Ecological Assessment in Semarang Coastal Area Based On Sediment Bioassay Approach Using Green Mussel Larvae

Rachma Puspitasari and Triyoni Purbonegoro

Research Centre for Oceanography, Indonesian Institute of Sciences
Jl. Pasir Putih 1, Ancol Timur, Jakarta 11048 Indonesia
Email: poespitsari@gmail.com

Abstract

Rapid developments of industry and population growth have lead to ecological pressures on coastal areas. Semarang as capital of Central Java and port city, has an environment sensitivity which is important to be investigated. Sediment is an important part in aquatic environment because acts as sink or source of pollutant and could be used to assess coastal environment health. Three locations had been studied, i.e. Tanjung Mas Port, estuaries of Banjirkanal Barat and Banjirkanal Timur. These locations were compared to assess their health of sediment through elutriate sediment toxicity test. Eighteen grams of sediment from each locations were weighed and placed in 1 L Beaker glass. Eggs and sperm are taken from spawning of adult green mussel in laboratory. Thirty mL of sperm was added to the eggs and fertilized eggs were observed microscopically. Fertilized eggs were exposure to elutriate water-sediment from each location for 48 hour. Statistical analysis showed that there are significantly different abnormalities larvae percentage of sites 5, 8 and 13. The highest percentage of abnormality larvae occurred in Port Tanjung Mas, followed by Banjirkanal Timur and Banjirkanal Barat. Lead concentrations in sediments of coastal Semarang was ranged from 10.9 to 15.62 mg.kg^{-1}, with an average of 13.69 mg.kg^{-1}. Lead concentration was decreased form Port, Banjirkanal Timur and Banjirkanal, respectively. The abnormalities of green mussel larva may related to lead contamination. It could be concluded that sediment quality in Port area was very poor compare than other site.

Keywords: *bioassay, green mussel larvae, sediment, Semarang coastal area*

Introduction

Semarang coastal area has valuable ecological and socio-economic importance to the Province of Central Java. It is one of areas which has progressive development in northern coastal of Java (Rositasari and Lestari, 2013). In 2010, Semarang consisted of 1.6 million inhabitants, and had growth rate 1.90 % per year. Administratively Semarang comprises 16 subdistricts and 177 villages. With the municipal administrative area at 37.4 hectares and the gross density is 43 inhabitants per hectare, Semarang does not have a high level of growth rate (Setioko *et al.*, 2013). During 2013, there were 300 large and medium scale industries with 84,227 labors (BPS Kota Semarang, 2015). The existence of the Port of Tanjung Mas, Banjirkanal Barat and Banjirkanal Timur in coastal Semarang has a consequence by increasing of anthropogenic activities like agricultural or domestic sewage, oil spill, PAH contamination through runoff water. Besides that, erosion and sea level rise also caused economic losses in the area like ponds and housing. Sedimentation that occurs rapidly in the Port, caused government to spend billions of rupiah to dredge (Pramudyanto, 2014). Wibowo (2009) examined the vulnerability of coastal Semarang based on rock composition, shape of the coastline, the potential for flood, subsidence speed, mangrove habitat and economic value. Port area has a highest vulnerability in Semarang. In addition, Semarang did not have a domestic waste water treatment, so liquid and solid waste from residents enter directly into the sea through flood canal, namely Banjirkanal Barat and Banjirkanal Timur, thereby potentially affect sea water quality in coastal (Wibowo, 2009).

Organic and inorganic contaminants especially heavy metal which enter aquatic system, will be accumulated in sediments (Luoma and Ho, 1993). Sediment is an important component of the aquatic environment and play a role in particulate and dissolved contaminant (Bat, 2005). Sediments are not only a reservoir for contaminants, but also a source of toxicants for marine animals (Beiras *et al.*, 2003).

Toxicity testing is used to manage the fate of discrete batches of sediment taken at a well-defined place and time, and evaluate risks in particular

*) Corresponding author

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circumstances (Luoma and Rainbow, 2008). Sediment toxicity can be interpreted as the ecological and biological changes caused by contaminated sediment or an adverse response observed in a test organisms exposed to contaminated sediments (Luoma and Ho, 1993). Sediment bioassay can be used in two separate ways to develop sediment quality criteria : (a) sediment bioassay and chemical analyses can be conducted with sediments collected from contaminated and reference areas. The bioassay responses can be compared quantitatively to identify whether problems exist and the levels of contaminants in sediments can be related to the bioassay responses; (b) dose-response relationships can be developed in the laboratory by spiking sediments with individual and mixed contaminants and then carrying out bioassays on these sediments (Bat, 2005) .

Sediment bioassays is an important step in the assessment of the marine environment quality, providing an integrated measure of toxicity and they are becoming widely used tools in monitoring program, permissions for dumping dredging material, and other regulatory activities (Long *et al.,* 1996). Elutriate sediment toxicity is possible for water-column organisms, such as early developmental stages of marine invertebrates in sediment toxicity testing (Beiras *et al.,* 2003). The use of oysters larvae (*Crassostrea gigas)* had been used to investigate the toxicity of elutriates sediment of Mediterranean Port as reported by Mamindy-Pajany (2011). Anthropogenic pressures on coastal give negative effects on benthic and pelagic community, fisheries and human health through direct contact between organism and sediment or resuspension of contaminant particles through the water column (Ingersoll, 1995). *Perna viridis(*Mollusca) (Romimohtarto and Juwana, 2004) has a commercial value as seafood and ecological value as filter feeder in tropic system. The objective of this study is to compare sediment quality of Port, Banjirkanal Barat and Banjirkanal Timur using bioassay approach.

Materials and Methods

Research was conducted in the coastal waters of Semarang, Central Java in August 2010. The sediment samples were taken from 13 sites in three location i.e Port of Tanjung Mas, the mouth of Banjirkanal Timur., and Banjirkanal Barat (Figure 1). Site 1-5, site 6-8 and site 9-13 were collected to represent sediment source for port of Tanjung Mas, Banjirkanal Barat and Banjirkanal Timur, respectively. Sediment was taken by Grab Smith McIntyre 0.05m^2. The top 2–3 cm of sediment was collected using a stainless steel spoon, placed in 1 L bottle and stored at 4°C prior to be used in the bioassays test. Generally, coastal water of Semarang was relatively shallow, is about 1-5 m, except in Port area is more than 10 m. Banjirkanal Timur was shallower than Banjirkanal Barat (Rositasari *et al*., 2010).

Eighteen grams of sediment from each locations were weighed and placed in 1 l beaker glass. Nine hundreds mL sterile seawater was added carefully to beaker and stirred so that homogenized. Water-sediment mixture was allowed to settle at least 4 hours. Nine ml of overlying water were distributed into 12ml tube reaction with automatic pipette (ASTM, 2006).

Spawning of Green Mussels

Adult green mussels (*P. viridis*) were obtained from Cilincing, North Jakarta. The mussels were transported to the laboratory in icebox to avoid spawning. The mussels were then placed in a water bath containing a sterile seawater. Spawning had been triggered by thermal stimulation with increasing water temperature. The initial temperature of water with thermostat is 28°C, and raised 2°C every 15 minutes. Spawning is stopped when the temperature reaches 34°C. Adult mussels that produce sperm and egg during the stimulation process, immediately were separated to prevent fertilization in the water bath. Eggs and sperm quality were observed microscopically. Sperm must be active in contact with seawater. Good eggs are rounded and full. Eggs are filtered through a 0.25 mm sieve, placed a 500 ml sterile seawater. Thirty ml of sperm was added to the eggs and observed microscopically so that there are 7-10 sperms surrounding the egg. The addition of the eggs must be done carefully to avoid polispermi. One ml of fertilized eggs was observed with Sedgewick Rafter Counting Cell until the formation of a polar body. Two hours after fertilization, the embryo divides into two cells. Embryo stock solution was made based on the number of two cells embryos (ASTM 2006).

Sediment Bioassay

One ml of 400 ml embryo of green mussel density inoculated into each labelled-test tube. Five tubes as zero time control were prepared and added with 1 ml of 50 % buffered formalin, cover with parafilm and mixed the tube carefully. This tube is useful to ensure the embryos inoculated density is 300-500 embryo/ml. Observation tubes containing sterile seawater and embryo were preserved every few hours (2-3 hours). This tube is useful for monitoring larval development until formation of a D -shape without disturbing negative control. Test were terminated after larvae in observation tube has reached ≥ 90 % D-shaped (prodissoconch I). One ml of 50% buffered formalin was given to each tube, the tube was covered with parafilm and shaken carefully. The test was considered to be valid, when the mean abnormality and normality was ≤ 10 % and ≤ 30 % in the negative control, respectively (ASTM, 2006)

Data Analysis

The normal and abnormal number of embryos mussels were counted from each test tube. Normal prodissoconch I larvae are large, smooth, translucent and distinctly "D" shaped with a straight hinge. Larvae are still considered normal as long as shell development is completed. The embryo is defined as abnormal if the round shape was irregular, rough or not perfectly formed shell or if the embryo is still in a phase of trocophore i.e., small, dark and ciliated under 100x magnification. Abnormality data was then compared with control. The endpoints of the bivalvia larvae test are based on adverse effetcs on larval development.

Percentage of abnormality had been transformed using arcsine of the square root. Normality and homogeneity of variances were analyzed using Shapiro-Wilks test and Bartlett test. The effect of the sediment elutriate from each site and difference with the control were tested with ANOVA and Kruskall-Wallis test.

Result and Discussion

Sediment Bioassay

Sediment bioassay can be used to asses aquatic ecosystem health by acute or chronic toxicity test. One of ecotoxicology method is elutriate sediment, where overlying water from mixture sediment-water exposed to aquatic larvae. Marine invertebrate embryos and larvae are more sensitive to toxicants than adults and have frequently been used for the assessment of marine pollution (His *et al,.* 1999; Southeward *et al.,* 1999;; Bellas, 2006; Puspitasari, 2011) because of their sensitivity, fast response, and ecological relevance. Contaminant in sediment affected the larval development of green mussel (Figure 2). In normal development, fertilized egg of green mussel will develop into D-shaped in 48 hour. Physical and chemical parameters in initiation test are recorded to make sure that they are in acceptable condition. Dissolved oxygen, pH and temperature are 4.37-5.85 mgl^{-1}, 7.7-7.94 and 23.9-24.6 ° C, respectively and all still in acceptable requirement for *P. viridis*.of standard method.

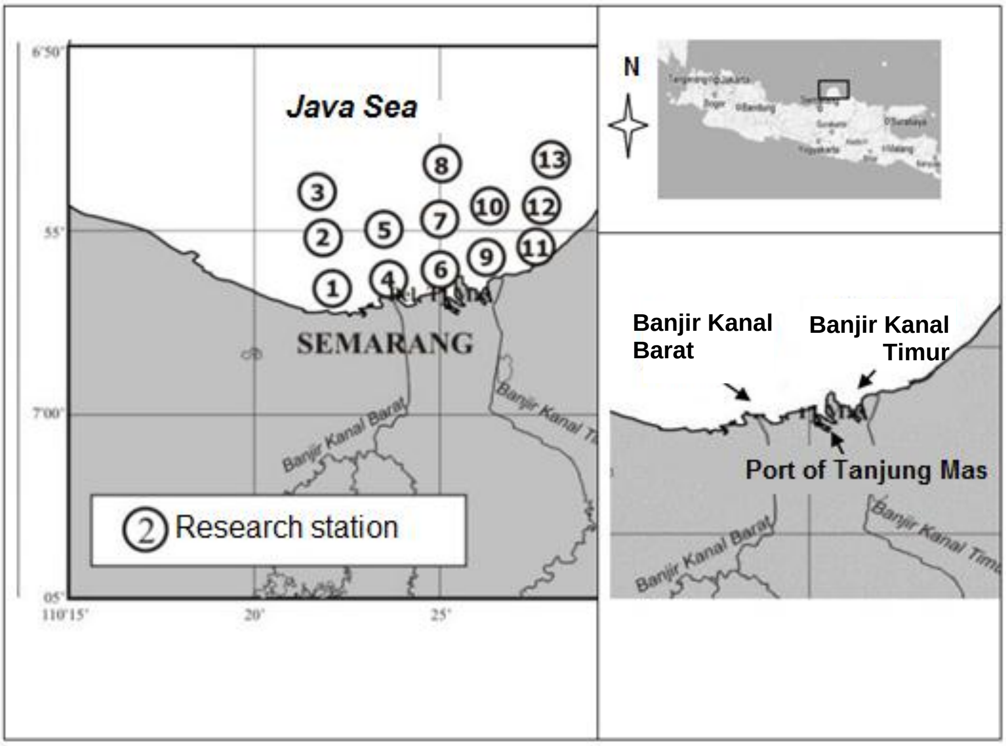

Figure 1. Sampling site in Semarang coastal area

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Table 1. Summary of elutriate sediment toxicity test after 48-h incubation from 3 location

Group	Sites	Abnormality (%)	Mean Abnormality (%)
	Control	8.76	
Banjirkanal Barat	1	17.79	
	2	26.53	
	3	9.93	23.24
	4	14.84	
	5	47.13*	
	6	18.30	
Port	7	23.74	28.51
	8	43.49*	
Banjirkanal Timur	9	18.60	
	10	18.46	
	11	17.37	27.52
	12	14.88	
	13	68.27*	

* Significance difference at 0.05

Percentage abnormality more than 50% was found in site 13 whereas in another station still below 50%. Statistical analysis (p=0.05) showed that there were significance difference among site 5, 8 and 13. This test was considered valid, because mean abnormality and mean normality were 8.76 % and 3.5 % in the negative control. The lowest abnormality in negative control is to make sure that larval was developednormally. Site 5 and 8 were located in outer side of Port Tanjung Mas and site 13 was located in outer side of Banjirkanal Timur. Table 1 explained the mean abnormality in three sites. From Table 1, it can be concluded that the highest abnormality was on Port area. Abnormality of larva depends on exposure to contaminant sediment. Re-suspension at sediment test preparation make contaminant binding sediment is dispersed into water column. It is likely happen in normal environment condition where in shallow habitat sediment was influenced by current and tidal. From elutriate sediment toxicity, what the main factor affect the abnormalities of larva was remained unknown. It was hypothesized that contaminant in sediment e.g. metal or organic contaminants were responsible for that result. In this research, the sediment quality of Semarang was assessed in corelation with sediment bioassay.

Water Condition Related to Sediment Bioassay

Bioassay results could not ignore the existing factors complexity of the sampling site. The research location was relatively shallow waters ranged of 1-5 meters, except in Port is deeper than 10 meters. Banjirkanal Timur was relatively shallower than Banjirkanal Barat (Rositasari and Lestari, 2013). In shallow waters, currents become the dominant factor that affects the stability and composition of surface sediments. In addition, current in August 2010 was influenced by monsoon, got a dominant influence of tides, in which mass of water come from northwest at high tide and to the northeast during low tide (Rositasari *et al.*, 2010). Mass of water was influenced by current, bring contaminant from Banjirkanal Barat and Port area to the outer side of Banjirkanal Timur. This explained why high percentage of abnormality happened in location far from mainland because mass of water influenced by current.

Rositasari dan Lestari (2013) reported that lead and zinc contaminant in surface sediment in Semarang were coastal 12,5 and 70 mg/kg, respectively. Zinc concentration in Semarang coastal was ranged from 84.14 to 131.74 $mg.kg^{-1}$, with an average of 97.11 $mg.kg^{-1}$ in August 2010. Lead concentrations in sediments of coastal Semarang was ranged from 10.9 to 15.62 $mg.kg^{-1}$, with an average of 13.69 $mg.kg^{-1}$. Lead concentration was decreased form Port, Banjirkanal Timur and Banjirkanal Barat, respectively. Metal contamination in Semarang coastal was higher than Klabat Bay, but lower than Siak waters, Banten Bay, Jakarta Bay and Malaka Strait (Rositasari dan Lestari (2013). Takarina *et al.* (2004) also found that Banjirkanal Barat had high levels of Zn and Ni (161–573 and 37–58 $\mu g.g^{-1}$, respectively) in sediments which was collected from downstream area of the wastewater outfall of electroplating industries.

From bioassay result (Table 1), the worst quality of sediment were in Port area, Banjirkanal

Timur and Banjirkanal Barat, respectively. The same result also found by Rositasari dan Lestari (2013). They found *Ammonia beccari*, benthic foraminifera which was used as a bioindicator for hypoxia/anoxia/eutrophic was abundance in Port of Tanjung Mas. This is indicated that Port of Tanjung Mas has the worst sediment compare the other.

Conclusion

Sediment bioassays are an important step in the assessment of the marine environment quality and providing an integrated measure of toxicity. Contaminant in sediment will affect the larval development of green mussel. The highest abnormalities of green mussel larvae were found in Port area, Banjirkanal Timur, and Banjirkanal Barat, respectively. This may related to the environment quality

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