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Mudskipper as an Indicator Species for Lead, Cadmium and Cuprum Heavy Metal Pollution in the Mangrove, Ambon, Indonesia

Kristin Sangur[1], Fredy Leiwakabessy[1], Hasan Tuaputty[1],
Laura Vintan Tuwankotta[2], Sisilya Vionetha Samloy[2], Costantina Ratila[2],
Olivia Basye Salakory[2], Chimberly Matulessy[2], Dominggus Rumahlatu[1*]

[1] Study Program of Biology Education, Faculty of Teacher Training and Education, Pattimura University, Jl. Ir. M. Putuhena, Ambon 97233, Indonesia

[2] Study Program of Biology Education, Graduate Students, Pattimura University, Jl. Ir. M. Putuhena, Ambon 97233, Indonesia

* Corresponding author's e-mail: dominggus_amq@yahoo.co.id

ABSTRACT

The heavy metal pollution in seawater and sediment can damage mangrove ecosystems and accumulate in mudskipper (*Periophthalmus* spp.) that lives in mangrove forests. The accumulation of heavy metal in mudskipper can affect the structure of its gill tissue, liver, and muscles. This research aims at (1) analyzing the levels of lead, cadmium and cuprum heavy metals in mudskipper, sediment, and seawater and (2) analyzing the damage to the gill, liver, and muscle tissue of mudskipper due to the exposure to heavy metals. The mudskipper samples were collected from the mangrove forests of Poka, Waai, and Rutong from May to June in 2019. The heavy metal analysis was conducted using the AAS method, while the gill, liver and muscle tissue staining was carried out with the HE (Hematoxylin Eosin) staining method with 40x magnification. The data were analyzed using descriptive analysis and correlational analysis. The results of the analysis showed that based on the mangrove locations, the order of Pb and Cu accumulation in mudskipper was Rutong> Poka> Waai; while the order of the Cd accumulation was Rutong> Waai> Poka. The changes in the gill tissue structure due to the exposure to heavy metals were in the form of teleangiectasia, secondary lamellar edema, hyperplasia, epithelial desquamation, clubbing, and primary lamellar edema. The changes in the liver tissue structure due to the exposure to heavy metals involved hemorrhage and fat degeneration. The changes in the muscle tissue structure due to the exposure to heavy metals include changes in muscle fibers, edema, and necrosis. The highest accumulation of Pb and Cd was found in gills and muscles from seawater, while low level of heavy metal Cu was found in the liver.

Keywords: heavy metals, lead, cadmium, cuprum; mangrove; mudskipper

INTRODUCTION

The heavy metal pollution in marine environment is caused by industrial and mining waste (Gümgüm et al., 1994), geographical, industrial, agricultural, pharmaceutical, domestic, and atmospheric wastes (He et al., 2005; Tchounwou et al., 2012). According to Şireli et al. (2006) and WHO (2008), heavy metals such as lead, cadmium and cuprum are toxic, and are mostly found in marine environments. In addition, the heavy metals in marine environments cannot be degraded, and can accumulate in organisms through industrial, atmosphere, soil, water cycles and food chain processes (Ziyaadini et al., 2017; Rodríguez et al., 2015; Monsefrad et al., 2012; Naghshbandi et al., 2007).

A lot of research has been conducted on the accumulation and distribution of heavy metals in marine environments and various biota, for example, the research on the heavy metal contamination in water and sediment conducted by (Bhuyan et al., 2017; Varol and Sen, 2012), and the research on heavy metal concentrations in Oysters (*Crassostrea* sp) in Setiu Wetlands, Terengganu (Shaari et al., 2016). Bioaccumulation of heavy metals in water and bodies of organisms depends on the ability of the organisms to accumulate metals and the heavy metal concentration in sediment, as well as the eating habits of these organisms (Eneji et al., 2011). The metal contamination in marine waters and sediments remains a global threat to biodiversity and humans (Edokpayi et al., 2015).

Heavy metals discharged by ship activity and gas-producing plants have contaminated marine environments. Therefore, monitoring on the water quality should be carried out to reduce health problems and to maintain the stability of aquatic ecosystems (Khan et al., 2018). Besides, Bhuyan et al. (2017) explained that the level of contamination and seasonal variations had an effect on the high levels of heavy metals in waters and sediments. Sediment is a source of heavy metal contamination in waters (Gu et al., 2016; Gu and Lin, 2016; Rosales-Hoz et al., 2003). Heavy metals can be absorbed and precipitated with sediment, along with the formation of heavy metal-sediment complexes. This can occur due to the physical and chemical properties of sediments (Pekey, 2006; Okafor and Opuene, 2007; Bibak et al., 2018). Bazzi (2014) explains that the threat of heavy metals in coastal waters can be analyzed through water analysis, sediment analysis, and marine organism analysis.

On the other hand, the use of organisms as pollution indicator species to monitor heavy metals in waters has become the current research trend. Rahmanpour et al. (2016) used the Asian stinging catfish or fossil cat (*Heteropnestis fossilis*) and crab (*Parasesarma persicum*) as pollution bioindicator species to monitor the Hg, As, Cd, Pb and Cu heavy metals in the Arvand River. Rumahlatu and Huliselan (2016) used *Apogon beauforti* as a bioindicator species to monitor the Hg pollution in Ambon Island waters. Vaidya (2017) reported that there were 19 species of zooplankton as pollution bioindicators in Nagpokhari Kathmandu. Moreover, Wickramasinghe et al. (2017) reported that the accumulation of the Cu, Cd and Pb heavy metals had an effect on the pigmentation and productivity of the *Fucus vesiculosus* and *Ulva lactuca* algae, and both types of algae can be used as bioindicators to monitor the metal pollution in coastal waters. Mbeh et al. (2019) reported that the level of heavy metal Cu in fish was higher than that in crustacean species.

One of the aquatic organisms that can be used as a pollution bioindicator in mangrove forests is the mudskipper fish (*Periophthalmus* spp.). This is because mud, one type of mangrove forest substrate, accumulates a lot of nutrients and minerals, including heavy metal pollutants. Consequently, the bodies of the mudskipper fish that live immersed in mud can also accumulate heavy metals. The mudskipper fish, which lives immersed in mud and jumps on the mud, is very tolerant to inorganic and organic pollutants. Therefore, mudskipper fish potentially accumulates the heavy metals disposed from industry, agriculture, domestic and transportation activities. Thus, this fish is very suitable for a marine bioindicator species (Polgar et al., 2010; Dabruzzi et al., 2011; Ansari et al., 2014). According to Bu-Olayan and Thomas (2008), the *Periophthalmus waltoni* mudskipper is prey for predators in mangrove ecosystems, lives in mud during both high tides and low tides, and is capable of accumulating the Zn, Cu, Cd and Fe heavy metals. Therefore, it can be used as a marine bioindicator species.

Mudskipper is abundantly found in the mangrove ecosystems of Poka, Waai and Rutong villages. The mangrove ecosystem of the Poka village represents the deep Ambon bay area. Meanwhile, the mangrove ecosystems of the Waai and Rutong villages represent the areas facing the open sea. The main sources of pollution in the mangrove forest in the Rutong village are household waste in the form of inorganic and organic waste, wastewater from laundry and car wash, and fishing activities in the form of fishing boats tied to mangrove trunks. Meanwhile, the main sources of pollution in the mangrove forest in the Poka village are organic and non-organic household waste, animal waste, and shipyard industrial activities. The main sources of pollution of the mangrove forest in the Waai village are household waste in the form of inorganic and organic waste, as well as fishing activities in the form of fishing boats tied to mangrove trunks. The research by Souisa (2017) and Male et al. (2017) also revealed that the accumulation of the Pb and Cd heavy metals was found in seawater and the sediments of the deep Ambon bay due to the community activities, residential areas, traditional transportation activities in the form of boats or speedboats, as well as the presence of *PLTD* (Diesel Power Electricity Generator) and shipyards. The source of Pb pollution comes from human activities which include burning fossil fuels and mining activity. Moreover, lead is also used in industrial activities, such as agriculture, paint making, battery manufacturing, ammunition, pipes, and devices to avoid X-rays, ship traffic, and lead-zinc smelting (Mulligan et al., 2001; Tchounwou et al., 2012). Gaetke et al. (2014) explained that the sources of the Cu pollution include water pipes, cooking utensils, and fungicides for swimming pools. Hasan et al. (2016) argued that industrial activities which included gas production, shipyard, port activities, urban waste, and metallurgical activities were the sources of the Cd pollution in the Bengal Bay. Therefore, the heavy metal accumulation analysis in the mangrove ecosystems of Waai and Rutong villages is essential.

According to Tanjung et al. (2019), aquatic organisms will absorb the heavy metals that accumulate in waters, but these heavy metals become toxic to the bodies of the aquatic organisms. The same thing will also occur to the body of mudskipper. The toxicity of the absorbed heavy metals can physiologically, morphologically, and genetically disrupt the homeostasis of mudskippers. According to the research results by Moslen and Miebaka (2016), this can happen because of the ability of mudskippers to accumulate and increase the levels of Cr, Ni, Pb, Ag and Cd heavy metals in the tissue without physical stress and season influences. In fact, the accumulation of Pb, Cd, and Cu heavy metals in the body of mudskippers can cause the structure of the fish tissue to be anomalous. The research results by Dange and Manoj (2015) also reported that the Cu, Zn and Ni heavy metals were found to accumulate in the digestive tract of mudskipper fish. Renieri et al. (2014) also reported that higher levels of Cd, Pb, and Hg heavy metals were accumulated in the liver and muscles, compared to fish gonads. This is affected with seasons and the age of the fish. Moreover, Thakur and Mhatre (2015) confirmed that the levels of heavy metal accumulation in fish depend on the species, age, and gender of the fish. The research conducted by El-Moselhy et al. (2014); Reyahi-Khoram et al. (2016) and Arantes et al. (2016) reported that the highest concentrations of heavy metals (Cu, Zn, Pb, Cd, Fe and Mn) were found in the liver and gills of benthic and pelagic fish in the Red Sea, Arabia. The liver tissue is often used as a sample of damage caused by the accumulation of heavy metals in the environment (Amaral et al., 2002). On the basis of the results of the previous research, the heavy metal pollution (Pb, Cd, and Cu) can accumulate in seawater, sediment, and mudskipper bodies in three mangrove areas in the Poka, Waai and Rutong villages. The accumulation of heavy metals can affect the tissue structure of mudskipper to become anomalies. Therefore, the purposes of this research were (1) to analyze the concentration of heavy metals in seawater, sediments and bodies of mudskippers; (2) to analyze the damage to the gill, liver and muscle tissue of mudskipper fish due to the exposure to heavy metals.

MATERIALS AND METHODS

Study area

This research was conducted in the waters of Ambon Island, Indonesia (Figure 1). The research locations were at Ferry harbor, Poka (station 1), DPEG (Diesel Power Electricity Generator), Poka (station 2), Waai (station 3), Waai (station 4), Rutong (station 5), Rutong (station 6).

Sample collection

The samples of water, sediment and mudskipper specimens were collected from each station. Two samples of mudskipper fish were randomly collected from each station. Afterwards, the samples were placed into plastic bags and labeled. The samples were stored in an ice box. All the samples were subsequently taken to the laboratory for analysis.

Sample preparation and analysis of the Pb, Cd, and Cu

The Pb, Cd, and Cu heavy metals from all samples were analyzed at Environmental Health and Disease Control Engineering Center – Ambon, Indonesia. The samples were prepared and reconstructed. Then, a calibration curve was created and then analyzed using an Atomic Absorption Spectrophotometer (AAS) to determine each heavy metal. Afterwards, the absorbance from the sample solution was put into the calibration curve, then the amount of μg Pb, Cd, and Cu could be calculated (Sirait et al., 2013). The heavy metal content in the sample (wet weight) was calculated by the equation:

$$\text{Content, ppm} = a/b$$

where: a – the amount of metal μg from the measurement results with AAS,
b – sample weight (5.0 g).

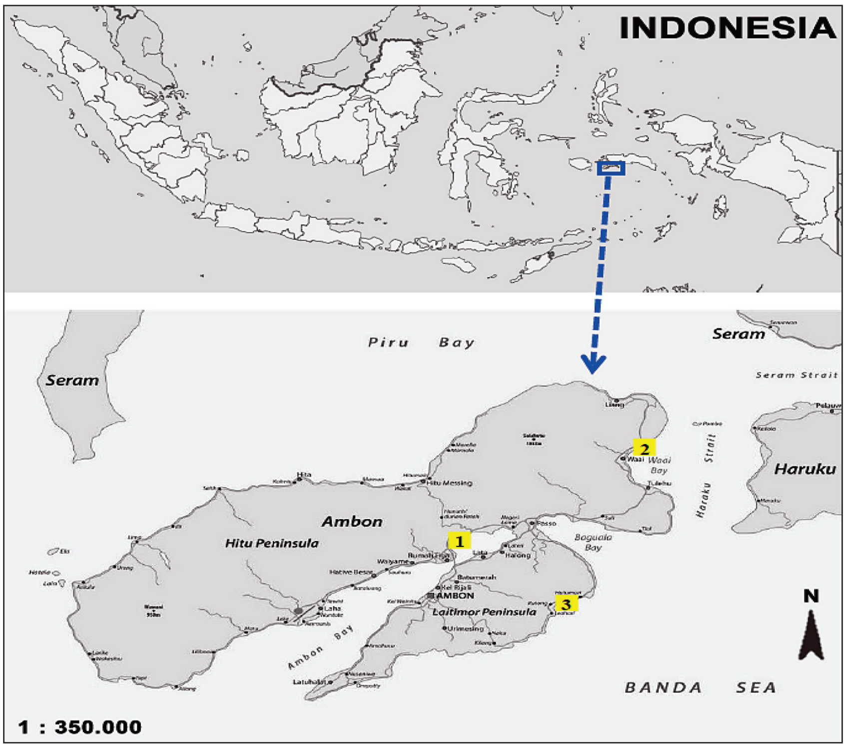


Figure 1. Location of sample collection: 1 – Poka, 2 – Waai, 3 – Rutong station

The histological analysis of the gills, liver and muscles of mudskippers

The histological preparation followed Chan (2014), with the following steps: (1) the samples were washed and then dissected to separate the gills, liver and muscle; (2) the gills, liver and muscles were then placed in 4% formalin, and then fixation was carried out for 24 hours; (3) after the fixation, the tissue was dehydrated to alcohol at 70%, 80%, 90%, absolute, xylol 1 and xylol 2 for 1 hour each; (4) the intestinal tissue was placed in a vacuum to remove air from the tissue; (5) the tissue was molded with a paraffin block, and then thinly sliced using a microtome; (6) the sections were subsequently placed on a waterbath and then removed using a glass object and placed on a hot plate; (7) afterwards, the tools and materials for staining were prepared, including xylol 1, xylol 2, absolute alcohol, graded alcohol, hematoxylin solution, eosin solution; (8) the sections were then arranged on a shelf, and placed in xylol 1, xylol 2, absolute alcohol, 90% alcohol, 80% alcohol, 70% alcohol, hematoxylin solution, rinsed with running water for 1 week, eosin solution, rinsed under running water for 1 week, 80% alcohol, 90% alcohol, absolute alcohol, xylol 1, and xylol 2. Each solution was added for 3 minutes; (9) the adhesive solution was dropped into a glass object and then covered with a glass cover and observed under a microscope at 40x magnification. Histology description of the mudskipper gills, liver and muscles used the Hematoxylin Eosin (HE) staining method, which was carried out at the Zoology Laboratory, Faculty of Mathematics and Natural Sciences, Pattimura University.

Data analysis

The data were descriptively analyzed to illustrate the condition of water, sediment and the body tissue histology of the mudskipper that had been exposed to heavy metals. Furthermore, to analyze the effect of heavy metal concentrations on the damage to the gill, liver and muscle tissue of the mudskipper in the Ambon Island waters, the research data were analyzed using correlational analysis to determine the effective contribution of each heavy metal towards the damage of the mudskipper tissue.

RESULTS AND DISCUSSION

Heavy metal concentrations in the mangrove area

Table 1 presents the results of the measurements on the Pb, Cd, and Cu heavy metals in the

Table 1. Heavy metal analysis in the fish, sediment and water samples (ppm)

Locations	Fish			Sediment			Seawater		
	Pb	Cd	Cu	Pb	Cd	Cu	Pb	Cd	Cu
Poka S1	0.0038	0.0060	0.0086	0.0055	0.0067	0.0086	0.0057	0.0061	0.0171
Poka S2	0.0019	0.0054	0.0076	0.0052	0.0050	0.0137	0.0055	0.0096	0.0078
Average	0.0029±0 .001	0.0057±0.000	0.0081±0.000	0.0054±0.000	0.0059±0.001	0.0112±0.003	0.0056±0.000	0.0079±0.002	0.0125±0.007
Waai S1	0.0020	0.0068	0.0102	0.0052	0.0055	0.0190	0.0051	0.0100	0.0096
Waai S2	0.0026	0.0052	0.0093	0.0048	0.0067	0.0183	0.0056	0.0089	0.0085
Average	0.0023±0.000	0.0060±0.001	0.0098±0.001	0.0050±0.000	0.0061±0.001	0.0187±0.000	0.0054±0.000	0.0095±0.001	0.0091±0.001
Rutong S1	0.0036	0.0059	0.0101	0.0051	0.0049	0.0137	0.0057	0.0086	0.0099
Rutong S2	0.0033	0.0064	0.0115	0.0055	0.0063	0.013	0.0054	0.0072	0.0084
Average	0.0035±0.000	0.0062±0.000	0.0108±0.001	0.0053±0.000	0.0056±0.001	0.0134±0.000	0.0056±0.000	0.0079±0.001	0.0092±0.001

fish, sediment and seawater samples in the mangrove area of the Ambon Island waters.

The results of the analysis showed that the heavy metals that accumulated in the fish body and sediment in the mangrove area of the Poka, Waai and Rutong villages from the highest to the lowest were Cu> Cd> Pb; while the heavy metals that accumulated in the seawater in the mangrove areas of the Poka and Rutong villages were Cu> Cd> Pb, whereas the heavy metals that accumulated at the two stations in the mangrove waters of the Waai village were Cd> Cu> Pb (Table 1). The Cu heavy metal which accumulated more in the fish body can be associated with the fact that Cu is an essential metal for organisms. Cu is classified as a transition metal group that has a certain function in the respiration of organisms, but it becomes toxic at high concentrations (Khaled, 2004). Anandkumar et al. (2017) reported that there was a higher increase in the accumulation of essential heavy metals in the bodies of shrimp than that of the accumulation of non-essential heavy metals. The accumulation of heavy metals in the body of fish is influenced by the nature of the heavy metals, the bioavailability, the feeding characteristics, the ecology and the physiology of fish (Perugini et al., 2014). Rainbow (2007) asserts that heavy metal toxicity can occur in living organisms when the accumulation of the heavy metals exceeds the level of metabolic excretion and detoxification of the organisms. Stern (2010) explains that the Cu heavy metal can be found as a transitional form between oxidized Cu (II) and reduced Cu (I) which is toxic because it produces superoxide and hydroxyl radicals. Frazier (1979) explains that Pb and Cd are toxic heavy metals, even in low concentrations because they cannot be degraded and can accumulate in the tissues of organisms. Similarly, Dange and Manoj (2015) explain that the concentration of the Cd heavy metal is lower than that of Cu heavy metal because of the toxicity and the nature of the Cd heavy metal, which is a non-essential metal for aquatic organisms. Elbay-Poulichet et al. (1987) states that Cd can be released from its organic complex, by increasing the concentration of Cl (chlorine), which forms a chloride complex. This research is also supported by the research by Tjahjono and Suwarno (2018) that the concentrations of the Pb and Cd heavy metal in the sediments of Demak waters was lower.

The order of the accumulation of the Pb heavy metal in fish body based on the locations was Rutong village> Poka village> Waai village; while the order of the accumulation of the Cd and Cu heavy metal was Rutong> Waai> Poka. The higher average concentrations of Pb, Cd and Cu were reported in the mudskipper fish originating from the mangroves in the Rutong village. This is related to the source of heavy metal pollution in the mangrove area of the Rutong village. Similar results were also reported by Nasr et al. (2006) that the areas with sources of pollution of household wastewater, industrial waste, fishing activities, and fiberglass boat factories tend to have higher accumulation of heavy metals. The research by Rijal et al. (2014) also reported that the Pb and Cd heavy metal accumulated in the sea waters of the Waai village. Male et al. (2014) emphasizes that the accumulation of the Pb heavy metal in the Waai waters may be caused by the Tetra Ethyl Lead (TEL) compound in the combustion residue released into the air and then descends into the waters through rain, the place where ships are anchored, oil spills and burning remains of motorboats, resident activities who dispose of garbage in the form of cans, plastics, electric cables, paint cans, used batteries. Meanwhile, the order of heavy metal accumulation in fish, sediment and sea water showed varied results.

In the mangrove waters of the Poka village, the Cu, Cd, and Pb heavy metal accumulated in seawater> sediment> fish. Meanwhile, in the mangrove waters of Waai village, the Cd and Pb heavy metal accumulated in seawater> sediment> fish, and the Cu heavy metal accumulated in sediment> fish> sea water. The mangrove waters of the Rutong Village also showed varied accumulations of heavy metals in seawater, sediment and fish bodies. The Cd heavy metal accumulated in seawater> fish> sediment, Cu accumulated in sediments> fish> seawater, and Pb accumulated in seawater> sediment> fish (Table 1). The high accumulation of Cu in sediments can be explained by the fact that the organic matter from household waste, leaves, mangrove litter, and carcasses of animals is degraded by detritivores and stays in sediments. This causes the sediments to accumulate high organic matter. Meanwhile, the Cu heavy metal binds more quickly to organic matter. Similar arguments are also stated by Rath et al. (2009)mineralogy, morphology and multivariate statistical technique for quantifying metal pollution in highly polluted aquatic sediments-a case study: Brahmani and Nandira Rivers, India.”,”type”:”article-journal”,”volume”:”163”},”uris”:[“http://www.mendeley.com/documents/?uuid=b1e05b46-8dcc-432f-8469-dc22d26de483”]}],”mendeley”:{“formattedCitation”:”(Rath et al., 2009, Mortatti and Probst (2010), and Yu et al. (2010) that Cu has a positive correlation with organic matter, so that it has high stability with organic compounds. Moreover, it is also explained that when Cu is in the form of Cu^{2+} ion, its affinity greatly increases metal mobility in the sediment. Meanwhile, the accumulation of the Cu, Cd, and Pb heavy metal in the mangrove waters of the Poka Village was higher than that in the seawater due to the waste disposal of PLTD (Diesel Power Electricity generator), shipyards and transportation. According to Lee et al. (2016), Sany et al. (2012) and Salleh and Halim (2018), the sources of heavy metals such as paint on ships, ship activity and transportation, and industrial waste can accumulate heavy metals in water. Coban et al. (2009) adds that the heavy metals dissolved in seawater vary and are not constant, depending on the heavy metal elements. The high accumulation of Cd and Pb heavy metal in seawater in the mangrove areas of the Waai and Rutong villages is influenced by the activities of motorized vehicles, boat traffic, motorized boats, and various kinds of inorganic waste. The findings of this research are also supported with the research results by Almiqrh et al. (2018) that the high accumulation of Pb heavy metal was influenced by the research location, which was close to manufacturing factories, so that a lot of factory waste was disposed into the water bodies, and shipping activity. Edokpayi et al. (2015) add that the emissions from cars and poor waste disposal are also sources of heavy metal contamination. Varol and Sen (2012) explain that the sediments containing heavy metals are a secondary source of heavy metal accumulation in aquatic biota.

Damage to gills due to accumulation to heavy metals

On the basis of the accumulation of heavy metals in the mudskipper fish, histological observations of the gills, liver and muscles were carried out to determine the tissue damage as a physiological response to exposure to these heavy metals. The damage to the gills can be seen in Figure 2. The damage to the gill tissue is characterized by primary lamellar edema, hyperplasia, epithelial desquamation, teleangiectasia, secondary lamellar edema, and clubbing. The types of the gill tissue damage differ among locations. This suggests that heavy metal accumulation can damage the gill tissue. The types of the damage can be visually seen in Figure 2, while the total damage in each location can be seen in Table 2.

It was known that the seawater in the three water locations contained the Cd, Cu, and Pb heavy metal. Meanwhile, gills are the respiratory organs of fish, which are immediately exposed to seawater. Therefore, gills are suitable for histological examination to determine the effects of pollution and xenobiotics, because they are the main organs that are directly exposed to seawater (Drishya et al., 2016; Rankin and Jensen, 1993). Camargo and Martinez (2007) add that the histological changes in the gills, kidneys and liver in *Prochilodus lineatus* indicate that the water flow has been contaminated causing stress effects on fish.

In addition to being a respiratory organ in fish, gills also function as osmotic regulators and excretion organs in the fish body. Evans et al. (2005) state that gills have a function as osmoregulation, gas exchange, excretion, and ion uptake or extrusion, therefore, these organs are ideal for analyzing toxins in aquatic toxicology studies. Thus, gills are representative organs for the analysis of tissue changes due to the heavy metal accumulation in

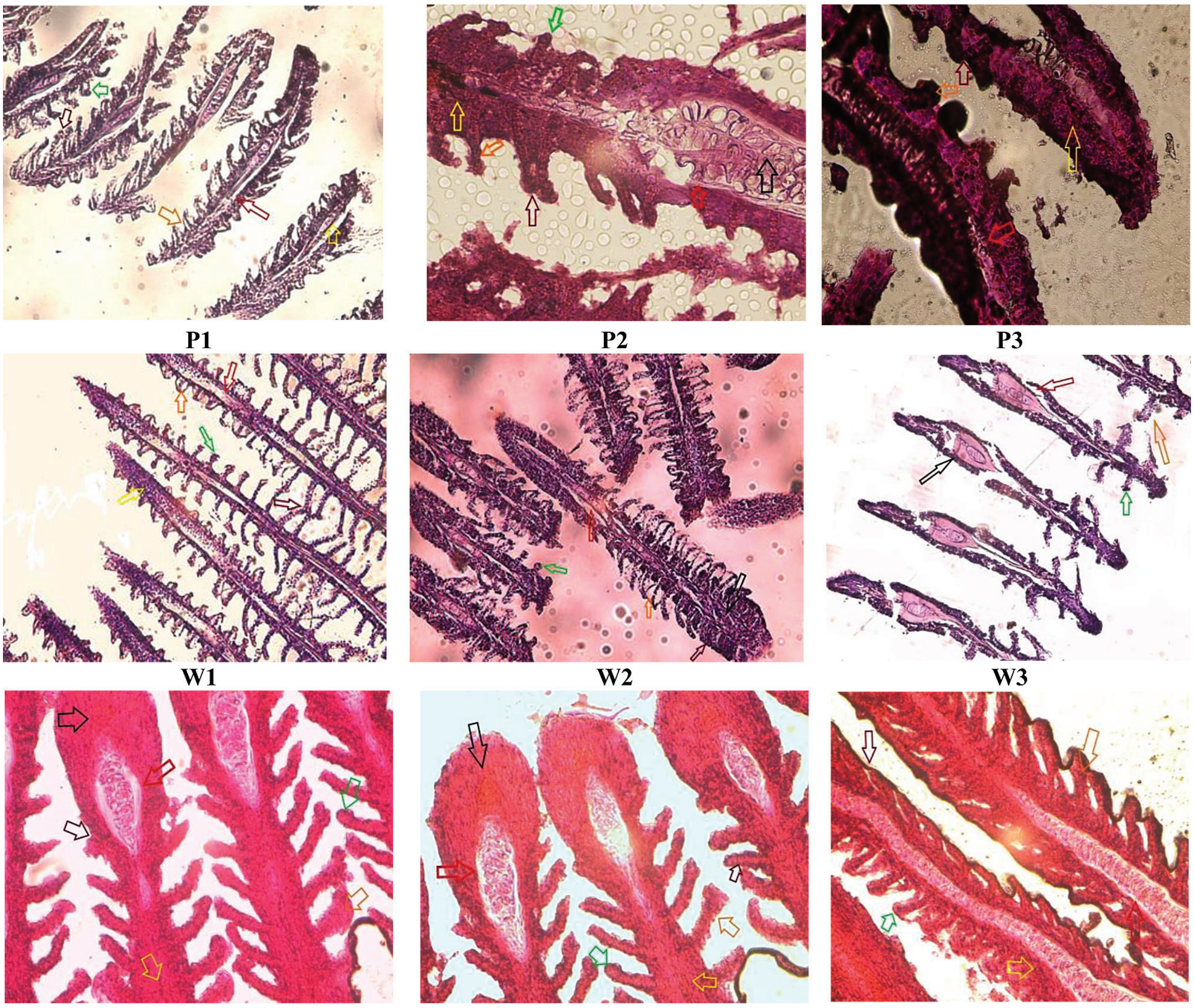


Figure 2. Histological overview of the gill tissue of mudskipper fish. P1, P2, P3 are the locations of Poka stations 1, 2, and 3. W1, W2, W3 are Waai stations 1, 2, and 3. R1, R2, R3 are Rutong stations 1, 2, and 3. Teleangiectasia (green arrows), Secondary Lamela Edema (orange arrows), Hyperplasia (dark red arrows), Epithelial desquamation (red arrows), Clubbing (black arrows), Primary Lamela Edema (yellow arrows). The staining used HE with a magn. of 40×.

Table 2. Calculation of the number of damaged gill cells

L	Picture	The number of damaged gill																								
		I					II						III					IV						V		
		PLE	H	D	SLE	T	PLE	H	D	SLE	T	C	PLE	H	D	SLE	T	PLE	H	D	SLE	T	C	PLE	D	C
P	1	6	5	1	0	2	6	4	0	0	4		0	22	0		1	3	3	1	0	2		1	1	0
	2	2	4	0	0	0	1	5	1	5	4		2	0	1		0	0	0	1	0	0		0	0	1
	3	1	3	0	3	0	0	3	0	4	4		1	6	0		0	1	3	0	3	3		1	0	0
	Average	3.0±2.65	4.0±1.0	1.0±0.6	3.0±1.7	2.0±1.1	3.5±3.2	4.0±1.0	1.0±0.6	4.5±2.7	4.0±1.0		1.5±1.0	14.0±11.4	1.0±0.9		1.0±0.6	2.0±1.5	3.0±1.7	1.0±0.6	3.0±1.7	2.5±1.5		1.0±0.6	1.0±0.6	1.0±0.6
W	1	1	0	0	7	7		0	0	0	7			3	0	0	5	0	0	1	5	6	0	0	4	
	2	0	15	2	0	0		7	0	6	4			7	0	7	6	0	25	1	3	6	1	0	4	
	3	1	30	0	0	0		10	1	0	0			10	1	0	0	1	5	0	3	0	1	1	2	
	Average	1.0±0.6	22.±15.0	2.0±1.2	7.0±4.0	7.0±4.0		8.5±5.1	1.0±0.6	6.0±3.5	5.5±3.5			6.7±3.5	1.0±0.6	7.0±4.0	5.5±3.2	1.0±0.6	15.0±13.3	1.0±0.6	3.7±1.2	6.0±3.5	1.0±0.6	1.0±0.6	3.3±1.2	
R	1		0		3		2	3	2	0		1	2	0		3	3	1	0		2	0		1	0	
	2		0		2		2	3	2	0		1	0	0		3	1	2	0		2	2		1	0	
	3		6		6		0	6	0	6		0	0	5		5	0	0	7		7	0		0	2	
	Average		6.0±3.5		3.7±2.1		2.0±1.2	4.0±1.7	2.0±1.2	6.0±3.5		1.0±0.6	2.0±1.2	5.0±2.9		3.7±1.2	2.0±1.5	1.5±1.0	7.0±4.0		3.7±2.9	2.0±1.2		1.0±0.6	2.0±1.2	

Note: PLE – primary lamella edema, H – Hyperplasia, D – epithelial desquamation, SLE – secondary lamella edema, T – teleangiectasia, C – clubbing, L – locations, P – Poka stations, W – Waai stations, R – Rutong stations.

seawater. One of the striking changes that occur in the gills of mudskipper fish is primary lamella edema and hyperplasia. Hyperplasia is a histological condition in fish gills characterized by secondary lamella thickening and secondary lamella cell fusion due to abnormal cell division. Lujic et al. (2013) stated that hyperplasia causes a reduction in the respiratory surface and removal of the epithelium which affects the water absorption and blood circulation, and it also triggers a gill defense mechanism when exposed to pollutants. However, these changes can lead to respiratory distress. Hyperplasia can cause disruption of the respiratory system of fish, because the secondary lamellae cells are fused and adjoined.

Primary lamellae edema is a condition of swelling in the primary lamellae of the gills which functions to supply blood to and from the secondary lamella. This swelling can disrupt the function of the primary lamellae. Fadaeifard and Azizi (2014) added that the thickening of the lamellae is a form of defense that reduces the surface area of the lamella branches exposed to the external environment. In addition, there is also a condition of teleangiectasia, where the tip of the secondary lamellae is ball-like, causing a buildup of red blood cells due to the damage to the pillar cells that make up the secondary lamellae. Bhagwant and Elahee (2002) added that the teleangiectasia condition observed in *Mulloidichthys flavolineatus* and *Mugil cephalus* could affect blood circulation, causing respiratory problems. The research by Oliva et al. (2013) showed that there was a correlation between the Pb heavy metal and changes in gill histology such as hypertrophy, hyperplasia, and epithelial thickening of the lamellae.

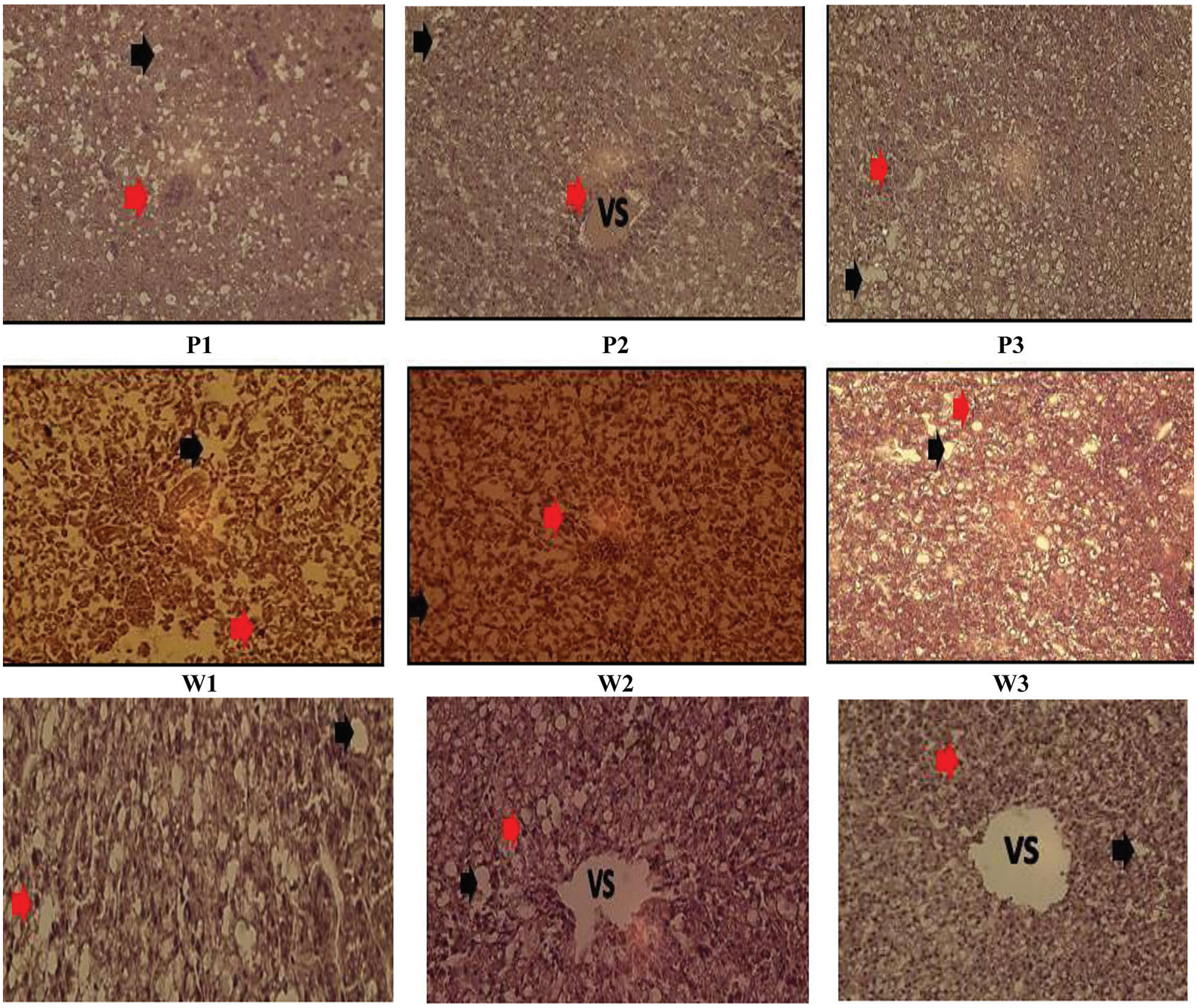
The research conducted by Rajeshkumar and Li (2018) reports that the gills of *Pelteobagrus fluvidraco* and *Cyprinus carpio* accumulate heavy metals due to the binding of metals with mucus which is difficult to separate. Similarly, the research conducted by Dane and Sisman (2015) shows that the accumulation of the Cd, Al, As, Pb, and Mn heavy metal may cause disturbance to the gills such as hypertrophy, hyperplasia, thickening of the lamellae, vasodilation, congestion and fusion. The research by Flores-Lopes and Thomaz (2011) shows that as a result of epithelial desquamation, there is an increase in the distance between water and blood, which disrupts the oxygen absorption. Similar research results were also reported by Khabbazi et al. (2015) that the absorption of copper can cause changes in the gills of rainbow fish, namely epithelial hypertrophy, hyperplasia, fusion of lamellae and aneurysms and edema in the gills.

Liver damage due to accumulation to heavy metals

The types of liver damage among locations vary. This shows that the accumulation of heavy metals can damage liver tissue. The type of damage can be visually seen in Figure 3, while the total damage for each location can be seen in Table 3.

Heavy metals can accumulate not only in the gills of mudskipper fish, but also in other fish tissues, such as liver and muscle. Therefore, the histological changes that occur in the gills can also occur in the liver and muscles. Dane and Sisman (2015) add that when absorbing heavy metals, fish can accumulate the heavy metals efficiently in various. The histological changes in the mudskipper liver are hemorrhage and fatty degeneration. Changes in the liver serve as a marker that fish have been exposed to a stressful environment (Velmurugan et al., 2009; Figueiredo-Fernandes et al., 2007). The liver is a multifunctional organ. The liver has a function to perform the metabolic processes of fat, protein, carbohydrates; nutrient distribution; and detoxification of toxic metabolites, drugs and xenobiotics, so that drugs, alcohol, and xenobiotics can damage liver cells (Chiang, 2014; Gulati et al., 2018; Mitra and Metcalf, 2009). The physiological function of the liver as detoxification causes this organ to be used as a biomarker of environmental quality. Bu-Olayan and Thomas (2008) argue that liver has the highest accumulation of heavy metals, because it functions as a detoxifier, while the accumulation of heavy metals in muscles is due to the absorption of heavy metal residues through the intestinal walls. In turn, the accumulation that occur in gills is due to the formation of metal complexes with mucus in the gill lamellae. Camargo and Martinez (2007) added that the detoxification and biotransformation processes of the liver make this organ the most vulnerable to the damage caused by various toxins.

As a biomarker of environmental quality, the liver of mudskippers in this research experienced some changes, such as fat degeneration and hemorrhage in hepatocytes. The research by Oliva et al. (2013) revealed that necrosis in the liver has a correlation with the accumulation of the As, Cd, and Cu heavy metal in water. The results of this



P1 **P2** **P3**

W1 **W2** **W3**

R1 **R2** **R3**

Figure 3. Histological overview of the liver tissue of the mudskipper fish. P1, P2, P3 are the locations of Poka station 1, station 2, and station 3. W1, W2, W3 are Waai station 1, station 2, and station 3. R1, R2, R3 are Rutong station 1, station 2, and station 3. Vena Sentralis (VS), Hemorrhage (red arrows), Fatty degeneration (black arrows). Staining used HE with a magnification of 40×.

Table 3. Calculation of the number of damaged liver cells

L	Figure	The number of damaged liver									
		I		II		III		IV		V	
		H	FT	H	FT	H	FT	H	FT	H	FT
P	1	82	38	42	24	51	28	62	28	40	47
	2	136	35	152	25	123	19	67	21	126	13
	3	77	34	85	23	66	62	69	44	93	47
	Average	98.33±32.72	35.67±2.08	93.00±55.43	24.00±1.00	80.00±37.99	36.33±22.68	66.00±3.61	31.00±11.79	86.33±43.39	35.67±19.63
W	1	55	30	33	32	42	34	42	32	80	35
	2	71	31	61	29	42	35	44	32	60	40
	3	44	14	50	13	50	14	25	21	46	21
	Average	56.67±13.58	25.00±9.54	48.00±14.11	24.67±10.21	44.67±4.62	27.67±11.85	37.00±10.40	28.33±6.35	62.00±17.09	32.00±9.85
R	1	59	19	78	7	46	17	43	7	49	18
	2	73	32	79	28	70	23	77	16	95	14
	3	118	14	82	31	147	28	130	24	159	17
	Average	83.33±30.83	21.67±9.29	79.67±2.082	22.00±13.08	87.67±52.77	22.67±5.51	83.30±43.8	15.67±8.51	101.00±55.24	16.33±2.08

Note: H – hemorrhage, FD – fatty degeneration, L – locations, P – Poka stations, W – Waai stations, R – Rutong stations.

research also indicated that the Cd, Cu, and Pb heavy metals were accumulated with varying concentrations at three locations. According to Godt et al. (2006), the chemical and physical characteristics of the Cd heavy metals are similar to those of the essential metals such as Fe, Zn or Ca, and thus it can be bound and incorporated into cells by "ionic" and "molecular mimicry" processes.

Several research results have shown changes in fish livers. The research by Hadi and Alwan (2012) also shows changes in the liver tissue of *Tilapia zillii* in the form of hypertrophy of hepatocytes, and blood congestion in the central veins. Ishibashi et al. (2009) adds that the tissue damage caused by various xenobiotics can cause fibrosis, inflammation, and necrosis, thus affecting the normal liver morphological structure and deteriorating the metabolic function of the liver. Ardeshir et al. (2017) the presence of heavy metals in the liver tissue causes oxidative stress and an increase in reactive oxygen species (ROS) through the induction of oxygen and the transformation of oxygen into superoxide, hydrogen peroxide and hydroxyl radicals. Meanwhile, these hydroxyl radicals are able to bind to various compounds. The research by Abalaka (2015) also reported that the liver histology of the *Auchenoglanis occidentalis* fish exposed to heavy metals showed hemorrhage. Mustafa et al. (2017) also reported that the liver of the *Cyprinus carpio* L. fish exposed to high concentrations of Pb showed a necrotic condition with nuclear picnosis.

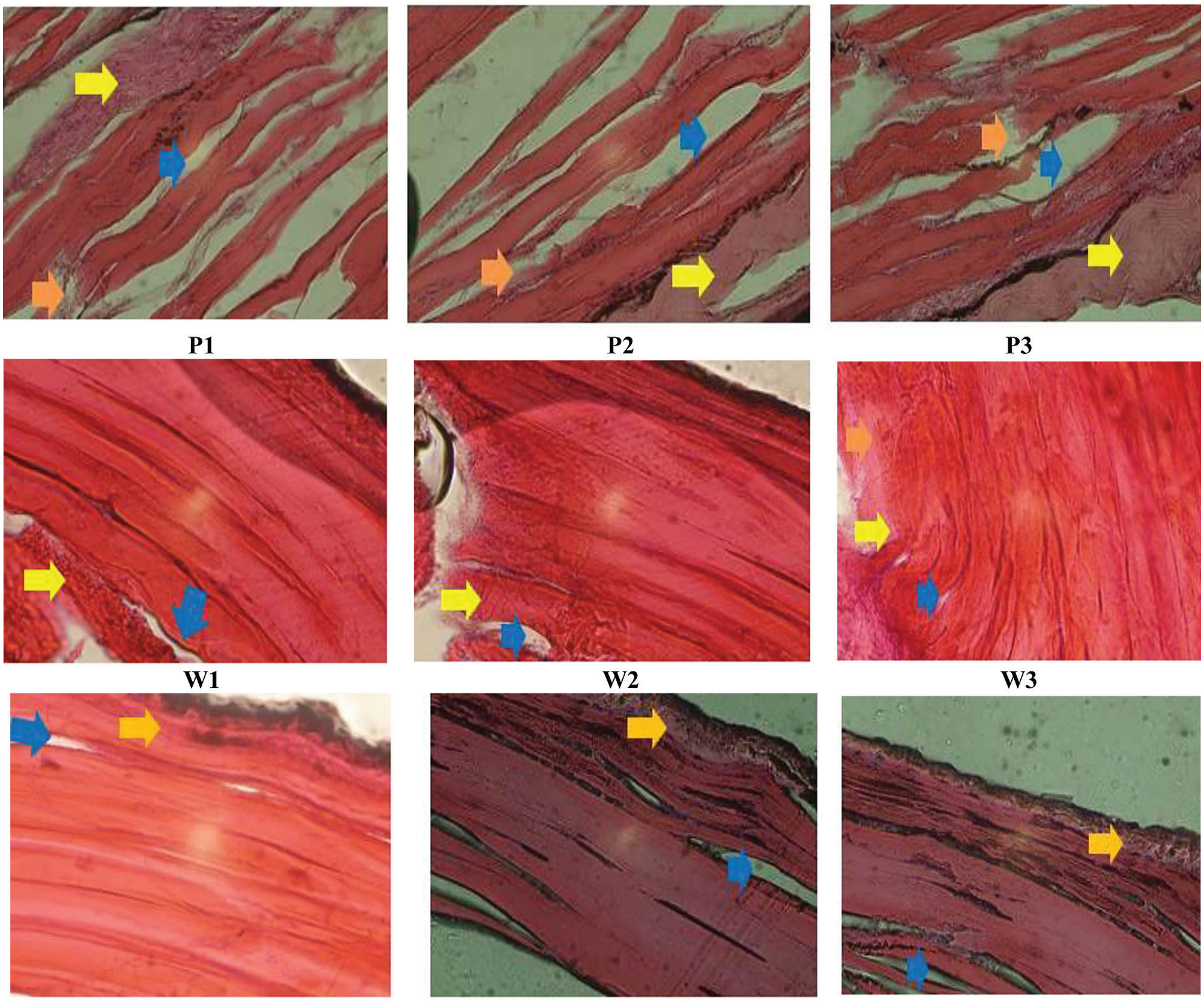
Jan et al. (2015) and Arroyo et al. (2012) added that after Pb and Cd were absorbed in the intestinal epithelium, the heavy metals were then bound with blood and circulated to the liver tissue. This condition causes changes in hemorrhage and fatty degeneration of the liver. The research by Kaur et al. (2018) also showed the histology of the liver with hemorrhage, necrosis, vacuolization of hepatocytes, degeneration of the nucleus, and the presence of macrophages. Fatima and Usamani (2013) reported histological conditions of liver tissue vacuolization, picnosis, and rupture of blood vessels and bleeding due to the exposure to heavy metals.

The higher accumulation of heavy metals in the liver of *Tilapia ziilii* than that in the liver of *Clarias gariepinus* and *Mugil cephalus* is likely because *Tilapia ziilii* has higher tolerance to heavy metals than the other species (Abdel-Satar and Shehata, 2000). Furthermore, Ahmed et al. (2017) reported that the accumulation of heavy metals in the liver, kidneys and gills of *Anodontostoma chacunda* was higher than that in the muscle tissue. The lowest accumulation of heavy metals was found in muscle, while the highest accumulation was found in liver or gills (Amundsen et al., 1997; Al-Yousuf et al., 2000; Yilmaz, 2009).

Muscle damage due to accumulation to heavy metals

Muscle damage can be seen in Figure 4. The damage to the muscle tissue is characterized by the changes in muscle fibers, edema and necrosis. The types of muscle tissue damage also vary among locations. This suggests that the accumulation of heavy metals can damage muscle tissue. Figure 4 shows visual damage types, while the total damage for each location can be seen in Table 4.

Although the accumulation of heavy metals in muscles was slightly lower than that in liver and gills, the exposure to heavy metals Cd, Cu, and Pb in this research also resulted in histological changes in the muscles of the mudskipper fish. The changes in muscles included the changes in muscle fibers, edema, and necrosis. Kaoud and El-Dahshan (2010) reported that muscle degeneration occurred in tilapia due to the exposure to Cu, Cd, Pb, and Hg heavy metals. Similar research results were also reported by Patnaik et al. (2011) that the accumulation of Pb and Cd causes the thickening and separation of muscle bonds and intramuscular edema. Krishnamoorthy and Nagarajan (2013) reported that the fish exposed to heavy metals showed the thickening and separation of muscle bonds characterized by severe intracellular edema. Bhuvaneshwari et al. (2015) vacuolar degeneration and atrophy in muscle fibers are caused by a mixture of various types and concentrations of heavy metals. Maftuch et al. (2018) argue that the necrosis that occurs in the muscle tissue can cause muscles to lose their function and nature. The results of this research are also supported with the research results by Sia-Su et al. (2013) stating that the accumulation of heavy metals can cause the disintegration of muscle fibers, causing gaps between each muscle bond. Kaoud and El-Dahshan (2010) added that the fish muscles exposed to heavy metals showed changes in muscle fibers through atrophy and separation of muscle fibers.



P1 P2 P3

W1 W2 W3

R1 R2 R3

Figure 4. Histological overview of the muscle tissue of the mudskipper fish. P1, P2, P3 are the locations at Poka station 1, station 2, and station 3. W1, W2, W3 are Waai station 1, station 2, and station 3. R1, R2, R3 are Rutong station 1, station 2, and station 3. The changes involved muscle fibers (yellow arrows), edema (blue arrows), necrosis (orange arrows). Staining used HE with a magnification of 40×.

Table 4. Calculation of the number of muscle cells experiencing damage

Locations	Figure	The number of damaged muscle											
		I			II		III		IV			V	
		PSO	E	N	PSO	N	PSO	E	PSO	E	N	PSO	N
Poka	1	1	0	0	1	0	1	0	1	1	0	1	0
	2	1	0	1	1	0	1	0	0	0	1	1	0
	3	1	2	0	1	1	0	2	0	0	1	0	1
	Average	1.00±0.00	2.00±1.16	1.00±0.58	1.00±0.00	1.00±0.58	1.00±0.58	2.00±1.17	1.00±0.58	1.00±0.58	1.00±0.58	1.00±0.58	1.00±0.58
Waai	1	1	1		1		1	1	1			1	
	2	1	1		1		1	0	1			1	
	3	1	2		1		1	0	1			1	
	Average	1.00±0.00	1.33±0.58		1.00±0.00		1.00±0.00	1.00±0.58	1.00±0.00			1.00±0.00	
Rutong	1	1	1		1		1	0	1	1		1	
	2	1	0		1		1	1	1	1		1	
	3	1	0		1		1	1	1	0		1	
	Average	1.00±0.00	1.00±0.58		1.00±0.00		1.00±0.00	1.00±0.58	1.00±0.00	1.00±0.58		1.00±0.00	

Note: CMF – changes in muscle fibers, E – edema, N – necrosis.

The correlation between the accumulation of heavy metals and damage to gills, liver and muscles

The results of the correlation analysis between Cu, Cd, and Pb towards the damage to gills, liver and muscles are presented in Table 5. These results showed that there was a correlation between fish, sea water and sediment towards the accumulation of heavy metals in the target organs of gills, liver and muscles (Table 4). On the basis of the data of the determination coefficient (R), the effective contribution of the medium of heavy metal accumulation such as fish, sea water and sediment (Table 4) can be explained. The results of the analysis showed that the sea water, sediment, and fish had a very high effective contribution towards the accumulation of Cu, Pb and Cd in the target organs of gills and liver. Meanwhile, the highest accumulation of Cu was due to the effective contribution of sea water. The results of this research indicate that the accumulation of heavy metals in target organs through sea water, sediment and fish varies. The effective contribution of sea water towards the accumulation of the three heavy metals was Cu in muscles> Pb in gills> Cd in the liver. The effective contribution of fish towards the accumulation of the three heavy metals was Cu in the liver> Pb in the gills> Cd in the liver. The effective contribution of sediment towards the accumulation of the three heavy metals was Cu in the gills> Cd in the liver> Pb in the liver (Table 5).

Sea water is a medium for the accumulation of Pb, Cd, and Cu in muscles, gills and liver. This is because gills are directly exposed to sea water. Javed and Usmani (2011) add that fish gills bind oxygen from sea water, so that the accumulation of heavy metals in the gills due to the sea water is very high. Therefore, the Pb which dissolves in water will indirectly accumulate in the body of fish through the gills. According to Hapsari et al. (2017), the highest accumulation of Pb was found in the gills of *Nemipterus* sp. compared to its muscle and skin. Gills take ions from seawater for respiration, making the gills to be the

Table 5. The correlation between the accumulation of heavy metals and the target organs

Heavy metals	Heavy metal medium	Target organs	R	R^2	Effective contribution (%)
Pb	Fish	Gills	0.635	0.403	40.3
		Liver	0.507	0.257	25.7
		Muscles	0.682	0.466	46.6
	Seawater	Gills	0.845	0.714	71.4
		Liver	0.616	0.379	37.9
		Muscles	0.675	0.456	45.6
	Sediment	Gills	0.296	0.087	8.7
		Liver	0.412	0.17	17
		Muscles	0.205	0.042	4.2
Cd	Fish	Gills	0.412	0.170	17
		Liver	0.369	0.136	13.6
		Muscles	0.186	0.034	3.4
	Seawater	Gills	0.544	0.296	29.6
		Liver	0.815	0.665	66.5
		Muscles	0.714	0.510	51
	Sediment	Gills	0.146	0.021	2.1
		Liver	0.666	0.444	44.4
		Muscles	0.509	0.259	25.9
Cu	Fish	Gills	0.320	0.102	10.2
		Liver	0.820	0.673	67.3
		Muscles	0.320	0.102	10.2
	Seawater	Gills	0.365	0.133	13.3
		Liver	0.642	0.412	41.2
		Muscles	0.920	0.846	84.6
	Sediment	Gills	0.828	0.685	68.5
		Liver	0.759	0.577	57.7
		Muscles	0.632	0.400	40

first organ to meet pollutants in the form of heavy metals through seawater, because they are stable and have a direct contact with the external environment, while the fish muscles are also in contact with the heavy metals dissolved in sea water (Padrilah et al., 2018; Kamaruzzam et al., 2008). The accumulation of heavy metals in the muscles can increase because the body of the mudskipper fish is submerged in sea water, so that Cu can bind to the fish's skin and can diffuse into the muscles of the fish. The research by Yunus et al. (2015) also reported that the high concentration of Cu in fish muscles was caused by high concentrations of Cu in seawater due to the contamination from factories near sea waters. Similarly, Jovanovic et al. (2011) state that heavy metals can be absorbed by the epithelial or mucosal surfaces of the fish skin. In addition, to the fact that the heavy metals can be absorbed through the surface of the skin, they can also enter the fish tissue through the process of eating in food chains and food webs. Heavy metals infiltrate into the fish body through the surface of the skin / epidermis, gills and the gastrointestinal tract of fish such as the intestines (Yousafzai et al., 2010; Sauliutė and Svecevičius, 2015).

Heavy metals are bound to water bodies and are absorbed through the food chain into the tissues. Meanwhile, fish is part of the aquatic food chain, so that heavy metals accumulate in fish bodies higher than in water and sediment (Chaphekar, 1991; Bakhiet, 2015). Canpolat and Calta (2003) suggest that the same heavy metal concentrations distributed in the same organs and tissues will also change depending on the season. The higher the sea water temperature is, the higher the heavy metal toxicity in an organism will be (Tupan and Uneputty, 2017). The heavy metals that enter through the process of eating can also increase the accumulation of Cd in the liver. Jarup and Akesson, (2009) explained that the bioaccumulation of Cd can occur through the food chain and trophic levels. The accumulation of Cd in the liver through sediment and fish accumulators is also associated with the liver's function as a metabolic organ that functions as an excretion. Akan et al. (2012) also argued that heavy metals accumulate in organs because of their function to support the metabolic processes.

Sediment is also the highest medium for the accumulation of Cu in the gills of the mudskipper fish, compared to Pb and Cd. The effective contribution of sediment is 68.5%. Saghali et al. (2014) state that sediment is a medium for the accumulation of heavy metals which have broken down and then immerse in the bottom of the water. The research by Reyahi-Khoram et al. (2016) reports that high concentrations of Cd accumulate in the liver, because the liver is one of the organs that functions as metabolism. In this research, it was found that the liver has the highest accumulation of Cd and Cu. This may be due to the function of the liver as a detoxification organ.

However, the highest accumulation of Cu in the liver is through the fish medium (the effective contribution was 67.3% higher than that of Pb and Cd and the other medium). The results of this research are also in line with those obtained by Huseen and Mohammed (2019) that the highest concentration of Cu in the liver was found in the *Ctenopharyngodon idella* fish. This was influenced by the binding of Cu with the sources of the fish food, which then entered the digestive tract and accumulated in the liver of the fish. Tunçsoy and Erdem (2014) report that the heavy metals that enter through the gills can be transferred to the liver through the circulatory system, heavy metal accumulation in the liver because the liver is one of the metabolic organs. Valavanidis et al. (2006) state that the liver has a high accumulation of heavy metals because heavy metals bind to metallothionein, which is a protein synthesized in the liver due to toxic buildup. Yosef and Ghada (2011) added that metallothionein is a protein that detoxifies the accumulation of heavy metals, which means that this protein functions to protect liver against the damage from heavy metal poisoning. Mohammadi et al. (2012) stated that the accumulation of heavy metals is adjusted to the tissue due to the influence of metabolic requirements, physic-chemical properties and heavy metal detoxification processes in certain tissues. This opinion supports the research findings that the liver is an organ that accumulates less heavy metals, except for the accumulation of Cu from fish with an effective contribution of 67.3%.

CONCLUSIONS

The results of this research provide important information about the levels of the Pb, Cd, and Cu heavy metals in three mangrove waters, namely Poka, Waai and Rutong. Mudskipper fish exposed to heavy metals has undergone histological changes in the gills, liver and muscles. Consequently, its metabolic processes in all three organs were inhibited. On the basis of locations of the mangrove, the accumulation of the Pb and Cu in fish was Rutong> Poka> Waai; while the accumulation of Cd was Rutong> Waai> Poka. The results of this research also prove that mudskipper, which lives in the mangrove ecosystem, can accumulate heavy metals, so that it can be used as a bioindicator species for heavy metals in waters.

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