IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

LEAD AND CADMIUM CONTENT AND GENETIC DIVERSITY OF RAZOR CLAM (*Pharella acutidens*) IN RUPAT STRAIT, INDONESIA

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***Abstract*:** Heavy metal Lead (Pb) and Cadmium (Cd) are toxic elements that accumulate in marine invertebrates, so these animals are often used as indicators of environmental pollution in marine waters. This research was conducted at the Rupat Strait, Indonesia. The aim of this research is analyzing the Pb and Cd content in the flesh of the razor clam (*P. acutidens*) and their correlation with the genetic diversity. The results show that the highest Pb and Cd contents were recorded in specimens from St.1 (0.0711 and 0.0020 mg/kg), followed by St. 2 (0.0693 and 0.0009 mg/kg), and from St. 3 (0.0008 and 0.0016 mg/kg). Reconstruction of the phylogenetic tree with the neighbor join model using the Kimura-2 model divides all samples into 2 clades. The first clade contains samples from St. 2 and St.3, which show closer genetic kinship compared to samples from St.2. Alignment of the sequencing results using the MEGA X application shows that there are differences in the arrangement of nitrogen bases in samples from St. 1 compared to St.2 and St.3. The results of the phylogenetic tree reconstruction showed that the clam from Selensing (St.1) with the highest Pb and Cd content, had distant relatives with clams originating from Bandar Bakau (St.2) and Darul Aman (St.3). Significant changes in the nitrogen base composition of Selensing (St.1) samples indicated that high heavy metal content may affect the genetics diversity of the clams.

Keywords: heavy metals, marine pollution, genetic diversity, clades, bioaccumulators

I. INTRODUCTION

The Rupat Strait, Riau, Indonesia, is a small strait between islands of Sumatra and Rupat Island which are connected to the Malacca Strait as an international shipping lanes. The waters are affected by the anthropogenic activities of Dumai City and its surroundings such as residential activities, industrial activities, and port activities which result in a decrease in water quality such as increasing the amount of heavy metal content (Amin et al. 2009; Ariani et al. 2016; Yoswaty et al. 2021).

Heavy metals undergo various processes, including transportation by tidal currents, dilution, association with suspended matter, coagulation and sedimentation to the bottom, association with sedimentary organic matter, and absorption by plankton. They are toxic and can have a variety of biological and ecological effects on the waters bioaccumulated in the body tissues of marine biota, which can damage the organs in the body tissues of the marine biota at a specific concentration (Hwang et al. 2016; Li et al. 2017).

Lead (Pb) and Cadmium (Cd) are heavy metals which are highly toxic and chronic to human. Domestic wastes, mining, industrial, agricultural activities, and depositional wastes from the atmosphere to the sea can increase the concentration of Cd metal in water and sediment. One of the entry point of Pb into the Rupat Strait is related to waste from ships passing and anchoring in the waters of Dumai. Motor boats and fishing fleets of local communities operating in this area of course also contribute. Generally, fuel oil is added with

tetraethyl additive containing Pb to improve its quality. Apart from ship waste, Pb can also enter the waters through industrial waste and household waste because Pb is widely used as a raw material in industry (Barokah et al. 2019; Botelho et al. 2017).

Bivalves, such as razor clam or sipetang (*Pharella acutidens*), are group of biota that are popularly used to detect environmental pollution. This is due to their associated life with sediment, their feeding habits as a filter feeder, which can filter all materials in the waters and accumulate pollutants (D'costa et al. 2018). Therefore, undetectable concentrations of chemicals in water can be found in the body of bivalves. Bivalves are known to be able to accumulate heavy metals in greater quantities than other aquatic biota (Riza et al. 2021). Bivalves whose lives tend to be sedentary, have a slow response to pollution and heavy metal pollution so that they can be used as an indicator for monitoring environmental pollution (Kesavan et al. 2010).

Cordova et al. (2011) reported that green mussels (*Perna viridis*) in Jakarta Bay waters were malformed due to Pb accumulation in their flesh. Malformation is a disruption of the shell structure, which results in a thicker shell, but with less meat content. Chen et al. (2021) also reported that there were variations in isozyme banding pattern which was indicated by the appearance and shape of the banding pattern that varies greatly in freshwater clams (*Anodonta woodiana*) exposed to heavy metal Cd on a laboratory scale. This study aims to determine the metal content of Pb and Cd in the flesh of the razor clam (*P. acutidens*) and their correlation with the genetic diversity of this biota in the waters of the Rupat Strait, Indonesia.

II. RESEARCH METHODOLOGY

2.1 Time and Place

Sampling of razor clam samples was carried out at 3 stations. Station 1 was located on the coast of Selinsing Village (1° 40′ 16″ N -101° 43′ 06″ E) and station 2 was in Bandar Bakau Village (1° 41′16″ N - 101 26′ 08″ E), both on the coast of Dumai City. Station 3 was located on the coast of Darul Aman Village (1° 55 38″ N - 101 23′ 35" E) on the coast of Pulau Rupat (Figure. 1). Razor clams were collected randomly in the intertidal zone at each observation station. The samples taken were consumption size, with a length of 50-70 mm (St. 1), 55-67 mm (St. 2) and 56-69 mm (St. 3). The number of samples from each station were 25 individuals. Water quality parameters were measured *in situ*, included temperature, salinity, brightness, pH, and dissolved oxygen (DO).

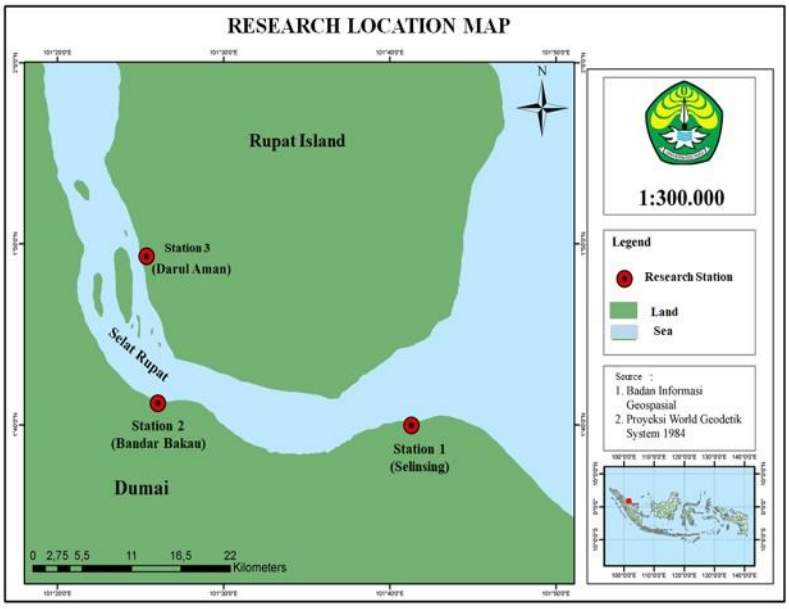


Figure 1 Research location map

2.2 Analysis of Heavy Metal Content

The method used to determine the heavy metal content was following the (APHA 2012; BSN 2011), procedures with the atomic absorption spectrometry (AAS) technique. The soft tissue of each individual razor clam is separated from its shell without separating the parts of its organs. A total of 25 individual razor clam samples per station (50 – 70 mm) were composited without separating the digestive and meat parts and dried in the oven. Furthermore, the sample was crushed using a mortar, then added 5 ml of HNO_3 and 0.5 ml of $HClO_4$ and allowed to stand for 24 hours. The sample was heated in the digestion block at 100ºC for 1 hour, then the temperature was increased to 150ºC. After the yellow vapor was exhausted, the temperature was

increased again to 200ºC. After the white smoke comes out and the remaining extract is ± 0.5 ml. The digestion tube was removed and allowed to cool. Then the extract was diluted using 49.5 ml of distilled water and stirred for 5 minutes until homogeneous. Heavy metal levels of Pb and Cd in the sample were measured using AAS. Measurements were carried out with 3 repetitions.

2.3 DNA Extraction and Amplification

DNA analysis included total DNA isolation, polymerase chain reaction (PCR), and sequencing. Total DNA isolation consisted of several stages, namely tissue dissociation, lysis, DNA binding, washing, elution, and electrophoresis. Total DNA isolation was carried out referring to the Geneaid Tissue Genomic DNA Mini Kit. The mussel flesh were taken and weighed as much as 0.4 g, the sample pieces were put into a 1.5 ml eppendorf tube and labeled. The samples were washed with TE solution (10 mM Tris-HCl; 1 mM EDTA pH 8) by: the sample was immersed in 200 µl TE pH 8.0, vortexed for 15 seconds, centrifuged at 3000 rpm for 2 minutes, and TE solution pH 8 the remaining was discarded (by pipetting). The washing process was repeated 3 times and the samples were dried on a piece tissue paper so that the remaining alcohol evaporated.

Tissue dissociation was carried out by taking 30 mg of mussel flesh into a 1.5 ml tube. A total of 200 µl GT buffer was put into the tube and the mussel muscles were crushed using a micropestle. Then 20 µl of proteinase K was put into the tube and then the tube was inverted. The sample was put into a water bath to be incubated for 30 minutes at 60°C. During incubation, the tubes were inverted every 5 minutes.

In the lysis process, as much as 200 µl of GBT buffer was poured into the tube and then the tube was inverted for 5 seconds. The sample was then incubated in a water bath for 20 minutes at 60°C and the tube was inverted every 5 minutes. The precipitate was centrifuged at 14000 g for 2 minutes and the supernatant is discharged into a 1.5 ml tube. For DNA binding, 200 µl of absolute ethanol was put into the tube and then the tube was inverted for 10 seconds. The GS column was placed on a 2 ml CT. The mixture contained in the tube was transferred to the GS column and then centrifuged at 14000 g for 2 minutes. The CT was then removed and the GS column was transferred to a new CT.

In the washing process, 400 µl of W1 buffer was added and then centrifuged at 14000 g for 30 seconds. The CT fluid was removed and the CT was reinserted into the GS column. A total of 600 µl wash buffer was put into the GS column and then centrifuged at 14000 g for 30 seconds. The liquid on the CT was discarded and the GS column was removed and then centrifuged at 14000 g for 3 minutes to dry the matrix.

The elution process was carried out as follows. The GS column was transferred to a 1.5 ml tube. A volume of 50 µl of warm elution buffer was added to the center of the matrix column and then incubated at room temperature for 5 minutes to ensure that the elution buffer was absorbed into the matrix. Elution was carried out twice.

2.4 Total DNA Electrophoresis

Preparation of 1.2% agarose gel solution for electrophoresis was as follows: Gel molds were assembled and combs were placed as tools for forming gel wells. A total of 1.2 g of agarose powder was dissolved with 100 ml of 1x TBE buffer in an erlenmeyer. Then heated on a hot plate until the solution boils and becomes clear. Volume of 3 µl ethidium bromide was added and then homogenized. The solution was allowed to stand for 15-30 minutes then the agarose gel was poured into the mold. After the gel solidified, the comb was removed and the mold was inserted into the electrophoresis chamber which already contained 1x TBE buffer solution.

The DNA sample was submitted for electrophoresis as follows: A piece of parafilm sheet was prepared. A total of 3 µl of DNA was mixed with 1 µl of loading dye on parafilm. The mixture is put into the well using a micro pipette followed by inserting a ladder. Electrophoresis was carried out at 50 volts for 20 minutes. The results were visualized on a UV lamp (WiseUV WUV-M20) and photographed.

2.5 Polymerase Chain Reaction (PCR)

Primer forward LCO1490, 25bp was used as primary forward and HCO2198, 26 bp was used as primary reverse. A set of PCR solution was prepared by mixing the ingredients for the PCR solution (Table 1) into a 1.5 ml tube. The mixture is then fed into the PCR machine. The PCR machine was turned on and pre-PCR was performed for 5 minutes at 94°C and continued for 35 cycles. Each PCR cycle consisted of denaturation of the template DNA for 45 seconds at 94°C, annealing for 45 seconds at 53°C, and primer elongation for 1

minute 30 seconds at 72°C. The PCR process ended with post-PCR for 10 minutes at 72°C. The store stage in the PCR machine was carried out at a temperature of 15°C. Checking the PCR results by electrophoresis was carried out as follows. A total of 2 µl of the PCR mixture was mixed with 1 µl of loading dye and then inserted into the well on the agarose gel. The PCR results were visualized on a UV lamp. The encoding genes in the mtDNA genome include the cytochrome oxidase subunit I (COI) gene. The presence of the target DNA band indicated the success of the PCR process.

Table 1 PCR solution components

No.	Components	(Quantity)	Vol (µl)	Vol (µl)
1.	10x buffer PCR	1x	5	48
2.	2 mM dNTPs	0.1 mM	2.5	24
3.	10 mM Primer Table F	0.4 mM	2	19.2
4.	10 mM Primer R	0.4 mM	2	19.2
5.	DNA	-	5	-
6.	5 unit/ml Taq DNA pol	1.5 u	0.3	2.88
7.	ddH2OH	-	33.2	318.72
	Total		50	

2.6 Data Analysis

The phylogenetic analysis was started by combining the results of primary and forward sequencing using BioEdit software. A blast was carried out on the NCBI website. Sequence alignment was performed using ClustalW on MEGA X software. The phylogenetic tree construction used MEGA X software with Construct/Test Neighbor Joining Tree (Saitou and Nei, 1987) and Bootstrap method with No. of Bootstrap Replications 1000.

III. RESULTS AND DISCUSSION

Results

Pb and Cd metal content

The highest Pb content in razor clam flesh was at St. 1 (0.0711 mg/kg) while the lowest content was at St. 3 (0.0008 mg/kg). The highest Cd metal content was at station 1 (0.0020 mg/kg) and the lowest one was at St. 2 (0.0009 mg/kg). More detailed data on the metal content of Pb and Cd at each station can be seen in Table 2.

Table 2 Pb and Cd metal content in *P. Acutidens* at each observation station strait of Malacca waters

Station	Replication	Pb content (mg/kg)	Average ± SD Pb content	Cd content (mg/kg)	Average ± SD of Cd
1	1	0.0549	0.071 ± 0.0237	0.0016	0.0020 ± 0.0005
	2	0.0600		0.0019	
	3	0.0982		0.0025	
2	1	0.0545	0.0693± 0.0288	0.0009	0.0009 ±0.0007
	2	0.0510		0.0002	
	3	0.1025		0.0016	
3	1	0.0007	0.0008 ± 0.0001	0.0001	0.0016 ± 0.0019
	2	0.0009		0.0037	
	3	0.0007		0.0008	

DNA Analysis

All samples of razor clam from the Rupat Strait have been subjected to a DNA isolation process. The results of DNA isolation were then electrophoresed to determine the success of the DNA isolation. Theresults

of electrophoresis is presented in Figure.2

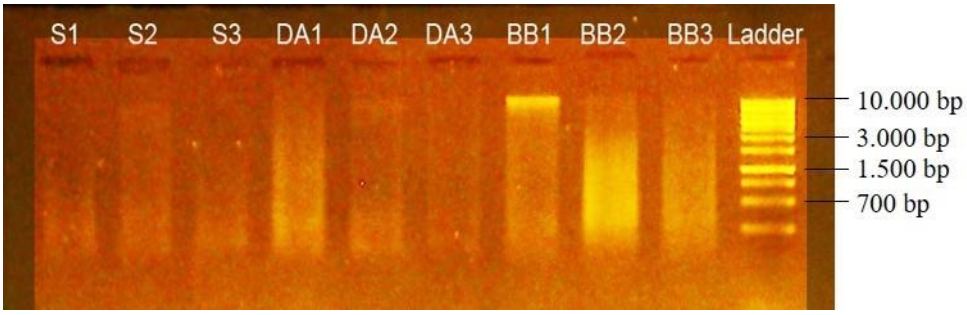


Figure 2 Electrophoresis results of razor clam DNA isolates with a ladder size of 1 kb. (S: Selinsing; DA: Darul Aman; BB: Bandar Bakau)

From the observations made on the extraction of all samples taken from the flesh of razor clam it was seen that the DNA bands from samples S1, S3, and DA3 look thin and vague. The DNA bands from samples S2, DA1, DA2, and BB3 were still relatively thin but were clearly visible compared to the DNA bands from samples S1, S3, and DA3. DNA bands from samples BB1 and BB2 were thick and clear with a length of 10000 bp. After the DNA was isolated, the next step was to perform PCR amplification and then electrophoresed again using agarose gel. The results of PCR amplification of 9 samples of razor clam from the Rupat Strait using primer pairs LCO-1490F and HCO-2198R can be seen in Figure. 3.

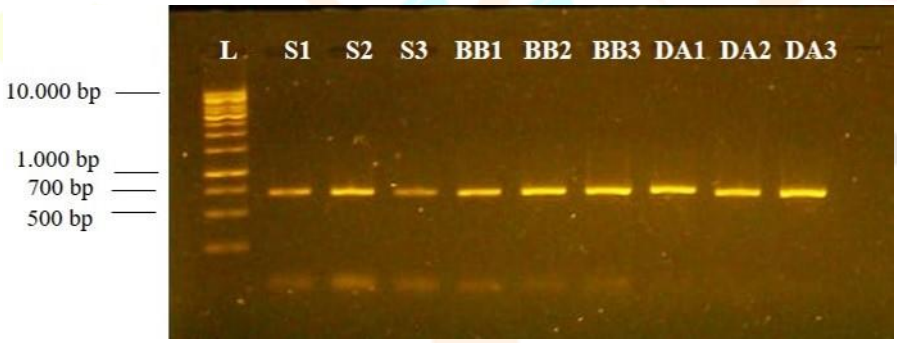
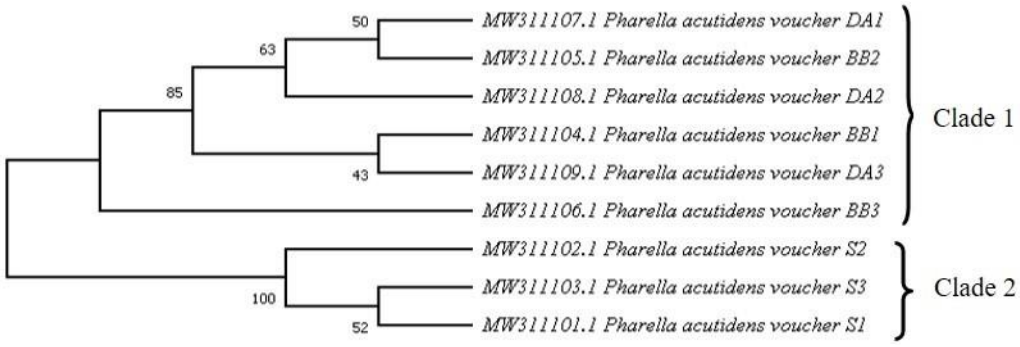


Figure 3 Results of PCR amplification of razor clam using primer pairs LCO-1490F and HCO-2198R. L= Ladder (Size 1 kb)

The success of the PCR amplification of all samples of razor clam was known through 1.2% agarose gel electrophoresis analysis. PCR amplification of all samples used annealing temperature of 53°C and was successful with the presence of a single band of DNA on the agarose gel as shown in Figure. 3. The DNA bands of 9 samples analyzed had the same length, which was about 650 bp. It has approximately the same thickness, but the DNA bands from samples S1 and S3 are slightly thinner than the DNA bands in other samples. Clear and thick bands indicate that optimal PCR conditions have been achieved, so it can be said that the PCR process was going well and can be processed at the sequencing stage.

The Relationship Among *P. Acutidens*

The results of the raltionship analysis using a genetic approach among all samples of *P. acutidens* from three stations showed that samples from Darul Aman station (DA1, DA2, and DA3) and samples from Bandar Bakau station (BB1, BB2, and BB3) had a closer relationship. compared to samples from Selinsing station (S1, S2, S3). More details can be seen in the phylogenetic tree in Figure. 4.



Figureure 4 Reconstruction of *P. acutidens* phylogenetic tree using neighbor joining method

Based on the reconstruction of the phylogenetic tree, it was known that the first sample from Darul Aman (DA1) was closer to the second sample from Bandar Bakau (BB2), the first sample from Bandar Bakau (BB1) was closer to the third sample from Darul Aman (DA3), and the sample from Darul Aman (DA3). The first and third of Selinsing (S1 and S3) have closer relationship. Meanwhile, the second sample from Darul Aman (DA2), the third sample from Bandar Bakau (BB3), and the second sample from Selinsing (S2) have their own branches.

Heavy Metal and DNA Nitrogen Base Arrangement

All samples of *P. acutidens* that have gone through the DNA isolation process, then to the sequencing process and finally the alignment using the MEGA X application. Each sample was composed of 620 base pairs of nitrogenous bases. The alignment of the sequencing results showed that there were differences in the nitrogen base arrangement of the all samples. The nitrogen base composition of samples from Bandar Bakau and Darul Aman was relatively the same (there was only a slight change). The three samples from Selinsing had quite a lot of difference in the nitrogen base arrangement compared to the other 6 samples from Bandar Bakau and Darul Aman, as seen in the nitrogen base arrangement in column 6. In the order of nitrogen bases in the sixth column, sample Selinsing 1, 2, and 3 were composed of the nitrogenous base guanine (G) while the other six samples were composed of the nitrogenous base adenine (A). Significant changes in the nitrogen base composition of Selinsing 1, 2, and 3 samples indicated that high heavy metal content may affect the genetics of the organisms.

Water Quality

The range of water quality parameters were as follows: seawater temperature 29-30 ºC, pH 6.0-7.0, salinity 29-33 ppt, brightness 17.5-97.5 and dissolved oxygen ranged from 4-8 ppt.

Discussion

In the study it was noted that the Pb content ranged from 0.0008 to 0.0711 mg/kg. Meanwhile, the metal content of Cd ranged from 0.0009-0.0020 mg/kg. This figureure is much lower than to that reported by previous researchers. Sijabat et al., (Sijabat, Trinuraini, and Supriyantini 2014) found that Pb levels in green mussel in Tanjung Emas, Semarang (Central Java, Indonesia) ranged from 1.42-1.64 mg/kg. Barokah et al. (2019) reported Pb and Cd levels in green mussels (*Perna viridis*) from Celincing, Kali Baru and Muara Kamal (Jakarta Bay) ranged between 0.20-0.29 mg/kg and 0.08-0.11 mg/kg, respectively. Another researcher Cordova (2011) also reported that in Muara Angke, Jakarta Bay, the levels of Pb and Cd in green mussel meat, aged 1-7 months, ranged between 17.13-41.94 mg/kg and 0.07-0.45 mg/kg.

The highest Pb and Cd contents were both found at station 1, namely Selinsing in Dumai City. One of the reasons for the high content of Pb at this station is the ships passing by in the waters of the strait so that the fuel that is also wasted along with the ship's waste water. While the high content of Cd metal at St.1, one of the causes is the distance between St. 1 which is close to oil palm plantations. The remaining use of phosphate fertilizers in oil palm plantations which is carried during the rain to these waters is estimated to play a role in increasing the concentration of Cd. One of the sources of Pb comes from anthropogenic activities in the form of fuel oil that pollutes marine waters. Fuel oil containing alkyl lead is non-degradable and easily accumulates in marine biota organs and eventually enters the food chain. Some organisms have the ability to control the

entry and exit of pollutants, but in organisms that do not have this mechanism, pollutants will accumulate in the body, one of which is bivalves (Kesavan et al. 2010).

Waste from shipyards such as fuel residue, oil, smoke from ships, to paint on ships contains Pb metal and has the potential to pollute coastal areas (Kinuthia et al. 2020; Yona et al. 2020) stated that Cd metal in nature usually comes from industrial waste metals, plastics, paints, fertilizers, and oil. In addition, atmospheric dust contributes to the entry of Cd into the waters.

Clam only get food from objects that are in the water. It is very possible to accumulate pollution in the water, especially pollution from Pb metal. Apart from how to eat, metals can also enter the body of shellfish through the respiratory tract. Shellfish obtain oxygen through water that enters the gills which if it contains heavy metals in the form of Pb, then the Pb metal can automatically accumulate in the shells' bodies (Chen et al. 2021). Heavy metals such as Pb with a density heavier than water will settle to the bottom of the water so that marine biota such as bivalves and gastropods have the potential to be directly exposed (Einsporn and Koehler 2008; Liu et al. 2021). Based on studies of Pb accumulation in *Strombus canarium* and *Perna viridis*, metal content was found to be higher in the body than in sediments (Barokah et al. 2019; Yap et al. 2004). However, the levels of Pb and Cd of razor clam from Rupat Strait, Indonesia are still below the maximum threshold set by FAO, BPOM of the Republic of Indonesia and the Ministry of Environment of the Republic of Indonesia.

The results of the phylogenetic tree reconstruction from all razor clam samples were divided into two clades. The first clade consists of 6 samples from Bandar Bakau and Darul Aman while the second clade consists of 3 samples from Selinsing. The grouping of samples contained in one clade is based on the similarity of nucleotide sequences. This shows that samples from Selinsing have a distant relationship with samples from Bandar Bakau and Darul Aman. The analysis of the neighbor joining method using the Kimura-2 model reconstructs the relationship between species by referring to the value of the evolutionary distance which assumes that each branching evolves at a different rate. The highest content of Pb and Cd in razor clam was also found at Selinsing station.

The high content of heavy metals in razor clam at station 1 is in line with the location of the sample in the phylogenetic tree which forms a separate clade from the six samples from St. 2 and St. 3. This is in accordance with the reports of Morales et al. (2016); Liu et al. (2010); Tran et al. (2007) who stated that the accumulation of heavy metals in shellfish can cause gene mutations and DNA damage and affect DNA transcription in shellfish. In Figure.4 also shows that samples DA2, BB3, and S2 are not on the same branch or node as other samples in the phylogenetic tree. Two samples that are on the same branch indicate the similarity of species.

Malformation is a disorder of shell structure, which results in green mussels that are thicker, but with less meat content. Exposure to heavy of *M. balthica* and *M. edulis* disrupted the development of the nucleus during cell division. As a result, the nucleus does not develop normally. In green mussels (*P. viridis*) it was reported that exposure to heavy metals such as Pb, resulted in a decrease in growth rate and death due to heavy metal disturbances in cilia during the process of taking and diffusion of food. The presence of heavy metals in living things can also inhibit major DNA repair systems resulting in genome instability and accumulation of critical mutations (Beyersmann and Hartwig 2008; Bower et al. 2005).

The composition of nitrogen bases that differed the most among the all samples were the samples from St. 1, the station with the highest concentrations of Pb and Cd among the three stations. This indicates that the high content of heavy metals in the mussel body may cause a genetic changes in the razor clam. Tchounwou et al. (2012) stated that heavy metal waste will cause direct impacts such as poisoning, paralysis, genetic disorders to death. Pb accumulation in mussels can cause disturbances in several genetic factors, spawning patterns, behavior, ability to orient, avoid enemies, migration, and decreased competition (Shen et al. 2020; Youssef et al. 2017). Evidence suggests that environmental disturbances such as heavy metal pollution during the growth phase of organisms can induce changes in the DNA methylation landscape (Bihaqi 2019). Accumulation of heavy metals in green mussels can cause gene mutations and DNA damage and affect DNA transcription in mussels (Liu et al. 2010).

Heavy metals such as Pb enter the cytosol through transporters or endocytosis channels, by inhibiting potential physiological import processes of the membrane. Heavy metals contaminate and bind to small molecules (eg. glutathione) and as carriers of chaperons (eg. ferritin, metallothionein) and apoproteins (eg. superoxide dismutase, cytochrome c oxidase). Chaperones and apoproteins can replace natural ligand processes from endogenous cations so that they disrupt the Ca++ homeostasis process and accumulate in metal-rich granules or minerals (Tran et al. 2007). With the redox reaction in the sulfur group, reactive oxygen

species/reactive nitrogen species are formed which results in disrupting gene expression and disrupting the antioxidant processes of cell organelles such as mitochondria and lysosol (Varotto et al. 2013). As a result of these disturbances, macromolecules and precursors can cause cell death either by necrosis or apoptosis, this makes DNA contain reactive groups and will trigger gene deregulation in cell cycle homeostasis resulting in uncontrolled and damaging replication.

Heavy metals are carcinogenic (Lanphear et al. 2018; Morales et al. 2016) and are widely distributed, including the marine environment. Heavy metal exposure can significantly change the way cells repair damage to DNA. Many epidemiological studies conclude that heavy metal exposure is associated with various types of diseases including cancer in humans. The study conducted by Morales et al. (2016) have shown that heavy metals such as cadmium, nickel and arsenic can trigger mutagenic changes by influencing the way cells repair double strand breaks (DSB). The effect of these metals on DNA repair pathways has an important influence in metal-associated carcinogenesis at lower than expected concentrasions

Many studies on Pb and Cd in marine invertebrates (Selpiani et al. 2015; Wang and Lu 2017) have shown that Pb can affect early life stages by inducing growth (inhibition or cessation of growth, morphological anomalies), and disturbances of growth, reproduction (quality of offspring, fertilization rate), which can cause negative effects on populations and communities. Botté et al. (2022) stated Lead (Pb) is a non-essential metal that occurs naturally in the environment and is often complexed with other elements (eg. copper, selenium, zinc). This metal has been used since ancient Egypt and its extraction has progressed in the last centuries. It has been used to date as a fuel additive and is currently used in the production of vehicle batteries and paints.

CONCLUSIONS

The highest Pb and Cd contents in *Pharella acutidens* were recorded at Selensing Village (0.0711 and 0.0020 mg/kg), followed by Bandar Bakau (0.0693 and 0.0009 mg/kg) and Darul Aman (0.0008 and 0.0016 mg/kg). The reconstruction of the phylogenetic tree with the neighbor joining model using the Kimura-2 model divided all samples into 2 clades. The first clade was filled with samples from Bandar Bakau and darul Aman and the second clade was occupied by samples from Selensing. This grouping of clades indicated that samples from Bandar Bakau and Darul Aman were more closely related genetically. The alignment of the sequencing results of all samples showed that there were differences in the nitrogen base arrangement of the samples. Differences in the composition of nitrogen bases were mostly found in samples from Selinsing. The results of the phylogenetic tree reconstruction also showed that the clam from Selensing, the highest Pb and Cd content, had distant relatives with clams originated from Bandar Bakau and Darul Aman. Significant changes in the nitrogen base composition of Selensing samples indicated that high heavy metal content may affect the genetics diversity of the clams.

ACKNOWLEDGEMENT

Our gratitude goes to the Chairman of the Institute of Research and Public Service, University of Riau, Pekanbaru, Indonesia, who has financed this research.

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