

# REVEALING THE CONDITION OF DARAH MUSSELS (Anadara granosa) AND KEPAH MUSSELS (Meretrix meretrix) ON BATU BELUBANG BEACH, BANGKA ISLAND

# Mengungkap Kondisi Kerang Darah (Anadara granosa) dan Kerang Kepah (Meretrix meretrix) di Pantai Batu Belubang, Pulau Bangka

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### ABSTRACT

Batu Belubang Beach has abundant biological resources of Darah Mussels (Anadara granosa) and Kepah Mussels (Meretrix meretrix) in season. On the other hand, Batu Belubang waters are a post-tin mining area, therefore it is a concern for people to consume shellfish from the area. This concern is caused by the results of previous studies that said that post-tin mining areas left heavy metals, one of which was non-essential heavy metals such as Pb and Cu. Therefore, this study aims to examine the heavy metal content of Cd and Pb in shellfish meat, and determine the condition of gill tissue and meat of Darah Mussels (Anadara granosa) and Kepah Mussels (Meretrix meretrix) from Batu Belubang. This research method is purposive sampling, and several analyses consisting of: analysis of heavy metals in sediment and meat, tissue observation from muscle/meat and gill histology. The results showed that the concentration of heavy metals Cd, Pb in sediment is still below the quality standard, this is due to the sediment texture at Batu Belubang Beach dominant with sand, so it has a low binding power to heavy metals. The concentration of heavy metals in meat is also still below quality standards, so it is still safe for consumption. This condition is also strengthened by the results of tissue observations that show the condition of the flesh and gill tissues has not been damaged. This condition shows that both shells have not experienced tissue disruption because the accumulation of heavy metals is still very low.

Keywords: Batu Belubang, Histology, Tissue, Shell, Heavy Metal

## ABSTRAK

Pantai Batu Belubang memiliki sumberdaya hayati Kerang Darah (*Anadara granosa*) dan Kerang Kepah (*Meretrix meretrix*) yang melimpah pada musimnya. Disisi lain, perairan Batu Belubang merupakan area pasca penambangan timah, sehingga menjadi kekhawatiran masyarakat untuk mengkonsumsi kerang dari daerah tersebut. Kekhawatiran ini disebabkan

oleh hasil penelitian sebelumnya yang menyampaikan bahwa daerah pasca penambangan timah menyisakan logam berat, salah satunya logam berat non essensial seperti Pb dan Cu. Oleh karena itu, penelitian ini bertujuan untuk mengkaji kandungan logam berat Cd dan Pb dalam daging kerang, dan mengetahui kondisi jaringan insang dan daging Kerang Darah (*Anadara granosa*) dan Kerang Kepah (*Meretrix meretrix*) dari Batu Belubang. Metode penelitian ini secara *purposive sampling*, dan beberapa analisis yang terdiri : analisis logam berat di sedimen dan daging, pengamatan jaringan dari histologi otot/daging dan insang. Hasil penelitian menunjukkan konsentrasi logam berat Cd, Pb di sedimen masih dibawah baku mutu, hal ini disebabkan tekstur sedimen di Pantai Batu Belubang dominan berpasir, sehingga memiliki daya ikat yang rendah terhadap logam berat. Konsentrasi logam berat dalam daging juga masih dibawah baku mutu, sehingga masih aman dikonsumsi. Kondisi tersebut juga diperkuat dengan hasil pengamatan jaringan yang menunjukkan kondisi jaringan daging dan insang belum ada kerusakan. Kondisi ini menunjukkan bahwa kedua kerang belum mengalami gangguan jaringan karena akumulasi logam berat masih sangat rendah.

Kata Kunci: Batu Belubang, Histologi, Jaringan, Kerang, Logam berat

#### **INTRODUCTION**

Bangka Island is an archipelago known as an area where tin mining can be done both on land and in water. One of the water areas that has been abandoned by illegal tin mining is Batu Be Lubang Beach. Several studies state that post-tin mining areas are known to still contain heavy metals in the sediment (Umroh et al., 2022; Umroh et al., 2023). This condition is a concern because Batu Be Lubang has benthic biological resources, one of which is the bivalve class. Blood cockles (*Anadara granosa*) and kepah cockles (*Meretrix meretrix*) are abundant in season. In general, the season for Blood Clams (*Anadara granosa*) and Kepah Clams (*Meretrix meretrix*) on Bangka Island is usually abundant in March – June. These two shells have important economic value, so many people collect them in the intertidal area of Batu Be Lubang Beach. This is a concern about the condition of shellfish from Be Lubang Beach if consumed by humans, due to their ability to accumulate heavy metals.

Research on the heavy metal Pb in *Anadara granosa* from the Musi River, Palembang ranges from Pb 2.41-2.85 mg/kg, and is classified as having exceeded the threshold (Putri & Anggraini, 2022). Research by Varotto et al., (2013) and Cordova (2016), shows that high Pb metal content in bivalves causes growth disorders and even malformations. We really need to be careful about the presence of heavy metals in waters because shellfish have a habit of eating from filter feeders, so heavy metals will enter with food. Heavy metals in sediment will be stirred when there is current, so that the heavy metals experience resuspension. This condition causes heavy metals to easily accumulate in the tissues of shellfish or other benthic biota.

Accumulation of heavy metals that exceed quality standard limits will inhibit the growth of shellfish (Riani *et al.*, 2018; Umroh *et al.*, 2021), and be dangerous for those who consume them, especially if lead (Pb) and cadmium (Cd) accumulate. Both metals are non-essential metals which are dangerous for living tissue. This is because the heavy metals Pb and Cd will deactivate the work of enzymes, so that these two heavy metals can induce damage to glands and organs through biochemical processes in the body (Mahalina et al., 2016). This condition impacts the morphology of the shellfish, damages tissue, and endangers the health of the shellfish. The negative impact on the condition of shellfish will also affect those who consume it. Some of these problems show that it is very necessary to study the heavy metal content in the bodies of shellfish in post-tin mining habitats, one of which is Batu Be Lubang Beach. Therefore, the aim of this research is to examine the content of the heavy metals Cd and Pb in shellfish meat, and determine the condition of the gill tissue and meat of Blood Clams (*Anadara granosa*) and Kepah Clams (*Meretrix meretrix*). The results of this research will be useful in

assessing the safety of the condition of highly economical shellfish found from Batu Be Lubang Beach, which are shellfish generally consumed by the public.

#### **METHODS**

#### **Place and Time**

The research was conducted in March 2023 in Batu Be Lubang Waters, Central Bangka Regency. Sampling locations were carried out at three points, as shown in Figure 1. Sediment samples were collected according to the presence/presence of benthos in the intertidal area. Analysis of Total Suspended Solid (TSS), sediment texture and heavy metals at the PT Global Quality Analytical Laboratory, Bogor. Making tissue histology preparations at the Histopathology Laboratory, Faculty of Veterinary Medicine, IPB University.

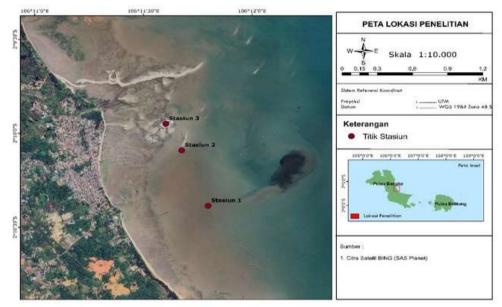


Figure 1. Map of sediment and shell sampling stations in Batu Be Lubang, Central Bangka Regency

#### Sediment and Water Sampling

Sediment samples were taken using an Eckman grab, and the samples were put into a flip plastic container and put in a cooler box at a temperature of  $\pm$  4oC to be taken to the laboratory. Physical - chemical water quality measurements in situ and in the laboratory. Parameters measured in situ include pH, DO, current speed, salinity and temperature. The parameters measured in the laboratory are total suspended solids (TSS), sediment texture, heavy metals in sediment and benthos and tissue histology.

#### Preparation of Total Heavy Metals (Cd, Pb) in Sediments and Benthos (APHA 2012)

Sediment samples are cleaned of litter and shell fragments, then sieving (50 micron size). Benthos samples were taken from the inside (soft) contents and collected at each station. Samples of sediment and benthic soft tissue were placed in a cup (sediment and benthos separated), and dried using an oven at 60°C until constant weight. The dried sediment samples were then ground and homogenized using a pestle and mortar (Riani et al., 2017; Suteja et al., 2020; Umroh et al., 2022). A 0.5 gram sample of fine sediment was placed in a vessel tube, then 5 ml of nitric acid (HNO<sub>3</sub>) and 1 ml of concentrated hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) were added, and left for 20-30 minutes. Next, the sediment samples in a closed vessel were destroyed using a microwave at 190°C for 1 hour. The sample is removed from the microwave and the vessel lid is slowly opened. The sample was poured into a 50 ml measuring flask, then dissolved

in distilled water to a limit of 50 ml. If there is sediment, it is filtered with 42 micron filter paper (APHA, 2012). Preparation and measurement of heavy metals in benthic soft tissue were carried out using the same method as sediment. Heavy metal concentrations were measured using Inductively Coupled Plasma - Emission Optical Spectroscopy (ICP-EOS).

# **Tissue Preparation and Histology**

Observation of tissue structure was carried out using the histology method, based on the method of Sanchez et al. (2016) and modifications. Samples were dissected for histological examination. The meat samples were placed in closed tubes labeled with information and filled with 10% formalin at room temperature for three days, then transferred to a container containing 70% alcohol. Meat samples were fixed in Bouin's solution for 24-36 hours and stored in 70% ethanol before making histological preparations. The meat is cut for dehydration with alcohol, clearing, infiltration, embedding, paraffinization, and with chloroform. In detail, it is explained as follows:

1. Dehydration

Dehydration is the stage of removing water, namely immersing the meat in stages in alcohol, starting from 80%, 90%, 95% to absolute alcohol. Soaking in alcohol in stages serves to attract water and prevent tissue shrinkage.

2. Clearing

Clearing is a purification, which aims to eliminate the influence of alcohol in the tissue, namely by immersing the tissue in xylol solution, so that it looks more transparent.

3. Infiltration

Soaking in paraffin in stages at a temperature of 60°C, and the paraffin is hard paraffin to make it easier to cut with very thin results.

4. Embedding

Embedding is planting or placing tissue in paraffin. This tissue implantation is carried out near Bunsen, the aim is to always keep it warm to prevent the paraffin from hardening before it is finished, then the container containing the tissue and paraffin is cooled so that it hardens and becomes a block, and stored in the refrigerator.

5. Paraffinization

Paraffinization is cutting paraffin blocks containing tissue, namely cutting paraffin blocks with a microtome machine with a thickness of 3-4 microns. The cut results are placed on the surface of water in a water bath at a temperature of 46°C to dissolve the remaining paraffin. Pieces in the form of paraffin sheets are placed on top of the preparation, and kept in an incubator (60°C) for 30-60 minutes.

6. Hematoxylin-Eosin staining process

## Data analysis

## **Comparative Analysis of Field Data with Quality Standards**

The results of measuring the concentration of heavy metals Cd, Pb in sediment were compared with several quality standards: Canadian Council of Ministers for the Environment (CCME, 2002) and (ANZECC & ARMCANZ, 2000). Results of measuring the concentration of heavy metals Cd, Pb in benthic soft tissue, compared with USEPA (2002), FAO (1989), and BPOM (2018) quality standards.

### RESULT

In this research, two types of clams were found, namely Blood Clams (*Anadara granosa*) and Kepah Clams (*Meretrix meretrix*), as in Figure 2. Both clams are clams with important economic value, so they are widely exploited by the coastal communities of Bangka Island

when the season arrives. The habitat of these two clams prefers to be immersed in sediment, so it is a concern if the sediment contains high levels of non-essential heavy metals.

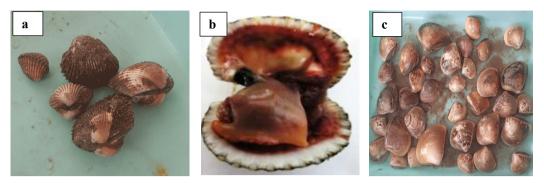


Figure 2. Macrobenthos found in the intertidal of Batu Be Lubang (a) Blood Clam (*Anadara granosa*); (b) opening of the Blood Clam (*Anadara granosa*); (c) Kepah Clam (*Meretrix meretrix*)

The results of the analysis show that in general the concentration of heavy metals Cd, Pb in the sediments of Batu Be Lubang waters is lower than in several studies in the sediments of the Matras waters of Bangka Island (Nugraha et al., 2022), sediments of Kelabat Bay (Umroh et al., 2022). The concentrations of heavy metals Pb and Cd in this study are still below the quality standards of ANZECC & ARMCANZ (2000), as shown in Table 1. This condition can be said to be that the sediments of Batu Be Lubang Waters are still suitable for the life of benthic organisms.

Station	S	Ε	Cd	Pb
1	02°10'23,1	106°11'47,8	0,0350	0,0010
2	02°10'05,4	106°11'40,5	0,0240	0,0008
3	02°09'56,9	106°11'36,1	0,0320	0,0007
ANZECC/ARMCANZ	Low		1.5	50
Guidelines (2000)	High		10	220

Table 1. Concentration of heavy metals in sediment (average - mg/kg in dry weight) in Sediment of Batu Be Lubang Waters, Central Bangka, Bangka Island

The results of the analysis of the accumulation of Cd, Pb metals in shellfish meat are also still low and below the quality standards of USEPA (2002), FAO (1989), and BPOM (2018) as shown in Table 2. This condition is possible that there has been no contamination of the tissue so that the growth of shellfish still in normal condition.

Table 2. Concentration of heavy metals in clam flesh (average	- mg/kg) from Batu Be Lubang
Waters, Central Bangka, Bangka Island	

Station	Spesies	Amount (individual)	Cd <sub>flesh</sub> (mg/kg)	Pb <sub>flesh</sub> (mg/kg)
1	Blood Clams (Anadara granosa)	102	0,002	0,117
2	Blood Clams (Anadara granosa	12*	-	-
3	Kepah Clams (Meretrix meretrix)	94	0,002	0,003
Quality	USEPA (2002)		1,4	1,7
standards	FAO (1989)		1	3
(mg/kg)	BPOM (2018)		0,10	0,20

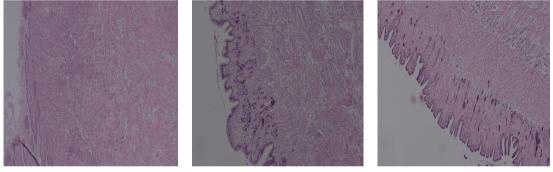
The impact of the accumulation of heavy metals (Cd, Pb) on tissue was also clarified by histological analysis of the flesh/muscle and gills, thus clarifying the influence of heavy metals on the tissue of Blood Clams (*Anadara granosa*) and Kapah Clams (*Meretrix meretrix*) (Figure 3–4).



Blood Clam (Anadara granosa) Station 1

Blood Clam (Anadara granosa) Station 2 Kepah Clam (*Meretrix meretrix*) Station 3

Figure 3. Histology of the gill tissue of the Blood Clam (*Anadara granosa*) and the Kepah Clam (*Meretrix meretrix*) with 10x magnification



Blood Clam (Anadara granosa) Station 1 Blood Clam (Anadara granosa) Station 2 Kepah Clam (*Meretrix meretrix*) Station 3

Figure 4. Histology of flesh/muscle tissue of Blood Clams (*Anadara granosa*) and Kepah Clams (*Meretrix meretrix*) with 10x magnification

The results of measurements of the physical and chemical quality of waters, consisting of temperature, salinity, DO, brightness, TSS and current speed, show that conditions are still normal, because they are still below the quality standards (Peraturan Pemerintah Republik Indonesia, 2021) as shown in Table 3.

Table 3. Results of physical and chemical measurements of Batu Be Lubang Waters, Central Bangka

St	Temper ature (°C)	Salinity (%)	DO (mg/L)	Brightness (%)	TSS (mg/L)	Current speed (m/s)	Depth (m)	Sedimentary texture
1	28,3	28	7,3	33,33	0,1280	0,163	2,4	Muddy sand
2	28,8	28	7,1	28,18	0,1316	0,110	2,2	Sand
3	29,6	28	6,8	12,88	0,1395	0,110	1,63	Sand
*BM	28-30*	33-34*	>5*	-	20			

\*Quality standards PP Number 22 of 2021

### DISCUSSION

Blood clams (Anadara granosa) and Kepah clams (Meretrix meretrix) have filter feeder eating habits (Haryono et al., 2017; Yaqin & Fachruddin, 2018). The habitat of these two clams is generally at the bottom of the water (surface of the substrate or buried in the substrate). The presence of live clams on the substrate will cause the accumulation of heavy metals from the sediment into the clam tissue. The results of the analysis of the heavy metal content Pb, Cd in the meat of Blood Clams (Anadara granosa) and Kepah Clams (Meretrix meretrix) from stations 1, 2 and 3 show that it is still below the quality standards of USEPA (2002). FAO (1989), and BPOM (2018). This shows that the two clam are still suitable and safe for consumption, however, this condition is spatial, meaning it only applies at the time of measurement. It is possible that the contents will differ if measured at different times according to the conditions of the aquatic environment. If measurements of heavy metals in meat are carried out and the heavy metal values are above the quality standard, then clam with a high metal content is not recommended for consumption, because it will be dangerous for consumers. The low accumulation of heavy metals Pb, Cd in the flesh of Blood Clams (Anadara granosa) and Kepah Clams (Meretrix meretrix), is due to the low content of heavy metals Pb, Cd in the sediment. The texture of the sediment at the three research stations shows that it is dominantly sandy (Table 3), so it has low binding capacity for heavy metals, this is what causes the heavy metal content in the sediment to be low. This is in accordance with the results of research (Umroh et al., 2023) which shows that the bulk sediment and dominant sand conditions are known to have low heavy metal content.

Heavy metals that accumulate in an organism's body will bind to enzymes and cell membranes through physiological reactions so that they can cause damage to the organism's cells and tissues if their content exceeds quality standard limits. The results of this study show that the accumulation of heavy metals Cd and Pb is very low (Table 2), so it has little or no effect on tissue damage. This is proven by the results of histological observations of the gill tissue and flesh/muscle of Blood Clams (*Anadara granosa*) and Kepah Clams (*Meretrix meretrix*), as shown in Figure 3 and 4. Both shellfish show that the condition of the gill tissue and flesh has not been damaged (Figure 3 and 4), so it can be seen that the two shellfish have not experienced tissue and metabolic disorders. Histology of the gill tissue has not shown hyperplasia, namely excessive tissue formation which results in thickening of the epithelial tissue at the ends of the filaments, like the shape of a baseball bat (Maftuch et al., 2015). The condition of the gill epithelial cells shows that they are still tight and quite well filling the gill lamella (Figure 3).

The results of observations of the gill tissue and flesh/muscle of Blood Clams (Anadara granosa) and Kepah Clams (Meretrix meretrix) showed that there was no damage to the tissue structure. This is also supported because the presence of these two shellfish in the environment does not cause pollution, and the physical and chemical water quality conditions are still in good condition. The results of measurements of temperature, salinity, DO and TSS of the waters at Batu Be Lubang Beach show that they are still below the quality standards of PP Number 22 of 2021. The temperature measurement values at 12 (twelve) research stations are between 28.3 oC - 30.39oC; salinity ranges from 28 - 31%; DO ranged from 6.7 - 7.3 (mg/l) and TSS ranged from 0.096 - 0.247 mg/l (Table 3). In general, the physico-chemical parameters of waters show a range of values that are not much different, because the measurements are still in the same season, namely transition season I. Physico-chemical factors of waters such as hydrodynamic conditions, current speed, salinity, pH, DO are important factors that play a role in distribution of metals in waters (Liu et al., 2016). By looking at the condition of the physical parameters which are still normal, the distribution of heavy metals is also relatively low, because the current speed is classified as moderate, the resuspension of heavy metals from the sediment is also only small.

# CONCLUSION

The content of heavy metals Pb, Cd in the flesh of Blood Clams (*Anadara granosa*) and Kepah Clams (*Meretrix meretrix*) is still in low concentrations. In general, *Anadara granosa* and *Meretrix meretrix* found on Batu Be Lubang Beach, Central Bangka are still below the quality standards of USEPA (2002), FAO (1989), and BPOM (2018). This low concentration of heavy metals Pb and Cd means that the meat tissue is still in good condition and no damage has occurred. The condition of the tissue that is still good indicates that the condition of the shellfish is relatively healthy and safe for consumption by the public.

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