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Ecological Asessment In Semarang Coastal Area Based On Sediment Bioassay Approach Using Green Mussel Larvae

Rachma Puspitasari* and Triyoni Purbonegoro

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

Abstract

Rapid development of industry and population growth have lead to ecological pressures on coastal areas. Semarang as the capital of Central Java and as a 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 that had been studied were Tanjung Mas Port, estuaries of West Canal Flood and estuaries of East Canal Flood. These locations were compared to assess health of sediment through elutriate - sediment toxicity test. Eggs and sperm are picked up from spawning of adult green mussel in laboratory. Thirty mili liter of sperm was added to the eggs and observed microscopically so that obtained fertilized egg of green mussel. Fertilized eggs were exposure to elutriate water -sediment from each location for 48 hour. Statistical analysis with ANOVA showed that abnormality larvae from sites 5, 8 and 13 are significantly different from other. The highest of abnormality larvae occurred in Port, followed by East Canal Flood and West Canal Flood. From other study had been reported that Ammonia beccari, bioindicator for hypoxia/anoxia/eutrophic was found in Port. Lead was decreased form Port, East Canal Flood and West Canal Flood, respectively. So, we suspect that abnormalities of green mussel larva are related to lead contamination. Overall, it could be concluded that sediment quality in Port area was very poor compare than other site.

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

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 sub districts 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, West Flood Canal and East Flood Canal in coastal Semarang has a consequence by increased 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, spends 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, 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 circumstances (Luoma and Rainbow, 2008). Sediment toxicity can be interpreted as the ecological and biological changes that are caused by

*) Corresponding author

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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 elutriate sediment 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* or *Mytilus edulis*, phyllum Mollusca (Romimohtarto and Juwana, 2004) has a commercial value as a seafood and ecological value as filter feeder in tropic system. The objective of this study is to compare sediment quality of Port, East and West Canal Flood 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, East Flood Canal, and the mouth of the West Flood Canal (Figure 1). Site 1-5, site 6-8 and site 9-13 were collected to represent sediment source for West Canal Flood, Port and East Canal Flood, 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 being used in the bioassays. Generally, coastal water of Semarang was relatively shallow, is about 1-5 m, except in Port area is more than 10 m in depth. East Flood Canal was shallower than West Flood Canal (Rositasari *et al*., 2010).

Eighteen grams of sediment from each locations were weighed and placed in 1 L Beaker Glass. 900 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 was obtained from Cilincing, North Jakarta. The mussels were transported to the laboratory with icebox to avoid spawning. The mussels placed in a water bath containing a sterile sea water. Spawning had been done 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 produces sperm and egg during the stimulation process, immediately separated to prevent fertilization in the water bath. Eggs and sperm quality were observed microscopically. Sperm must be active by contact with seawater. Good eggs are rounded and full. Eggs are filtered through a 0.25 mm sieve, placed in Beaker and matched into a 500ml volume with the addition of a sterile sea water. Thirty mili liter 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 milliliter 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 form a D -shape without disturbing negative control. Test were terminated after larvae in observation tube has reached ≥ 90 % D-shaped (prodissoconch I). Give 1 ml of 50% buffered formalin to each tube, covered with parafilm and shake the tube carefully. The test to be considered valid, if mean abnormality and normality must be ≤ 10 % and ≤ 30 % in the negative control, respectively (ASTM, 2006)

Data Analysis

The number of embryos mussels which are normal and abnormal 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 of irregular, rough or not perfectly formed shell or if the embryo is still in a phase of trocophore i.e., small and dark and still can be found cilia at 100x magnification. Abnormality data 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 (Southeward *et al.,* 1999, His *et al,.* 1999; Bellas, 2006; Puspitasari, 2011) because of sensitivity, fast response, and ecological relevance. Contaminant in sediment will affect the larval development of green mussel (Figure 2). In normal development, fertilized egg of green mussel will form 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 mg L^{-1}, 7.7-7.94 and 23.9-24.6 ° C, respectively and all still acceptable requirement of standard method.

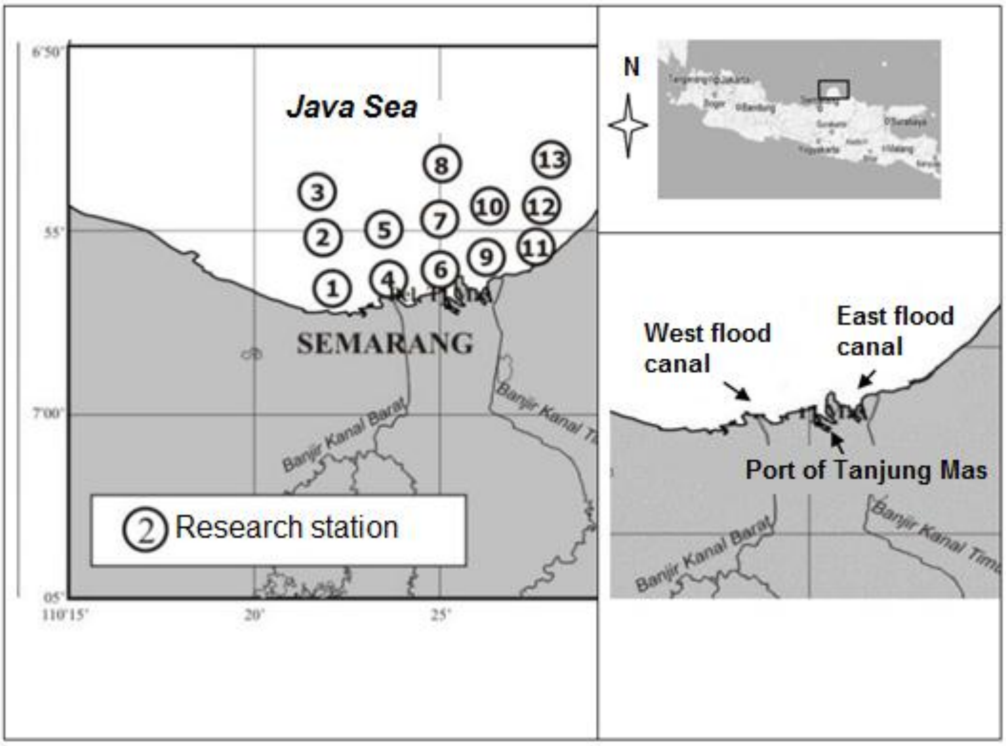

Figure 1. Sampling site in Semarang coastal area

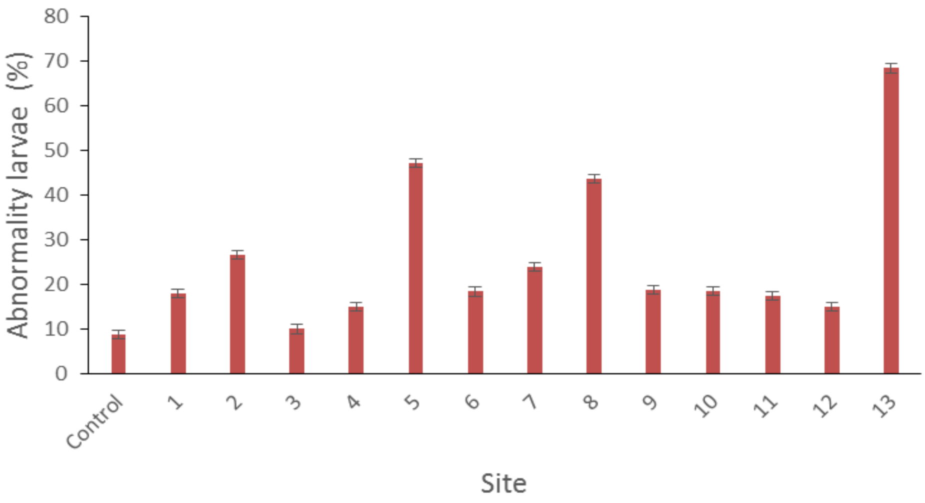

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Figure 2. Percentage of *P. viridis* larvae after 48-h incubation with elutriates sediment

Table 1. Summary of elutriate sediment toxicity from 3 location

Group	Sites	Abnormality (%)	Mean Abnormality (%)
	Control	8.76	
West Canal Flood	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*	
East Canal Flood	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% founded in site 13 whereas in another station still below than 50%. Statistical analysis ($p=0.05$) showed that significance difference are in 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 making sure that larval development occurs normally. Site 5 and 8 were located in outer side of Port Tanjung Mas and site 13 was located in outer side of Eastern Canal Flood. Table 1 explaining about mean abnormality in three sites. From Table 1, we can conclude that the highest abnormality was on Port area. Abnormality of larva depends on contaminant in exposure sediment. Re-suspension at sediment test preparation make contaminant binding sediment are dispersed into water column. It is likely normally condition in natural where sediment in shallow habitat, will influenced by current and tidal. From elutriate sediment toxicity, we cannot know what is the main factor affecting the abnormalities of larva. We hypothesized that contaminant in sediment e.g metal or organic contaminant were responsible to do that. In this research, we only asses the sediment quality of Semarang from sediment bioassay but what is main factor affecting cannot be known clearly.

Water Condition Related to Sediment Bioassay

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

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

From bioassay sediment related to lead and zinc contamination and current characteristic of Semarang water, the worst quality of sediment were in Port area, East Canal Flood and West Canal Flood, respectively. Rositasari dan Lestari (2013) found *Ammonia beccari*, benthic foraminifera as a bioindicator for hypoxia/anoxia/eutrophica, in Port of Tanjung Mas. This is indicated that Port of Tanjung Mas has the worst sediment compare the other.

Conclusion

Sediment bioassays is 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, East Flood Canal, and West Flood Canal, respectively. This is similar with other research that distribution of lead was decreased from Port area, East Canal Flood and West Canal Flood, respectively. In addition, abundance of *A. beccari*, benthic foraminifera as bioindicator of hypoxia environment was found in Port area.

To manage sediment contamination in Semarang coastal area, it is important to consider a regular program of sediment quality monitoring. The monitoring itself must be
based on scientifically sound questions and be capable of distinguishing between environmental damage and natural variability.

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