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A study of selected biomarkers for monitoring effects of pollution in coastal areas of Indonesia

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Selected biomakers, Cholinesterase (ChE) and phagocytic activities have been investigated with the exposed green mussel *Perna viridis* in Indonesian coastal waters. An operative effect-based monitoring on two polluted sites and one reference area were investigated for aquaculture enterprises and human health aspects. Between two heavily polluted sites, green mussels from Cilincing indicated a lower level of the ChE activity than those from Kamal Muara. The phagocytic activity of green mussels from the polluted sites demonstrated significant higher activity than that of green mussels from the pristine site, Pangkep. However, there were no significant differences of phagocytic activities between the polluted sites. This might indicate that the existing pollutants in Jakarta Bay were more neurotoxic rather than immonotoxic substances. The results showed clearly that both selected biomarkers were potential valuable tools for effect-based monitoring and pollution impacts in coastal zones of Indonesia.

Key words: Green mussel, biomarkers, coastal zone management, Indonesia.

INTRODUCTION

A biological approach has been used as a counterpart of a classic chemical approach for surveying marine pollution effects in many international programs (Cajaraville et al., 2000; Devier et al., 2005; Lehtonen et al., 2006; Orbea et al., 2006; Minier et al., 2006). A chemical analysis solely is considered as an invaluable analysis for interpretation of the pollutant impact in marine ecosystem since it does not illustrate the harmful effects (Walker, 1998; Damiens et al., 2004) and the fate of chemical compounds on living organism through biotransformation of xenobiotic substances within living organism body (Nicholson and Lam, 2005). In many cases, the biotransformation may increase xenobiotic substances toxicity on organism via producing reactive metabolite compounds that are more toxic than original

parent compounds (Belden and Lydy, 2000). Moreover, the chemical approach is costly, usable to only a small proportion of the xenobiotic compounds in the environment, produces a little biologically meaningful data, and consequently simplifies the complexity of the ecosystem under monitoring (Butterworth, 1995). For those reasons, the classic chemical analysis should be accompanied by the biological approach which is so called "biomarker" that elucidates biological responses of environmental pollution.

Biomarkers have been considered as sensitive and suitable tools for detecting either exposure, or effects of, pollutants (Hansen, 1995; Narbonne et al., 2001; Lagadic, 2002) since they can provide more comprehensive and biologically more relevant information on the potential impact of pollutants on the health status of organism (Van der Oost et al., 1996; Picado et al., 2007; Galloway, 2006). In respect to pollutants that has a lower stability in water such as organophosphate and carbamate pesticides,

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biomarkers are reliable tools for assessing the impacts of the pollutants on biota even if the existing of the pollutants in water cannot be detected (Sturm et al., 1999). It is because biomarkers can detect persistence responses and/or effects of the pollutants in such duration of biota lifetime (Depledge and Fossi, 1994). Therefore, they have been used enormously in biomonitoring to assess the risk of marine ecosystem pollution (Cajaraville et al., 2000; Martin-Diaz et al., 2004).

Mytilid mussels have received tremendous concerns as a sentinel organism when applying biomarkers in many pollution monitoring programmes (Cajaraville et al., 2000; Dizer et al., 2001a; Livingstone et al., 2000; Castro et al., 2004; Nesto et al., 2004; Leinio and Lethonen 2005; De Luca-Abbott et al., 2005; Halldórsson et al., 2007: Verlecar et al., 2008). As sedentary and filter-feeder animals, marine mussels do not escape from contaminated water where they are living and can accumulate many contaminants to the level higher than existing in water (Widdows and Donkin, 1992). Hence, the behaviors are providing realistic sentinel organisms that indicate the biologically available concentrations. The realistic bioavailability of contaminants in mussels is also demonstrated by the fact that they have inefficient detoxifying enzymes permitting small portion of contaminants that can be transformed within their body (Nicholson and Lam, 2005). Consequently, mussels have been considered as notable eco-sentinel organisms for effect-based monitoring activity and have represented the sensitivity of detection harmful effect of pollutions (Goldberg et al., 1978; Kim et al., 2008).

The extensive use of mussels and biomarkers for that purpose were carried out in temperate region by using blue mussels, *Mytilus edulis* (Halldórsson et al., 2007; Gagné et al., 2008; Tedesco et al., 2008; Yaqin and Hansen 2010). However, compare to the use of blue mussel in temperate regions there are few studies conducted concerning biomarkers in tropical region by using native species, green mussels, *Perna viridis* (Nicholson and Lam, 2005). It has been postulated that genetic and ecosystem differences of two marine mussels generated complicated inherent difficulties, when an extrapolation of *M. edulis* data to *P. viridis* was conducted. Therefore, a hot spot investigation of biomarkers in tropical regions by employing *P. viridis* is required to enhance the understanding of biological response of indigenous species toward contaminants to enforce biomonitoring of marine pollution effects programs in tropical region.

This study applied selected biomarkers which are cholinesterase (ChE) and phagocytic activities to monitor effects of pollution in coastal areas of Indonesia. ChE activity has been widely used as a biomarker (biochemical response) for neurotoxic effects of organophophorous and carbamate pesticides. There are some influences of ChE activity by several metals, PAH and surfactants exposure (Tabche et al., 1997; Guilhermino et al., 1998; Akcha et al., 2000; Moreira et al., 2004). Moreover, the immune system is a vital part of the organism and associates intimately with the function of many organs and organ system (Fournier et al., 2000). In invertebrate, the phagocytic activity which is part of the immune system can be induced by wide range of xenobiotics. Hence, the phagocytic activity is considered as a less specific early indicator of immunotoxicity or as a biomarker (Oliver and Fisher, 1999; Blaise et al., 2002). The two selected biomarkers were employed in the current study based on microtiterplate techniques in order to provide a rapid, cost-effective, justifiable (Blaise et al., 2002), and well-adapted application in developing countries.

MATERIALS AND METHODS

Chemicals

Acetylthiocholine iodide, 5,5'-Dithio-bis-(-2-Nitrobenzoic acid) (DTNB), γ-globuline, Bovine Serum Albumin, Fluoresceinisothiocyanate were purchased from Sigma. Bradford reagent was purchased from Bio-Rad Laboratories GmBH, Germany. All others reagents used were analytical grade products.

Study area

The study was conducted on three different areas of Indonesian coastal zone. A coastal area of Pangkajene Kepulauan (Pangkep) regency in South Sulawesi was chosen as reference site (station 1; Figure 1) because there are relatively minimal anthropogenic activities that were performed in this place such as traditional fisheries, which use static fishing equipment. On the other hand, two sites of Jakarta Bay, Kamal Muara and Cilincing (Figure 2) were chosen and considered as heavily anthropogenic polluted sites (station 2 and 3) since they received almost domestic and industrial wastes from Jakarta and neighboring cities of Jakarta. Moreover, some studies based on chemical analysis indicated that Jakarta Bay was under threatened by anthropogenic pollutants (Williams et al., 2000; Sudaryanto et al., 2002; Munawir, 2005). Whilst, many traditional fisheries activities such as green mussel aquacultures are situated along Jakarta Bay. Hence, Jakarta Bay is considered also as highly valued fisheries resources of coastal area, which plays indispensables role for preserving marine food resources and economic basis of small scales fishermen.

Sample collection and preparation

Thirty two mussels (5 to 6 cm) were handpicked on traditional green mussel cultures along Jakarta Bay at Kamal Muara and Cilincing, and from the Pangkep wild reference population attached naturally on traditional static fishing equipments.

The collected living mussels were directly transferred to the laboratory using cool box under humid condition. Prior to dissecting out of the mussels, 1 ml of hemolymph was withdrawn from posterior adductor muscle (PAM) sinus using 1 ml syringe and 0.4 mm needle followed by phagocytosis assay as described thus. Gill, foot, mantle and PAM were cut off, blotted dry and weighted before placing them in 2 ml potassium phosphate buffer in Eppendorf tube (0.1 M/pH 8.0). Prior to transferring the tissues to Ecotoxicology Department Laboratory (Technische Universitaet of Berlin,
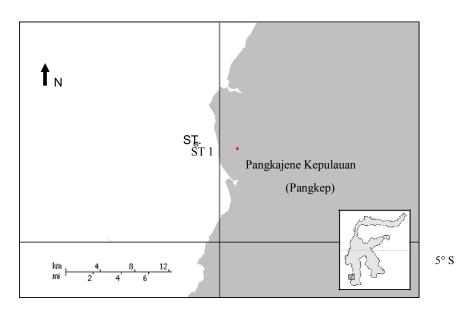
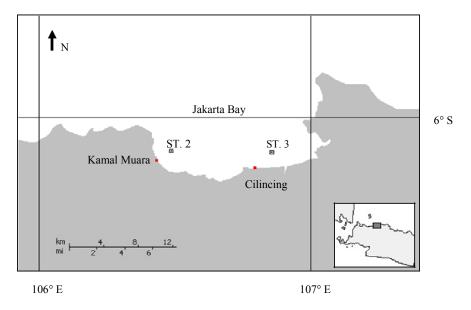**Figure 1.** Sampling station (ST) in Pangkajene Kepulauan (Pangkep).

Figure 2. Sampling stations (ST) in Jakarta Bay.

Germany) using cool box that were filled by dry ice, the tissues were stored at -70°C.

Cholinesterase activity

The enzyme activity was measured following the modified Ellman method (Ellman et al., 1961) for a 96-well plate. A Dounce homogenizer was used to homogenize 0.3 g of each tissue in 2 ml potassium phosphate buffer (0.1 M/pH 8.0). The homogenate was centrifuged for 10 min at 10000 × g and the supernatant was harvested and stored at -80°C prior to the analysis of ChE activity and protein content. The supernatant was diluted in 1:2 of

potassium phosphate buffer (0.1 M/pH 8.0) following the enzyme measurement.

The enzyme measurement was carried out by placing 50 µl of the diluted sample into each well of the microplate. A blank was made by putting 50 µl of potassium phosphate buffer into the blank section of the microplate wells. The plate was incubated for 5 min in 25°C with 200 µl of 0,75 mM 5,5'-Dithio-bis-(-2-Nitrobenzoic acid) prior to the reaction which started by addition of 50 µl of 3 mM Acethylthiocholine iodide. Accordingly, the plate was read by photometry for microtiter plate (Spectra Thermo TECAN) in an interval of 30 s for 5 min at 405 nm. Four independent measurements of the ChE activity were carried out for each individual of *P. viridis*, and the average activity were calculated.

Protein |measurement for cholinesterase assay

Protein content measurement was carried out by diluting the gill extract 1:10 with distilled water. It was measured previously by placing 10 µl of the diluted extract and 10 µl of serial dilutions of γ-globuline protein standard into a separate well section of the microplate. A blank was made by placing 10 µl of distilled water into the blank section of the microplate. After the addition of 5% Bradford-reagent solutions (200 µl) into the microplate, the samples were left in room temperature for 20 minutes to allow color development. The absorbance was read at 620 nm using photometry (Spectra Thermo TECAN).

The ChE activity is expressed as nmoles of product developed per minute per mg of protein (nmol/min/mg protein). The ChE activity was measured on each tissue to recognize which tissue has the highest ChE activity.

Phagocytic activity

Phagocytic activity of hemocytes was determined by a microplate-based fluorescence measurement method (Hansen, 1992; Anderson and Mora, 1995). This method is based on the number of fluorescence labeled yeast cells that were phagocytosed by mussel hemocytes. The yeast cells were treated and labeled by Fluoresceinisothiocyanate (FITC) (Anderson and Mora, 1995) and kept in aliquots at -80°C. After withdrawing hemolymph from the PAM sinus of the mussels, 100 µl of hemolymph was dropped into 96-microplate wells. Five replicates were used to analyze the phagocytic activity and 3 replicates were used for the protein analysis. The density of hemocytes from each mussel was calculated by using a hemocytometer under a light transmission microscope. After the incubation of the plate for 30 min to allow hemocytes deposition at the bottom of the microplate wells, 25 µl of the FITC-labeled yeast was added into each phagocytic activity section of the microplate wells. A standard was made by adding 100 µl of phosphate buffer saline (PBS) and 25 µl of the FITC-labeled yeast into the microplate wells. One column (8 wells) was used as a blank section by adding 125 µl of PBS. The plate was incubated for 90 min in 21°C at dark condition. At the end of the incubation, 50 µl of 1 % glutaradehyd was added into each phagocytosis section of microplate wells, while 50 µl of methanol was dropped into the protein section of microplate wells. Before transferring to the laboratory in Germany, the plates were covered by a film and wrapped in aluminum and stored at 5°C in darkness. Accordingly, the fixatives were removed carefully and replaced by 125 µl of PBS when samples have arrived in the laboratory. For quenching the fluorescence background of unphagocytosed cells, 25 µl of 0.6 mg/ml trypan blue dissolved in PBS was added to each well of the microplate. The plate was incubated for 20 min prior to removing of all supernatants. The fluorescence of the ingested FITC-labeled yeast cells were read at excitation of 485 nm and emission of 535 nm using a microtiter plate fluorometer (Dynatech, Fluorolite 1000).

Protein measurement for phagocytic assay

A protein content measurement was carried out using hemocytes only. Prior to the measurement, the buffer was removed carefully and hemocytes were lysed with 50 µl of 0.1 N NaOH. After incubating the lysed hemocytes for 10 minutes in a shaking chamber, 10 µl of the lysed hemocytes and the serial dilutions of protein standard (Bovine Serum Albumin) were added to 96-microplate wells. Accordingly, 200 µl of 5% Bradford-reagent solution were added into the plate and incubated for 20 minutes to allow color development. The absorbance of protein was measured at 620 nm using photometer (Spectra Thermo TECAN).

Accordingly, the phagocytic activity was expressed as Relative Fluorescence Units (RFU) and finally calculated as a Phagocytic Index: RFU/mg hemocyte protein.

Statistical analysis

The statistical analyses were performed using non-parametric test, Kruskall-Wallis to distinguish the differences of ChE and phagocytic activity among the sites. If there were differences among the sites (p < 0.05), the test was continued by Dunn's multiple comparison test to determine the different between two sites. The statistical analyses were conducted using GraphPad Prism trial version 5.0 for Windows, GraphPad Software, San Diego California USA.

RESULTS

Cholinesterase activity

It has been reported that the ChE activity level differed among organs in marine mussels (Bocquené et al., 1990; Brown et al., 2004). The current study was started by recognizing which organ of green mussel, *P. viridis* that posses the highest ChE activity. It has been performed using *P. viridis* tissues from expected clean area. The results presented in Figure 3 demonstrated the median of the ChE activity in the gill which had the significant highest ChE activity namely 83.56 ± 12.19 nmol/min/mg protein followed by the foot (46.16 ± 4.18 nmol/min/mg protein), the mantle (27.35± 2.50 nmol/min/mg protein) and the PAM (4.94 ± 4.08 nmol/min/mg protein). Accordingly, the gill was used as a tissue target for measuring the ChE activity since the highest ChE activity of such organ should be the most suitable for measurement of the ChE activity inhibition (Bocquené et al., 1990; Escartin and Porte 1997; Valbonesi et al., 2003; Lau et al., 2004; Brown et al., 2004; Damiens et al., 2007; Taleb et al., 2009; Yaqin 2010).

Statistical analysis showed the difference ChE activity in the gills of the samples (p < 0.05) (Figure 4). The animals collected from the reference site had the significant highest ChE activity (83.56 ± 12.19 nmol/min/mg protein) followed by the green mussel collected from heavily polluted areas, Kamal Muara (49.92 ± 3.29 nmol/min/mg protein) and Cilincing (27.20 ± 1.80 nmol/min/mg protein). Between two heavily polluted sites, the animals inhabited in Kamal Muara showed significant less inhibition of the ChE activity than those from Cilincing (p < 0.05).

Phagocytic activity

In the present study, the phagocytic activity expressed as phagocytic index, and hemocytes numbers and total cell protein content were measured simultaneously. The results were represented in Figures 5 and 6. Statistical analysis of the median of circulating hemocytes numbers exhibited no difference numbers of hemocytes ranging
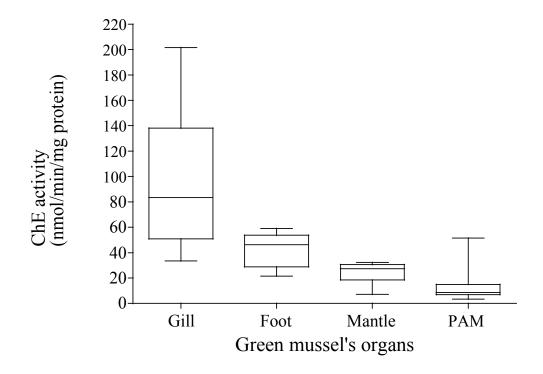
Figure 3. Cholinesterase activity of different organs of green mussel, *Perna viridis* from Pangkep Indonesia. Data were expressed as median (25 and 75 % quartile, 5 and 95 % confidence interval).
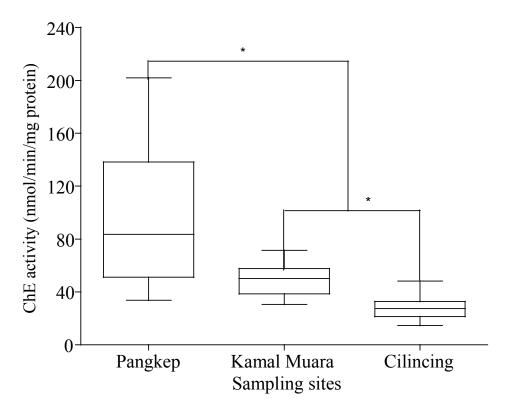

Figure 4. Gills ChE activity of green mussel, *Perna viridis* collected in the selected areas of Indonesian waters. Data were expressed as median (25 and 75 % quartile, 5 and 95 % confidence interval). * indicate significant difference ($p < 0.05$) of the gills ChE activity.
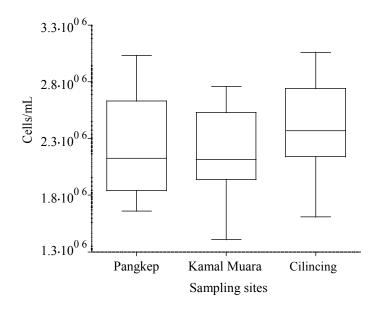
Figure 5. Circular hemocytes of green mussel *Perna viridis* collected in the selected areas of Indonesia waters. Data were expressed as median (25 and 75% quartile, 5 and 95% confidence interval).

Figure 6. Phagocytotic Index of green mussel *Perna viridis* collected in the selected areas of Indonesia waters. Data were expressed as median (25 and 75% quartile, 5 and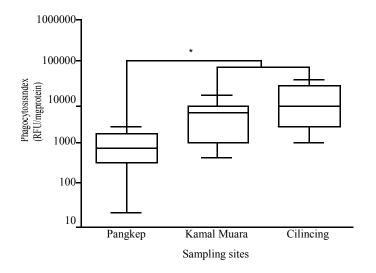 95% confidence interval). *indicate significant difference (p < 0.05) of the hemolymph phagocytic activity. Y-axis is logarithmic scale.

from 2,1250,000 to 2,370,000 cells/ml. In contrast, the median of the phagocytotic index demonstrated significant different phagocytic activities of *P. viridis* collected from gradient pollutions of Indonesian coastal waters (p < 0.05). The animals collected from the two heavily polluted sites in Jakarta Bay showed significant higher phagocytic index than those collected from reference sites. Nevertheless, there was no significant

different phagocytic index within the polluted site (p = 0.118). The highest phagocytic index was demonstrated in hemocytes of *P. viridis* from Cilincing (23410.10 RFU/mg protein) which followed by Kamal Muara (7566.84 RFU/mg protein) and reference site, Pangkep (1714.19 RFU/mg protein).

DISCUSSION

Cholinesterase activity

Cholinesterases (ChEs) are enzymes that hydrolyses and inactivates neural transmitter acetylcholine (ACh) for regulating neural transmission impulse in the synaptic gap of cholinergic synapses and neuromuscular junctions (Soreq and Seidman, 2001). ACh play an important role both as excitatory and inhibitory transmitters of the gill muscle of bivalve (Gainey et al., 2003). In blue mussel, *Mytilus edulis*, ciliary movement of the gill is controlled by acetylcholine, dopamine and 5-hydrotryptamine (Aiello, 1990). Organophosphorous and carbamate pesticides inhibit ChE activity which may lead to severe physiological impairment of marine animals (Dauberschmidit et al., 1997) such as reduction in feeding efficiency of marine mussels (Donkin et al., 1997).

Since ChEs was purified by Wachtendonk and Neef (1979) in marine mussels hemolymph, a measurement of ChE activity in marine mussels has been used as a biomarker in laboratory test (Galloway et al., 2002; Rickwood and Galloway, 2004; Canty et al., 2007; Yaqin and Hansen 2010) and several international monitoring programs in the field (Narbonne et al., 1999; Cajaraville et al., 2000; Dizer et al., 2001a,b; Roméo et al., 2003; Bocquené et al., 2004; Gagné et al., 2008).

Characterization of ChEs in bivalve has been conducted in some bivalves e.g. in *M. galloprovincialis* the ChE specific activity was predominantly localized in the gills compare to others organs (Mora et al., 1999; Porte et al., 2001; Taleb et al., 2009). Moreover, the ChE activity from *M. galloprovincialis* gill was observed more sensitive to organophosphorous pesticides than that from the digestive gland (Escartin and Porte, 1997). In *M. edulis*, Bocquené et al. (1990) found that the highest ChE activity occurred in the gill compare to others organs such as the hepatopancreas, the mantle and the adducent muscle. By characterizing and comparing the ChEs in different organ of *M. edulis*, Brown et al. (2004) found that 'mitochondrial' fraction of foot had the highest ChE specific activity with very low recovery of activity.

Accordingly, the gill 'microsomal' activity had the second highest ChE specific activity with useful level of recovery and therefore was the most suitable fraction for biomarker application. The highest ChE activity in the gill compared to others organs such as the adducent muscle and the digestive gland were observed in the Antarctic scallop, *Adamussium colbecki* (Corsil et al., 2004).

Compared to the foot, the gill of the bivalve, *Scapharca inaequivalvis*, demonstrated the higher specific ChE activity level as well (Romani et al., 2005). Eventually, Bonacci et al. (2008) observed that the highest ChE activity also occurred in the gill of scallop, (*Pecten jacobaeus*) compared to others organs which were the adducent muscle and the digestive gland. Kopecka-Pilarczyk (2010) observed that the ChE activity from gill of *Mytilus trossulus* was the most sensitive enzyme activity compared to the activity from others organs and whole body tissue when exposed to carbaryl and metals.

The current study compared the ChE activity of green mussel, *P. viridis* in different organs such as the gill, the foot, the mantle and the PAM. The results demonstrated that the gill of *P. viridis* had significant higher of the ChE activity compared to the foot, the mantle and the PAM. Porte and Albaiges (2002) demonstrated that the ChE activity from the gill of blue mussels, *Mytilus galloprovincialis* was more sensitive enzyme activity than that of digestive gland and it revealed a certain correlation with the concentration of fenitrothion in whole mussels. It has been reported that the gill of *P. viridis* collected from Hong Kong waters had the higher ChE activity than that of the whole tissue and this ChE activity was not size-dependent (Lau and Wong, 2003). This is conceivable because mussels use their gills not only as a respiratory apparatus but also as filter feeder organ thereby ambient water filtered and managed for gaseous exchanges and sifting food (Bayne et al., 1976). Since the gill are the front line of contact with contaminants and the first line of defense (Lau and Wong, 2003), detoxification compounds such as ChEs are necessary to be produced to protect other organs. Consequently, the production of ChEs not only provides as the control of neurotransmission, but also serves as contaminants detoxification particularly for organophosphorus and carbamate pesticides (Soreq and Seidman, 2001). In addition, it has been reported that the protein level of *P. viridis* gill was not seasonal dependent which lead to reduce the intrinsic variability of the biochemical responses in different growth phase throughout the year (Lau et al., 2004). Those evidences set up the gill as a *par excellence* tissue for biomarkers application to minimize effects caused by the natural reproductive cycles and the dilution effect due to large variation in the total tissue protein (Lau et al., 2004). The selection of the gill as tissue target for conducting biomarkers were also shown by the nature of the gill, which comes into contacts with relatively large volumes of seawater compared to the rest of the animal so that conferring them with the potential for being a suitable target tissue for xenobiotic substance exposure.

The present study used the gill of *P. viridis* to investigate pollutants effect to ChE activity in some coastal areas of Indonesia. The results suggested that the ChE activity was a sensitive tool to detect neurotoxic effects of pollutants since it could discern different levels

of two heavily polluted areas. It was supported by the evident that the ChE activity of the gill of *P. viridis* from reference site was significantly higher than that from the gill of *P. viridis* which inhabit two polluted sites. The inhibition of the ChE activity from the gill of *P. viridis* collected from Kamal Muara was about 49.2%. Statistically, the greatest inhibition of the ChE activity was indicated in mussels from Cilincing, which was about 72.41%. By exposing brown mussels, (*Perna perna*) to furadan (carbamate pesticide), Alves et al. (2002) observed that the ChE activity of the gill was suppressed by 35%.

Ludke et al. (1975) classified the percentage of ChE activity inhibition based on comparison of the individual value with the activity of the normal population for providing the interpretation of the environmental risk. The following are the risk criteria of inhibition percentage of ChE activity that were proposed by Ludke et al. (1975):

0 to 20% = zone of normal variation
20 to 50% = presence of exposure or zone of reversible effects
50 to 100% = life-threatening situation or zone of irreversible effects

In respect to estuarine fishes, Coppage (1972) suggested that inhibition level of the ChE activity in the range of 20 to 70% could be classified as an indication of organophsophorous exposure. Subsequent studies observed that the inhibition of the ChE activity in the fish brain, which reached 70 to 90% indicated mortality (Coppage et al., 1975; Coppage and Matthews, 1975). Sandahl et al. (2005) observed that the inhibition of the ChE activity in brain and muscle from juvenile coho salmon (*Oncorhynchus kisutch)* was correlated well with the behavior disruption, that is, feeding and swimming ability when the fish were exposed by chlorphyrifos. At the lowest concentration (0.6 µg/l), chrlophyrifos caused 12% inhibition of the muscle ChE activity reducing 27 % of the swimming rate, while no mortality was observed when fish exposed by the high concentration (2.5 µg/l) inhibiting 67% of muscle's ChE activity. By conducting microcosm study using mixtures of selected organophosphorous pesticides, Sibley et al. (2000) observed that 10% mortality was correlated with approximately 50% inhibition of AChE activity, while 50% mortality was correlated with approximately 90% inhibition of AChE activity of fathead minnows. Fleming et al. (1995) found the die-off freshwater mussels (*Elliptio steinstansana*) from sites that were influenced by agricultural activities with the inhibition of ChE activity from 65 to 73% compared to the reference site. Based on the criteria and the results of those studies, it is suggested that discharged pollutants into coastal area of Jakarta Bay indicated neurotoxic compound causing from reversible to irreversible effects of the neurological activity of the green mussel population. By compiling the

data of ChE activity from the research above which ranging from bird to freshwater mussel it is suggested that the green mussels which populated in Kamal Muara indicated reversible effects, while those from Cilincing showed irreversible conditions.

The link between the inhibition of ChE activity of sentinel organism and the discharged neurotoxic compounds from agricultural, urban and industrial activity to aquatic environment has been suggested by many studies (Fulton and Key 2001; Printes and Callaghan 2004; Galloway et al., 2002; Crane et al., 2002; Rickwood and Galloway, 2004; Canty et al., 2007; Warberg et al., 2007). However, the relationship between ChE activity and higher level biomarker such as feeding rate in green mussel has not been studied yet. Therefore, a chronic *in vivo* study on the response of ChE activity in green mussel and other behavioral biomarkers such as feeding rate to the serial concentrations of pollutants, which picturize suspected pollution area concentrations, is indispensable to translate the inhibition of ChE activity induced by pollutants into ecological perspective. The translatable of ecological consequence of the suppressed ChE activity is a vital consideration in ecological risk assessment in the coastal zone. It is because an appropriate ecological relevance of biomarkers can eliminate the primary source of uncertainty in application of ecological risk assessment (Sibley et al., 2000).

Phagcytosis activity

Green mussel hemolymph contains both hemocyte and humoral defense factors which are responsible for the defense system. Hemocytes circulating in hemolymph are the principal cellular effectors of invertebrate immunity (Mitta et al., 1999) which have a capability to perform phagocytosis of foreign materials (Cheng, 1984; Carballal et al., 1997) and cytotoxycity via the production of radicals (Winston et al., 1996).

Phagocytosis of mussel hemocytes can be affected by various chemical stressors in the aquatic environment (Anderson and Mora, 1995). Biphasic patterns of mussel phagocytic responses induced by xenobiotic have been demonstrated in many laboratory studies (Cole et al., 1994; Pipe et al., 1999; Parry and Pipe, 2004). Theoretically, the phagocytic activity will be stimulated when mussels are exposed to low level of contaminants, while it will be suppressed when mussel are exposed to high level of contaminants. Consequently, measurement of the phagocytic activity, which is as part of immune system of mussel, has been used as a biomarker of xenobiotic substances effect (Anderson and Mora, 1995; Oliver and Fisher, 1999; Blaise et al., 2002; Gagné et al., 2002).

In spite of mussel hemocytes playing an important role in the phagocytic activity, it is difficult to depict the correlation pattern between circulated hemocytes number

and the phagocytic activity of mussel. The current study showed that there was no different numbers of circulating hemocytes of green mussel, which were collected from both polluted and clean sites. However, significant differences of the phagocytic activity between the collected green mussels from polluted sites and those from clean site were evident. The data showed that discharged pollutants in Jakarta Bay have stressed cultivated green mussels, which stimulated significantly their phagocytic activity compared to the phagocytic activity of the green mussels collected from the clean site. The modulation of mussel phagogytic activity was in accordance with Luengen et al. (2004) who observed the elevation of phagocytic activity of mussels that collected from polluted sites. The elevation of phagocytic activity induced by the pollutants may be a part of mussel's strategy to sequester the toxic materials from vulnerable organs (Oliver et al., 2001). Nevertheless, Dizer et al. (2001b) found that high number of circulating hemocytes of mussels collected from control site followed by relatively low phagocytic activity, while relatively low number of hemocytes from polluted sites had a high phagocytic activity. They could not depict clearly the relationship between hemocytes number and the phagocytic activity of mussels.

The complicated relationship between hemocytes number and the phagocytic activity of mussels may result from dynamic association/dissociation between hemocytes and bivalve tissues that enable to change the total size of the hemocytes population within bivalve body over short time (Ford et al., 1993). The population could not be simply depicted by circulating number of hemocytes, which were drained from the PAM sinus as the mussel has the open circulatory blood system, which circulate the blood to whole organs. In addition, commonly the mussel hemocytes are composed by phagocytotic and unphagocytotic hemocytes which can be altered by xenobiotic substances (Pipe et al., 1999). Unfortunately, most of the techniques to measure the phagocytic activity including the technique used in the present study were based on the mixture of hemocytes sub-population so that an estimation of capability level of each sub-population of hemocytes was not possible.

Although, the present study enabled to distinguish the phagocytic activity of green mussels dwelled in polluted and clean sites, the difference of the phagocytic activity within the polluted site could not be differentiated significantly. Having taking into account the data from the ChE activity, which enable to distinguish the magnitude effects of released pollutants within the polluted sites, it is tempting to suggest that released pollutants in Jakarta Bay seem to be ChEs inhibitors, which raised greater impact on the ChE activity rather than the phagocytosis activity. For that purpose, the chemical analysis of water/sediment samples and relevant pollutants within mussel's tissue should be taken into account. Regardless of the chemical analysis

approach, the ChE activity indicated a more responsive tool compared to the phagocytic activity so that it could distinguish between two heavily polluted sites. However, it is hard to justify that the ChE activity is more sensitive compared to phagocytic activity as was observed by Perez et al. (2004) in ChE activity of invertebrates, *Scrobicularia plana* (clam) and *Nereis diversicolor* (marine worm). The authors delineated higher sensitivity of ChE activity compared to others biomarkers that were used in biomonitoring of Spain waters. Therefore, the useful results that recorded by the current study are the information on neurotoxicity and immunotoxicity compounds which were present in Jakarta Bay and the magnitude impact of neurotoxicity contaminants to induce an effect is greater than the immunotoxicity contaminants.

Conclusion

Conclusively, the results suggested that the use of the selected biomarkers is a reliable and preferential strategy in the ecological risk assessment of released xenobiotic compounds in coastal waters due to their ability to elucidate bio-effects of neuro-immuno systems disruptors.

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