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Research Article
Toxicological Analysis of Gonad Development in Green Mussels (*Perna viridis*) in Jakarta Bay, Indonesia

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Abstract

Background and Objective: The accumulation of heavy metals such as cadmium and lead in mussels is very high compared to that in another marine biota. The mussels are sessile, widely distributed filter-feeding organisms, with the ability to sequester many lipophilic organic compounds, absorb anything in their surroundings. The very low mobility allows heavy metal bioaccumulation to occur and cannot avoid pollutants, which increase over time. This bioaccumulation can be toxic to mussels. This study aimed to evaluate the effect of different toxic chemicals and histological changes in green mussels. **Materials and Methods:** All archive gonad sample of green mussels in 2015 was fixed in paraformaldehyde 4% solution and were sliced by a rotary microtome at 8 µm thickness and finally, the slides were stained with a solution of hematoxylin-eosin. **Results:** The obtained results demonstrated that developmental disorders in the testes are characterized by the arrangement of follicle cells in a relatively less dense state and some follicles are not fully filled with spermatozoa. It means that the male gonad samples of green mussels in the port of Muara Angke undergoing toxicity and the process of gonad developmental was disrupted. **Conclusion:** The effects of toxicity of the male gonad of green mussels were more sensitive and were more susceptible than the female gonad of the green mussels.

Key words: Follicle cells, green mussels, male gonad, sensitive, susceptible, toxic, bioaccumulation

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Jakarta Bay has become contaminated by both organic and inorganic pollutants, including heavy metals. The heavy metal pollution can effect on shells malformation of the green mussels cultured in Jakarta Bay[1]. Bioaccumulation of mercury, cadmium and lead has been detected in the hepatopancreas, gills and muscles of mussels and bioaccumulation has also been detected in the gonads from mussels in ponds and ports of Muara Angke. In male and female gonads, concentrations of lead and cadmium are still below the threshold, but mercury concentrations in male gonads ($0.06 \geq 0.01$) are relatively high[2]. Bioaccumulation of heavy metals is influenced by the amount of uptake and excretion of mussels. Bioaccumulation and contamination of heavy metals in mussels are of considerable interest because mussel consumption is an important source of metals for humans.

Mercury is easily oxidized to divalent mercury ions (Hg^{2+}). This form can bind to nucleosides, thiol groups, glycine and other amino acids under certain physiological conditions to form complex compounds[3,4]. The methyl mercury is produced through the methylation of Hg^{2+} by bacteria in the digestive system of marine organisms before absorption can bind to amino acids[5]. Mercury is usually used in the gold extraction process. This process has occurred since antiquity and is still used mainly by artisanal or small-scale gold miners in Indonesia, even in most of South America, Africa and the Philippines[6,7]. This metal is released into the environment at approximately one to two grams of Hg per gram of gold produced. During the tailings process, mercury is released onto land or into rivers. The mean mercury concentration of tailings is approximately 4600 mg kg^{-1} [8]. Cadmium concentration increases 3,000 fold when it binds to cysteine-rich protein such as metallothionein. In the liver, the cysteine-metallothionein complex circulates and then to gets accumulated in the renal tissue. The same oxidation states on cadmium and zinc cause the cadmium can replace zinc, to binds the metallothionein protein. this condition can lead to the deficiency of iron[9]. The low level Pb exposure can affect for DNA damage, where is chronic exposure to heavy metals may act alone or in combination over time to cause disease[10]. Cadmium and lead have long been known to accumulate in the aquatic food chain. Because Cd, Cu, Pb and Zn are widely distributed in coastal environments, due to both natural geological processes and anthropogenic activities, these metals are well-known in terms of public health and these metals easily accumulate in mussels. Mussel natural habitats are intertidal areas, usually close to an estuary. Possible exposure to heavy metals and contaminants from land-based activities through river systems is very high and exposure can also occur due to sea-based sources. The Impact of bioaccumulation of cadmium and lead in class bivalves can cause DNA damage[11,12].

Bioaccumulation of lead, mercury and cadmium can cause a decrease in the bodyweight of green mussels, which can indirectly cause interference with gonad development[2]. The lead and cadmium can cause a reduction in luteinizing hormone and follicle-stimulating hormone binding, which significantly alters steroid production and exerts a direct influence on granulosa cells[13]. Low-level exposure to cadmium in both males and females can interfere with the biological effects of steroid hormones in reproductive organs[14], such as abnormal spermatocytes and oocytes and shrinkage in the size of gamete cells and lysis cells[15]. The bioaccumulation of mercury, cadmium and lead in the body has reached the level of tissue, even at the cellular level. The complex of nonessential metal ions with ligands can disturb the balance of essential metal ions in the body. This can disrupt the balance of metabolic processes. Finally, cell division and protein synthesis can be disrupted. However, whether the effects bioaccumulation of mercury, cadmium and lead on histological changes on both male gonads and female gonads of mussels are persistent or not, it is still unknown. Therefore, it is necessary to conduct research to determine the effect of lead, mercury and cadmium bioaccumulation on the toxicity and histological changes of gonads in green mussels.

MATERIALS AND METHODS

Study area: All archive gonad sample collections of the green mussels were from the port and the pond of Muara Angke, the pond of Panimbang, Jakarta, Indonesia, in 2015 and they were stored in a freezer at -10°C in block paraffin form. The experiment was done in Animal Developmental Laboratory, Faculty Mathematics and Natural Science, Universitas Negeri Jakarta, Jakarta, Indonesia. The research was conducted from September-October, 2015 and gonad incision in August, 2020.

Materials: All archive gonad sample collections of the green mussels consisted of 30 individuals of similar sizes (shell length 7-8 cm). The sex of P. Viridis was distinguished based on the color of the gonads.

Methodology: All archive gonad sample collections of the green mussels were fixed in paraformaldehyde (PFA 4%) solution. Dehydration in alcohol gradually increased the concentration. Clearing was performed in a solution of alcohol-xylol and xylol-pure. Paraffin infiltration was

performed in an oven at a temperature of 57-60°C. Embedding the specimens was carried out in rectangular-metal containers, containing paraffin. The archive sample collections of the green mussels were from the ponds of Panimbang and the port and ponds of Muara Angke in Jakarta Bay, Indonesia, in 2015 and they were stored in a freezer at -10°C in block paraffin form.

Histological sections (8 μm thick) were cut on a rotary microtome and then taped on glass slides that had Mayer's albumen poured on them. The slides were stretched and dried in a hot plate at a temperature of 42°C. To remove the paraffin, the slides were immersed in a xylol solution, hydrated in alcohol, whose concentration decreased gradually and then washed with distilled water. Finally, the slides were stained with a solution of hematoxylin-eosin[16].

RESULTS

The histological structure of the male gonad of the green mussels in the pond of Panimbang is composed of large follicular cells, the spermatozoa were radially arranged and the connective tissue between the follicle cells (Fig. 1a). The arrangement of the follicles of the male gonad of the green mussels in the port of Muara Angke was relatively looser (Fig. 1b) than the arrangement of the follicles of the male gonad of the green mussels in the pond of Panimbang (Fig. 1a). Some follicles were not fully filled with spermatozoa and the size of follicles was relatively smaller than the size of follicles of the green mussel in the pond of Panimbang. The follicles were also relatively decreased in number (Fig. 1b).

While the arrangement of the follicles of the male gonad of the green mussels in the pond of Muara Angke was relatively denser (Fig. 1c) rather than the arrangement of the follicles in the male gonad of green mussels in the port of Muara Angke (Fig. 1b). The size of the follicles of the male gonad of the green mussels in the pond of Muara Angke (Fig. 1c) was relatively bigger than the follicles of the male gonad of the green mussels in the port of Muara Angke (Fig. 1b), but was relatively smaller than the follicles of the male gonad of the green mussels in the pond of Panimbang (Fig. 1a). The development stage of the male gonad of the green mussels in the pond of Muara Angke and the port of Muara Angke are in the second stage of development. The characteristics of the male gonad in this stage are marked by the follicles, which are filled the spermatozoa, although there are some follicles were not fully filled with spermatozoa.

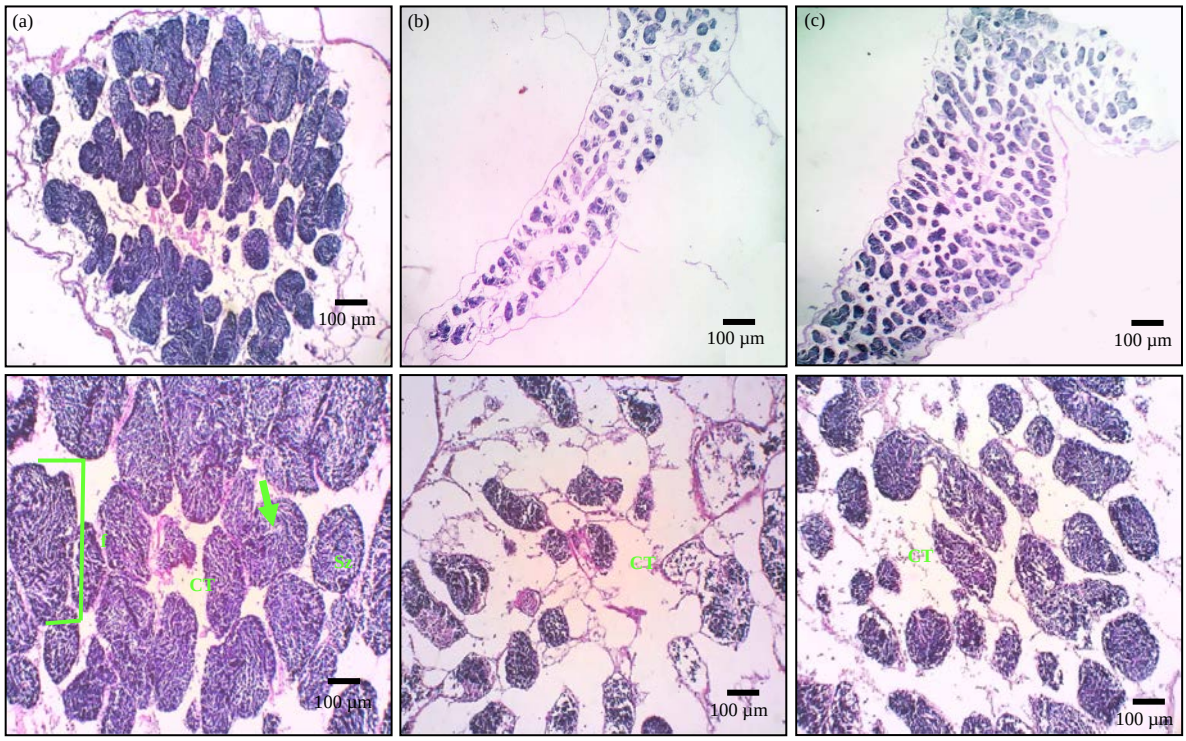

Fig. 1(a-c): Cross section of male gonad of green mussels in the pond (a) Panimbang, (b) Port Muara Angke and (c) Pond Muara Angke

From the top row down are magnifications of 4×10 and 10×10. f: Follicle, Green arrow: Follicle lumen, Sz: Spermatozoa, CT: Connective tissue

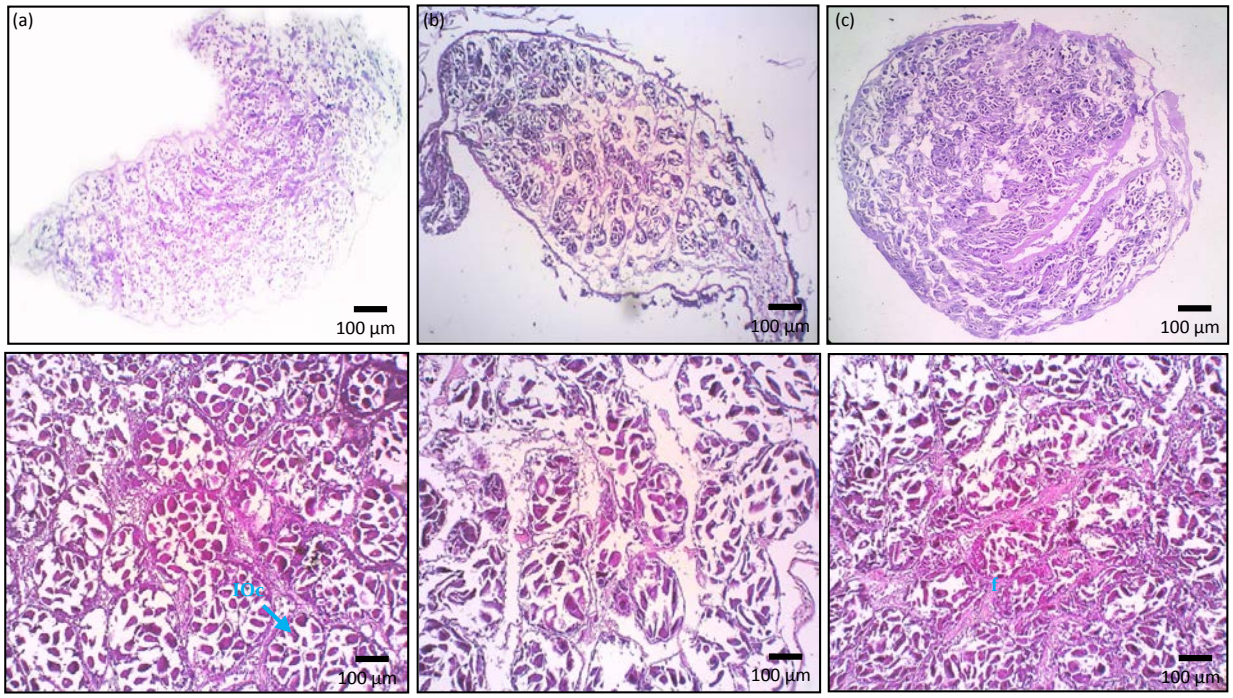

Fig. 2(a-c): Cross section of female gonad of green mussels in the pond (a) Panimbang, (b) Port Muara Angke and (c) Pond Muara Angke
From the top row down are magnifications of 4×10 and 10×10. f: follicles, IOc: Immature oocyte

The histological structure of the female gonad of the green mussel in the pond of Panimbang is composed of the follicles surrounded by connective tissue on the outside and oocyte of the follicles. There is a darker purple polygonal oocyte. The arrangement of follicles and oocyte of the follicle was relatively rather dense and there are no spaces in the follicle. The maturity stage of the female gonad of the green mussels in the pond of Panimbang is the second stage development, which is characterized by the presence of a mature oocyte in the follicles (Fig. 2a).

The histological structure of the female gonad of the green mussels in the port of Muara Angke (Fig. 2b) is different than the histological structure of the female gonad of the green mussels in the pond of Panimbang. Although the female gonad of the green mussels in the port of Muara Angke is also in the second stage of development (Fig. 2c), but the arrangement of follicles of the female gonad of the green mussels in the port Muara Angke and the oocyte of the follicles are relatively less dense than the oocyte of the follicles of the green mussels in the pond of Panimbang. While the histological structure of the female gonad of the green mussels in the pond of Muara Angke (Fig. 2c) is no different from the histological structure of the female gonad of the green mussels in the pond of Panimbang (Fig. 2a).

DISCUSSION

The gonads of green mussels have separate sexes, where the gonads are located in the dorsal body, usually fused with mantle tissue. In the developing stage, the color of the gonads in male mussels is cream; however, the color of the gonad in female mussels is pink. In the ripe stage, whereas the color of the male gonad is milky white and the color of the female gonads is orange. The process of gametogenesis is very season-dependent and varies among green mussels in the same location. In this study, both male and female gonads of the green mussels in the pond of Panimbang were in stage two of development. These five maturity stages in the reproductive cycle in mussels were the resting stage (0), developing stage was subdivided into three substages (I, II, III), ripe stage (IV), spawning stage was divide into two substages (III, II) and spent stages (I) and resulting in a total of eight stages[17]. The characteristics of a mature gonad in this research are a mantle layer thicker than the first stage mantle layer, the mantle of the male gonad is milky white and the mantle of the female gonad is orange. The gonads are composed of follicles and connective tissue between the follicles. Oocyt and sperm are found in the follicle and the shape of the ovum is polygonal. This study also shows that the

male gonads of green mussels are in the third stage of development. The characterized in this stage by radially arranged spermatozoa, the follicles have expanded, displacing most of the gonadal connective tissue. In male gonad the follicles are mostly filled with spermatozoa, while on the follicle wall there is a small layer[17].

In general, an adult green mussel has a length of approximately 47 mm and a width of 32 mm. The life span of a green mussel is 3 years[17]. Based on previous research shows that green mussels is significant decrease in weight and it was influenced by the bioaccumulation of lead, cadmium and mercury in their bodies[2]. Based on the result of research on archive samples gonad of green mussels in 2015 that the mercury concentration on the male gonad of the green mussel in the port of Muara Angke (0.06) and the pond of Muara Angke (0.03) was relatively higher than the mercury concentration on the male gonad of the green mussels in the pond of Panimbang (0.00), as the control[2]. Therefore, the disorder of the process of gonad development in the port of Muara Angke in this study also caused by bioaccumulation of lead, mercury and cadmium, because all the sample were archive sample collection in 2015. This influence can also an impact on the development of the gonad of green mussel, especially in the male gonad was undergone toxicity. Additionally, the water condition in the port of Muara Angke is polluted; the waters are dirty, smelly, blackish and much closed to the residential area[2]. The bioaccumulation of lead, cadmium and mercury were also detected at the tissue level, in the gonad, the gills and hepatopancreas through the expression of the metallothionein protein[2]. It is suspected that lead, mercury and mercury in the body of green mussels will compete with the essential metals, which is needed by the body. The availability and continuity of essential metals in cells depends largely on the function of the metallothionein protein. The lead, cadmium and mercury in the body of green mussels will bind metallothionein rather than essential metals. This complex can disrupt metabolism, especially cell division and protein synthesis[18] and can disruption of endocrine activity[19], which has an impact on the process of gametogenesis.

Cell division is very important during gonad development and the process is influenced by several factors. One factor is essential metals, such as zinc metal. This metal acts as a catalyst and cofactor in enzymatic processes. Zinc has a low affinity for metallothionein protein[20], while the lead, cadmium and mercury have a strong affinity against metallothionein protein. Metallothionein-heavy metal protein complexes can interfere with enzymatic ligase, which plays a role in the processes of replication, transcription, translation and protein synthesis. If the zinc metal is not sufficient during the process of cell division, then the process will be disrupted. Therefore, impaired gonad development has an impact on decrease in the number of follicle of the male gonad, in which the follicle was relatively less dense and some follicles were not fully filled with spermatozoa. The results of this study also supported by research of Jalius[17], that show that heavy metals can cause in the decrease in the number of follicle and spermatozoa of follicle of the green mussels in Jakarta Bay is fewer than spermatozoa in follicle of the green mussels in those in the pond of Panimbang .

The water condition in the port of Muara Angke is very polluted, black color and many the passenger ships in and out. The lead, mercury and cadmium were detected in the water of the port of Muara Angke and also detected on the tissue of the green mussels[2]. Although the concentrations of lead, mercury and cadmium were not exceeding the specified quality standard but the effect of the heavy metals is impact on the male gonad development of the green mussels in the port of Muara Angke. This data supported by Bayen and Goldberg research, showed that the male gonad is the most sensitive organ to acute Cd-induced damage with irreversible necrosis, because acute exposure to Cd caused blood–testis barrier (BTB) disruption, germ cell loss, testicular edema, hemorrhage, necrosis and sterility[21,22]. The mussels are sessile, widely distributed filter-feeding organisms, with the ability to sequester many lipophilic organic compounds[23]. Therefore, the male of gonad in the green mussels was more sensitive and the main target organ of environmental estrogens[22]. Our findings reported that the male gonads of the green mussels are more susceptible against bioaccumulation of the lead, mercury and cadmium than the female gonad of the green mussels.

The implication of this research is very important therefore to have an overall picture of the information on the effect of bioaccumulation of heavy metals in green mussels, that consumed for daily food as a protein source. The villagers of Muara Angke consume these mussels for daily food as a protein source. Consumption of large quantities of the green mussels can pose a potential health hazard for consumers. People who live in coastal areas generally consume more seafood than people from inland areas. Therefore they potentially exposed to metals and should be more concerned about intoxication from heavy metal. Understanding health risks due to seafood consumption, therefore seems to be essential.

The analysis of lead, mercury, cadmium of the green mussels of the port and pond in Muara Angke should be particular attention. Over consumption of lead causes renal

failure and liver damage in humans[23]. Because of the limited funding in this study, so this study only investigates analysis of toxicity on gonad development of the green mussels. This research can apply for an assessment of the exposure risk to heavy metal from the consumption of green mussels, knowing the amount of heavy metal ingested is important

The previous research findings that the bioaccumulation of lead, mercury and cadmium is just an amount small[2], but the all the heavy metal can impact the development of the male gonad of the green mussels. This study discovers the effect of heavy metal against the green mussels, which were consumed by local people. The possibility accumulation of the heavy metal can occur by biomagnification, by which heavy metal is passed from one trophic level to the next within a food web. Therefore, the further studies are needed to examine the above matters. We recommend calculating the Provisional Tolerable Weekly Intake of heavy metal and implication on local people.

CONCLUSION

The development of the male gonad of the green mussels in the port Muara Angke undergoing toxicity and are characterized by a relatively less dense follicular cell arrangement. The effects of toxicity of the male gonad of green mussels were more sensitive and were more susceptible than the female gonad of the green mussels.

SIGNIFICANCE STATEMENT

Studies about heavy metals in the port of Maura Angke have been carried out from time to time, but the government does not apply policies regarding the dangers of heavy metals in marine biota which are consumed. This study is a basis for future research by examining the impact of consuming mussels, that contain heavy metals by the villagers in the port of Muara Angke. This study will help the researcher to uncover for an assessment of the exposure risk to heavy metal as an impact consumes of green mussels that many researchers were not able to explore. Maybe a new theory on the toxicity of heavy metal in level tissue may be arrived at. It does not only explore the toxicity of heavy metals in green mussels but also explores the toxicity of heavy metals in humans uses blood analysis.

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