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Antioxidant Enzyme Activities and Malondialdehyde Level in Green Mussel (*Perna viridis* L.) at Jakarta Bay, Indonesia

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Abstract. Jakarta Bay water has endured severe pollution load, and the water is being severely polluted; however, green mussels (*Perna viridis*) can survive. This study aimed to evaluate whether green mussels at Muara Angke, Jakarta Bay have endured oxidative stress by measuring enzyme activities, including catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx), and malondialdehyde (MDA) level. The control population was green mussels obtained from Panimbang, Pandeglang, Banten. The measurement of SOD, catalase, GPx, enzymes, and MDA level was performed using a spectrophotometer. Data were analyzed using Mann Whitney test, Spearman Rank correlation test, and followed with the Z-Fisher test. Results of the study showed that: (1) No significant different activity of SOD enzyme in green mussels of both area; (2) there was the significantly higher activity of catalase enzyme in green mussels of Muara Angke, Jakarta Bay compared to those of Panimbang, Banten area; (3)there was a significantly lower activity of GPx enzyme in green mussels of Muara Angke, Jakarta Bay; (4) the MDA level was significantly higher in green mussels of Muara Angke, Jakarta Bay; (5) the correlation coefficient for enzyme activities of SOD, catalase, GPx, and MDA level was not significant in green mussels in Muara Angke, Jakarta Bay and Panimbang, Banten. The high MDA level and low SOD and GPxactivities indicate that the green mussel of Muara Angke, Jakarta Bay have experienced oxidative stress. MDA level and GPx enzyme activity can be used as biomarkers for oxidative stress in green mussels of Muara Angke, Jakarta Bay.

INTRODUCTION

The growing number of citizens and industrial development has increased the pollution load in the marine ecosystem of Jakarta Bay. Widdows (1985) said that monitoring should be performed about various physiological responses, which occurs as the impact of pollutants on the marine environment [1]. According to Arifin et al. (2012), monitoring of pollution load on heavy metal in Indonesian marine regions has been performed since 1979 [2]. Results of measurement in 2004 showed that the level of Pb in the seawater of Jakarta Bay was 0.0002 ppm; while in the sediment, it was 18.7 mg/Kg dry weight. According to Cordova (2016), the level of Pb in sediment increased into two folds in 2016, i.e. 35.65±12.71 mg/Kg dry weight [3]. Suratno (2020) said that the average concentration of arsenic in clam *Periglypa reticulata* in Lancang Island, Jakarta, was higher than the limit of permissible standard National Agency of Drug and Food Control [4]. Asaduzzaman (2019) said that the gonadosomatic index of green mussel (*Perna viridis*) showed a strong correlation with the parameters of water quality [5].

Measurement of physiological responses on the affected organism should be done to identify the impact of pollution. The measurement must fulfill some criteria such as sensitivity to pollution, can predict its correlation with pollutants, can be precisely measured, and it should be correlated with the survival of an organism [1]. The pollution

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occurs at Muara Angke, Jakarta Bay affects the life of organisms; however, green mussels (*Perna viridis*) can have an adaptive life there.

A polluted environment can affect the life of green mussels, as has been mentioned by Cheung and Cheung (1995) that exposure of Cd and Zn for 21 days can reduce oxygen consumption significantly in green mussels (*Perna viridis*) [6]. Moreover, Gorinstein (2003) measured antioxidant activities in black mussels (*Mytilus galloprovincialis*), and he found that there was higher antioxidant activity in the polluted areas compared to a relatively clean area [7]. Mocheva et al. (2004) suggested that antioxidant activities in mussels can be used as a physiological biomarker for the water environment [8]. Shuhong et al. (2005) found that there was a significant negative correlation between the scope for growth (SFG) and dichlorodiphenyltrichloroethane (DDT) levels in green mussels [9]. It indicates that in polluted water, the growth of green mussel becomes slow.

Vlahogianni (2007) concluded that the activity of an antioxidant enzyme, superoxide dismutase (SOD), increased two folds, while the activity of catalase increased 2 to 3 folds and lipid peroxide levels increased two folds in black mussel (*Mytilus galloprovincialis*) living in polluted water [10]. Richardon et al. (2008) exposed green mussels to polycyclic aromatic hydrocarbons (PAHs) and measured the activity of the antioxidant enzyme after four weeks. From the experiment, it can be concluded that catalase and glutathione (GSH) are biomarkers that can be used to detect physiological responses against pollutants [11]. Krishnamoorthy et al. (2019) said that *Perna viridis* showed antioxidant capacity [12].

Lushchak (2011) suggested that heavy metal pollution such as Hg, Pb, Cu, and Cr, and pesticides such as insecticides, herbicides, and fungicides can induce the development of oxidative stress [13]. Increased free radicals and reduced antioxidant characterize oxidative stress. Vosloo et al. (2012) also found that copper exposure (Cu) to brown mussel (*Perna perna*) increased reactive oxygen species (ROS) level and caused deoxyribonucleic acid (DNA) damage [14]. Verlecar (2012) suggested that green mussel (*Perna viridis*) that endured oxidative stress due to exposure to water-accommodated fractions (WAFs) of petroleum had increased levels of lipid peroxides and hydrogen peroxides [15].

Chainy (2016) and Chakraborty (2016) suggested that invertebrates have an antioxidant defense to protect themselves from damaging effects caused by increased reactive oxygen species (ROS) [16,17]. Free radicals that are formed inside the organism's body will react with the lipid of the cell membrane that develops into lipid peroxides, and it will be further degraded into malonaldehyde or malondialdehyde (MDA) [18]. The development of MDA is an indicator of cell damage, which subsequently causes tissue damage, and it can cause the death of the organism.

In order to overcome the effect of free radicals, cells will increase the production of antioxidant enzymes such as SOD, catalase, and GPx. Organisms that can deal with the impact of free radicals by increasing the activities of antioxidant enzymes have also been assumed to be able to have adaptive life in polluted water, such as the Jakarta Sea. Green mussels can adapt well in the Jakarta Bay water. Therefore, a study that analyzes the activities of antioxidant enzymes, which are SOD, catalase, GPx, and MDA level, should be carried out.

The present study aimed to analyze the activity of antioxidant enzymes of SOD, catalase, GPx, and MDA level in green mussels and to identify whether there is any correlation between antioxidant enzymes activities (SOD, catalase, GPx, and the MDA level in green mussels at Muara Angke, Jakarta Bay with the control population of green mussels in Panimbang, Pandeglang, Banten. The mussels living in Panimbang, Pandeglang, Banten were selected as a control since they live in clean seawater, and the land is still covered by many forests, gardens, rice fields; moreover, the human population is not crowded. There are relatively only a few fisherman boats, and it is quite far from the industrial area.

Meanwhile, in the Muara Angke Sea at Jakarta Bay, there is a heavy environmental load, and the land is crowded with the urban citizen. The estuaries are full of domestic sewage, industrial, and transportation waste. There are many fisherman boats and other boats for water transportation. With such a condition, Muara Angke at Jakarta Bay is an environment with severe environmental load, and it is highly polluted.

This study assumes that green mussels that live at Jakarta Bay have experienced oxidative stress, i.e. they have high oxidant levels and low antioxidant levels. Pollution causes the development of reactive oxygen species (ROS) such as superoxide radical ($O_2^{\bullet}$), hydroxyl radical ($OH^{\bullet}$), and hydrogen peroxide ($H_2O_2^{\bullet}$) in green mussels. Radical oxygen and ROS can react with the main components of the cells, which will cause tissue damage leading to oxidative stress. Radical oxygen can oxidize double-bond unsaturated fatty acid on the cell membrane that develops lipid peroxides, and it can be degraded into malondialdehyde [18]. However, the green mussel can be adaptive to the environment of Muara Angke, Jakarta Bay. Therefore, it is assumed that green mussels at Muara Angke, Jakarta Bay can overcome the effect of oxidative stress by increasing the activities of the antioxidant enzyme, i.e. SOD, catalase, GPx and to deal with the high levels of lipid peroxides that forming MDA.

METHOD

Green Mussels Sampling Site

Green mussels (*Perna viridis*) were obtained from 2 sites, i.e. Muara Angke, Jakarta Bay (with location coordinate of 6º 6'20.50" S, 106º 46'41.26"E) and Panimbang, Pandeglang, Banten (6º 31'58.31"S, 105º 37'13/23"E). The green mussels were put into a cool box containing ices, and they were taken to the Animal Physiology laboratory, Faculty of Mathematics and Science, Universitas Negeri Jakarta, and the samples were kept in a freezer at -20ºC.

(a) (b)

FIGURE 1. (a) Sampling site at Muara Angke, Jakarta Bay (6º 6'20.50"S, 106º 46'41.26" E) and Panimbang, Banten (6º 31'58.31"S, 105º 37'13.23"E).(b) Green mussels sampling at Muara Angke, Jakarta. The samples were obtained from dolosse, while in Panimbang, Banten, they were obtained from ropes and bamboo of the green mussel culture sites.

Sample Preparation

The tissues of green mussels were crushed using Elvejehm voter into fine cuts. About 3 grams of fine tissue was weighed, and 5 mL of tris-buffer saline solution (TSS) was added, and the specimen was crushed further using voter into very delicate pieces. After it became a very delicate specimen, another TSS was added so that the volume became 30 mL. It was centrifuged at 4500 RPM for 15 minutes at 4º C. The supernatant was taken as a sample, and it was put into a reaction tube. The tube was securely sealed and kept in a freezer at -20º C until the measurement of the parameter was conducted.

Measuring Parameters

Superoxide Dismutase (SOD)

The principle for measuring SOD was using xanthine and xanthine oxidase to produce superoxide anion radicals that reacted with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form red formazan dye. SOD inhibited the reaction by altering superoxide radical into oxygen.

SOD activity was measured using a spectrophotometer with 505 nm wavelength using Superoxide Dismutase Ransod Kit, Cat. No. SD 125 by Randox. The measurement of SOD was performed at Biochemistry Laboratory, Faculty of Medicine, University of Indonesia.

Catalase

The principle for measuring catalase enzyme activity was the Mates method using a spectrophotometer at 210 nm wavelength. The measurement was performed at Biochemistry Laboratory, Faculty of Medicine, University of Indonesia.

Glutathione Peroxidase (GPx)

The activity of glutathione peroxidase (GPx) was measured with Ransel Glutathione Peroxidase Cat. No. RS 504 Kit from Randox using a spectrophotometer at 340 nm wavelength. The measurement of GPx was performed at Biochemistry Laboratory, Faculty of Medicine, University of Indonesia.

Malondialdehyde (MDA)

The principle for measuring malondialdehyde (MDA) was on a reaction that two molecules of TBA bound with one molecule of MDA, resulting in TBA-MDA-TBA complex, which had pink color. MDA was measured using a spectrophotometer at 532 nm wavelength. The sample was previously heated at a boiling temperature in 72% trichloracetic acid (TCA) to precipitate the protein, and 0.67% thiobarbituric acid (TBA) served as a chromogen. The measurement of MDA was performed at Animal Physiology Laboratory, Faculty of Mathematics and Science, Universitas Negeri Jakarta.

Data Analysis Technique

Since our data had no normal distribution, the data were analyzed using Mann Whitney test to identify the mean difference of SOD, catalase, GPx, enzyme activities, and MDA level. Spearman rank correlation test and Z-Fishertest to determine the correlation coefficient difference between SOD, catalase, GPx antioxidant enzyme activities, and MDA level.

RESULT AND DISCUSSION

Based on the results of measurement, the following data were obtained: The sample size of each group were nine green mussels (*Perna viridis*) from Muara Angke, Jakarta, and Panimbang, Banten. The activity of superoxide dismutase in green mussels from Muara Angke was 1.656±0.881 unit/mg protein, and it was 1.173±1.173 unit/mg protein in the green mussels from Panimbang, Banten. The activity of the catalase enzyme in green mussels from Muara Angke, Jakarta was 0.017±0.007 unit/L, and it was 0.010±0.004 unit/L .in green mussels from Panimbang, Banten. The activity of gluthation peroxidase enzyme in green mussels from Muara Angke, Jakarta was 4,182.630±1,543.390 unit/L, and it was 8,972.800±515.130 unit/L in the green mussels from Panimbang, Banten. The malondialdehyde level in green mussels from Muara Angke, Jakarta was 31.926±2.646 mMol/g of tissues, and it was 12.927±2.533 mMol/g of tissues in the green mussels from Panimbang, Banten.

Results of Mann Whitney test showed that SOD activity in green mussels of Muara Angke and Panimbang, Banten was not significantly different ($p = 0.222$). Catalase activity in green mussels of Muara Angke, Jakarta was significantly higher compared to those of Panimbang, Banten ($p = 0.031$). The activity of gluthation peroxidase enzyme in green mussels of Muara Angke, Jakarta, was lower than those of Panimbang, Banten in a very significant way ($p = 0.000$). The MDA level in green mussels at Muara Angke was higher, very significantly compared to those at Panimbang, Banten ($p = 0.000$). The result Mann Whitney test of SOD, catalase, GPx enzyme activities, and MDA level in green mussels (*Perna viridis*) at Muara Angke, Jakarta, and Panimbang, Banten are shown in the following Table 1.

TABLE 1. Results of Mann Whitney on mean difference of catalase, glutathione peroxidase, superoxide dismutase enzyme activities and MDA level in green mussels (Perna viridis) at Muara Angke, Jakarta and Panimbang, Banten, n=9

Variables	Muara Angke, Jakarta mean ± SE	Panimbang, Banten mean ± SE	Sig (p)
Superoxide dismutase activity (Unit/mg Protein)	1.656 ± 0.881	1.173 ± 1.173	0.222^{ns}
Catalase activity (Unit/L)	0.017 ± 0.007	0.010 ± 0.004	0.031*
Gluthation peroxidase activity (Unit/L)	4,182.630 ± 1,543.390	8,972.800 ± 515.130	0.000**
Malondialdehyde level (mMol/gram tissue)	31.926 ± 2.646	12.927 ± 2.533	0.000**

Note: n.s. = non significant
* = significant
** = highly significant

Increased catalase enzyme activity and MDA level significantly and reduced GPx activity indicate that green mussels at Muara Angke, Jakarta Bay, had experienced oxidative stress. SOD enzyme activity in green mussels at Muara Angke, Jakarta Bay, increased; however, the rise was not significant when it was compared to the increased of SOD enzyme activity in green mussels of Panimbang, Banten.

GPx enzyme activity in green mussels (*Perna viridis*) at Muara Karang, Jakarta Bay, was significantly lower compared to green mussels at Panimbang, Banten. It also indicates that green mussels at Muara Angke, Jakarta Bay, had experienced greater oxidative stress. In an oxidative stress condition, which has increased oxidant and reduced antioxidants, the survival of an organism is threatened, and it may cause death. However, green mussels have interestingly survived at Jakarta Bay. Increased catalase enzyme activity is a physiological adaptation to overcome the high level of lipid peroxidation of the cell membrane, which is further degraded into MDA. It is obvious from the results of the MDA level in green mussels at Muara Angke, Jakarta, which was extremely high.

To identify whether the increase of MDA was followed by increased activity of the antioxidant enzyme, an analysis was performed on the correlation between SOD, catalase, GPx activities, and MDA level. Based on the review of Spearman's rank correlation coefficient, a result was obtained that SOD and catalase enzyme activities and MDA levels in green mussels at Muara Angke, Jakarta Bay, had a positive correlation; while the GPx activity and MDA level had a negative relationship. Moreover, the relationship between catalase and GPx enzyme activities and MDA level in green mussels at Panimbang, Banten was negative, while SOD activity and MDA level had a positive relationship. However, all of those correlation coefficients were not significant. Results of Spearman's rank correlation coefficient test are presented in Table 2 as follows:

TABLE 2. Spearman's Rank correlation coefficient on SOD, catalase, glutathione peroxidase enzyme activities, and MDA level in green mussels (Pernaviridis), n = 9.

Enzyme activities	Muara Angke, Jakarta Malondialdehyde (MDA)	Sig (p)	Panimbang, Banten Malondialdehyde (MDA)	Sig (p)
Superoxide Dismutase (Unit/mg Protein)	0.510	$0.160^{n.s}$	0.368	$0.330^{n.s}$
Catalase (Unit/L)	0.399	$0.287^{n.s}$	-0.156	$0.689^{n.s}$
Gluthation Peroxidase (Unit/L)	-0.462	$0.211^{n.s}$	-0.405	$0.279^{n.s}$

Note: n.s. = non significant

Z-Fisher test was performed to identify whether there was any difference in the correlation coefficient between antioxidant enzyme activities and the MDA level. From the results of the Z-Fisher test, it is concluded that there was no significant difference in the correlation coefficient between SOD, catalase, and GPx enzyme activities, and MDA level in green mussels at Muara Angke, Jakarta and those at Panimbang, Banten. Results of the Z Fisher test are presented in the following Table 3.

TABLE 3. Results of the Z Fisher test on the difference of correlation coefficient of SOD, catalase, GPx enzyme activities, and MDA level in green mussels (Pernaviridis) at Muara Angke, Jakarta, and Panimbang, Banten, n = 9.

Enzyme activities	Muara Angke, Jakarta Malondialdehyde (MDA)	Panimbang, Banten Malondialdehyde (MDA)	Z Fisher	Sig (p)
Superoxide Dismutase (Unit/mg Protein)	0.510	0.297	0.31	0.757$^{n.s.}$
Catalase (Unit/L)	0.399	0.115	1.00	0.317$^{n.s.}$
Gluthation Peroxidase (Unit/L)	-0.462	-0.600	- 0.12	0.905$^{n.s.}$

Note: n.s. = non significant

This study results that there was a significantly higher level of MDA in green mussels of Muara Angke, Jakarta Bay compared to those of Panimbang, Banten (p = 0.000). Increased MDA level was followed by a significant increase in catalase enzyme activity. However, there was a significantly lower GPx enzyme activity in green mussel at Muara Angke, Jakarta Bay. The SOD enzyme activity was not significantly different. This result was relevant to Gurkan (2020) [19]. This condition indicates that the green mussels at Muara Angke, Jakarta Bay have experienced oxidative stress. The results are consistent with those of Gorinstein's study (2003), which concluded that antioxidant activity in black mussel (*Mytilus galloprovincialis*) was higher in polluted areas than in the cleaner area[7]. Nevertheless, the activity of GPx enzyme in green mussels of Muara Angke, Jakarta, was significantly lower compared to those at Panimbang, Banten. GPx is an enzyme that catalyzes the reaction of 2GSH + ROOH into ROH + GSSG + H_20. It is consistent with Pena-Llopis's findings (2002), which suggested that in mussels exposed to organophosphate pollutants, there is an increased level in reduced glutathione (GSH) and oxidized glutathione (GSSG) in the digestive gland, muscle, and gill of the mussels [20].

Pollution at Jakarta Bay causes a reduced oxygen level in the water. Depleted oxygen level induces the development of hypoxia in green muscle tissues. Rusdi et al. (2012) suggested that hypoxia conditions may enhance the development of reactive oxygen species (ROS) through the hypoxanthine reoxygenation pathway [21]. Pena-Llopis (2001) also suggested that ROS, such as superoxide radical ($O_2^{\bullet}$), hydroxyl radical ($OH^{\bullet}$), and hydrogen peroxide ($H_2O_2^{\bullet}$) are very reactive.[20] ROS is a standard physiological product, which is produced through cell respiratory, pathological condition, and toxicological effect due to oxidative stress conditions. Rathod (2009) and Radovanovic (2010) suggested that ROS can react with the main cellular component; therefore, it will cause damage to the tissues [22,23]. Radical oxygen can oxidize protein, DNA, and including reacting with double-bond unsaturated fatty acid on the cell membrane producing lipid peroxide, and it is further degraded into MDA. Hardiani (2019) suggested that oxidative stress is a condition of imbalance between ROS and antioxidants [24]. Prego-Faraldo et al. (2017) said that when the cell in a situation of experiencing oxidative stress, i.e., when there is increased oxidant and reduced antioxidant, then the cell will increase the activity of antioxidant enzymes such as SOD, catalase, and GPx, and reduced lipid peroxidation [25].

Box et al. (2008) suggested that mollusks have an antioxidant defense mechanism, that has a function for neutralizing endogenous and exogenous ROS [26]. Radovanovic (2010) said that free radicals in the cells would be neutralized by an antioxidant enzyme such as SOD, which alters superoxide anion (O2•-) into H_2O_2. Furthermore, H_2O_2 will be changed into H_2O and O_2, which is catalyzed by catalase[23]. GPx detoxifies H_2O_2 and hydroperoxides (ROOH) using reduced glutathione (GSH) as a cofactor. The oxidant-antioxidant balance is managed; therefore, cellular damage can be minimized. The high level of MDA in green mussels of Muara Angke, Jakarta Bay, can be balanced with increased catalase activity to overcome the effects of oxidative stress.

Increased MDA level, which is the final product of lipid peroxidation of cell membrane into lipid peroxide that is further degraded into MDA, is a biomarker of oxidative stress. Therefore, it can be concluded that green mussels at Muara Angke, Jakarta Bay have experienced oxidative stress, which is proven by increased MDA levels. However, the green mussels can increase antioxidant enzyme activities such as catalase, which enable them to have an adaptive life in the polluted water of Muara Angke, Jakarta Bay.

CONCLUSION

The activity of the catalase enzyme is significantly higher, and SOD activity is higher but not significantly in green mussels living at Muara Angke, Jakarta Bay, with a condition of polluted seawater compared to green mussels of Panimbang, Banten with cleaner seawater condition. Furthermore, the MDA level is also significantly higher in green mussels living at Muara Angke, Jakarta Bay than those of Panimbang, Banten. GPx level is significantly

lower in green mussels living in Muara Angke, Jakarta Bay, compared to those of Panimbang, Banten. Therefore, it can be concluded that green mussels at Muara Angke, Jakarta Bay have experienced oxidative stress. Yet, they can survive in the polluted sea as they can increase antioxidant enzyme activities, which are the catalase and SOD. Catalase activity and MDA level can be used as biomarkers of oxidative stress in green mussels.

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