLUSION

This study presents a coherency between the concentration of heavy metals and the percentage of larval abnormality in Jakarta Bay sediments particularly in the nearshore (river mouth and 5 km) sites. The finding corroborates their links to anthropogenic activities from Jakarta particularly due to untreated waste water. This research assumes the influence of heavy metal contamination in the sediments on the tested biota, however, future works should also take into account other factors such as organic pollutants and microorganisms that may also cause larval abnormality.

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