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Bioaccumulation of Pb in the Gills of *Perna viridis* (Bivalvia: Mytilidae) in Jakarta Bay, Indonesia and its Histological Alteration

Rony Marsyal Kunda[1*], Maman Rumanta[2], Leonard Raden Hutasoit[2]

[1] Biotechnology Study Program, Faculty of Mathematics and Natural Sciences, Universitas Pattimura, Ambon, Indonesia.
[2] Biology Education, Study Program, Faculty of Education and Teacher Training, Universitas Terbuka, Banten- Indonesia.

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Corresponding Author:
Rony Marsyal Kunda
ronykunda14@gmail.com

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Abstract: Pollution in Jakarta Bay associated with terrestrial run-off and eutrophication. Bivalves have been recognized as aquatic organisms that play the most dominant role in aquatic ecosystems. Pb is an element that in many cases causes health problems in the liver, kidneys, hematopoietic system and nervous system. This study aims to analyze the bioaccumulation of Pb in the gills of *P. viridis* in Jakarta Bay, and its histological changes. Bioaccumulation analysis using the AAS and histology changes using transmission electron microscopy (TEM) methods. The results of AAS, showed that the value of Pb concentration in gill tissue at the two seasons did not show a noticeable difference. Pb concentrations in gills were lower at 1, 4, and 5 than at 2 and 3 stations. The results of ultrastructure analysis as an impact of Pb accumulation showed that thickening of the nucleus membrane, and a decrease in the number of microvilli. The thickening of the nucleus membrane, and the decrease in the number of microvilli are cellular responses to the entry of the Pb heavy metal into the cell. Besides, the results of this study showed inflammation, a decrease in the number of mitochondria, as well as causing cristae to be empty.

Keywords: Bioaccumulation; Gill; Histological; Jakarta Bay; Lead (Pb)

Introduction

Pollution of aquatic biota is a serious issue worldwide, and it is mainly caused by increased industrial wastes, agricultural, commercial, and human activities (Mustafa, 2020). Recently, pollution in Jakarta Bay with toxic substances or chemicals has become a major environmental problem and a serious public health problem in Jakarta (Baum et al., 2016; Rumanta, 2019). Moreover, Baum et al. (2016), stated that, pollution in the Jakarta Bay associated with terrestrial run-off and eutrophication. The majority of industrial and domestic wastes with significant levels of pollutants are not appropriately handled; instead, are dumped directly into Jakarta Bay (Baum et al., 2016; Rumanta, 2023a). The toxic hazard of heavy metals represents the danger of all pollutants that accumulate in the environment because they are non-biodegradable, and undergo biomagnification processes through the food chain (Ali et al., 2019). Mustafa (2020) stated that the heavy metal bioaccumulation in aquatic biota is dependent on the amount of the metal in the marine, the ability of the organisms to consume the metal, and the organism feeding habits.

Bivalve have been identified as the primary aquatic species that accumulate significant amounts of various metals above the levels seen in the aquatic ecosystem (Phuong, 2014). Heavy metals impacts are mostly determined by their potential for bioconcentration and biopropagation within aquatic species, which then spread to humans (biomagnifications) (Georgescu et al., 2017). The identification of Pb concentration in bivalves useful as an *ecosentinel organism* in marine environmental monitoring programs aimed at a variety

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of aspects of risk assessment. Younis et al. (2015) reporting that several species of aquatic Macrophytes, and the clam *P. viridis*, Yaqin et al. (2015) have been used as bioindicators and water quality assessments.

Currently study have revealed that bivalve can retain and perhaps accumulate heavy metals in their soft tissues, included in gills (Hamed et al., 2014). The primary organs for metal accumulation are gills, which also have the highest amounts of pollution. This is as a result of their function in respiration and digestion as well as their close contact with entering water. Moreover, the primary defenses against pathogenic agents and environmental pollutant damage are the gill and mantle epithelia (Zannella et al., 2017). The study of microscopic structure and functions of inner organs of bivalve can be utilized to analyze the physiological of marine organisms. Moreover, Attaallah et al. (2020) reported that similar to the epithelial cells of the mantle and gills, the epithelial cells of a bivalve act to various environmental stimuli. As a result, information of these cells structure could be valuable in determining animal physiological circumstances. Smooth muscle fibers make up the majority of the organs. The sarcoplasm contains mitochondria and sarcoplasmic reticula, while thin and thick myofilaments make up the smooth muscle fibers. Their compositional proportions and ultrastructural properties vary according to species and habitat (Shin et al., 2022).

Presently, very little information about the level of heavy metal pollution in Jakarta Bay has been correlated with the ultrastructure organ of clams. The Jakarta Bay is thought to have lately experienced an upsurge in heavy metals influx as a result of increased industrial activity spills in the area. The study aimed to observed ultrastructure change of the gill in *P. viridis* (Bivalvia: Mytilidae) exposed to lead (Pb) polluted. The determination of *P. viridis* based on its abundant population and even distribution in Jakarta Bay, as well as its ability to accumulate Pb and translocate the compound to various organs including the gills. The results of this study will provide implicit solutions regarding species that have great potential as bioaccumulator agents in the Muara Angke ecosystem, North Jakarta. Another advantage that will be provided from this research is that the identified mussel species that have great potential as bioaccumulator agents will be utilised to annul the concentration of Pb and other heavy metals in Muara Angke, especially in areas close to industrial areas.

Method

The Study Site and Sampling

This study was conducted in Jakarta Bay, Indonesia in two periods based on the division of seasons, i.e the rainy season (November to March) and the dry season (April to October). Preliminary survey results concluded that sampling survey sites between 10-30 kilometres offshore were selected to cover most of the Jakarta Bay area (Figure 1). Pb testing of *P. viridis* mussels was conducted at the Soil Research Institute laboratory in Bogor, while histology of gill samples was conducted at the Eijkman Institute Molecular Biology Laboratory.

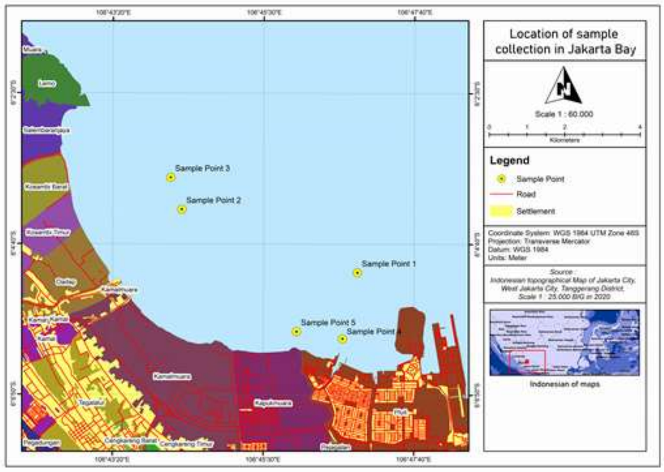

Figure 1. Sampling area of green mussel in Jakarta Bay, Indonesia

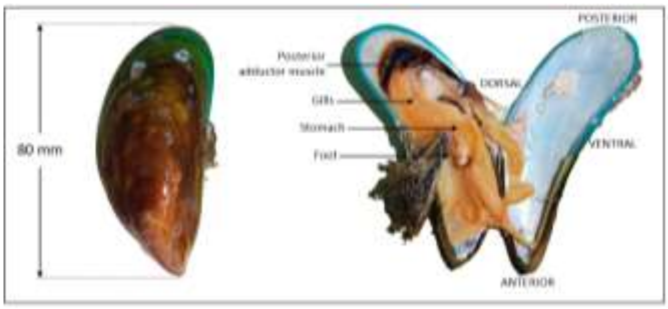

Figure 2. Morphology of *P. viridis*: the external morphology of the mussel (*left*); the internal morphology (*right*)

A total 103 green mussels similar in size and morphologically mature were chosen at random from the study areas. Sampling was carried out at low tide conditions with a depth of 2 meters because this species was attached to wooden substrates and nylon nets planted at the location. Each sample was measured along its maximum anterior-posterior axis (length), then opened and macroscopically evaluated to determine its condition of productivity. The morphology of *P. viridis* is presented in figure 2. The color of the mantle mesosoma, the appearance of follicles in the mantle, the texture of the mantle, and the amount to which the genital tissue had penetrated the mantle tissue were all observed. Samples were collected by diving to the bottom of the marine, transported to the laboratory, and processed the same day. Each mussel gills tissue was removed, cleaned, weighed, put in polyethylene bags, and refrigerated at -20^{o}C. To avoid metal contamination of the materials by laboratory equipment, tissues were dissected with a sterile scalpel at room temperature and all laboratory gear was immersed in 2M HNO_3 for 24 hours, before use, rinsed with distilled water, then finally rinsed with deionized water.

Pb analysis in gills

This study was conducted by direct observation technique in Jakarta Bay (In situ Measurement) and laboratory analysis. At the study site, five stations (ST1 to ST5) were selected for data collection based on information from fishermen about the existence of bivalves (Figure 1). Each sample was collected in three replications (triplo), dried, then crushed, and weighed. Tissue samples were then ashed using muffle furnace at 435°F and digested using 1 ml of HNO3 and 0.5 ml of HClO3. These were filtered and made up to volume 25 ml using 25% HNO3 and analyzed using flame AAS. The research data were analyzed using one way ANOVA to determine difference of Pb concentration at three species bivalves with software of SPSS version 21.

Ultrastructure Analysis in Gills

A total 65 gill tissue was carefully removed and preserved in 5% buffered formalin shortly after trapping (40%, UK). Ultrastructure analysis performed at the Transmission Electron Microscopy Laboratory, Eijkman Institute for Molecular Biology (EIMB), Indonesia. Samples preparation for TEM analysis is carried out referring to the Hamed *et al.* (2014) methods. Samples were fixed in 2.5% glutaraldehyde solution (pH 7.2, buffered 0.1 M phosphate buffer) for 2-4 h at 4^{o}C and rinsed in 0.1 M phosphate buffer and then post-fixed in 1% osmium tetroxide (OsO4) solution for 2 h at 4^{o}C. After fixation, the samples were washed with 0.1 M phosphate buffer 4 times for 2 h and dryed with ascending grades of ethanol. Ultrathin parts (± 60 nm thickness) were collected on copper grids (Ted Pella) (200 mesh) and stained with uranyl acetate and lead citrate. Observations were conducted using an FEI EM 208S TEM with an accelerating voltage of 200 kV. Comprehensively, the flow of this research is presented in Figure 3.

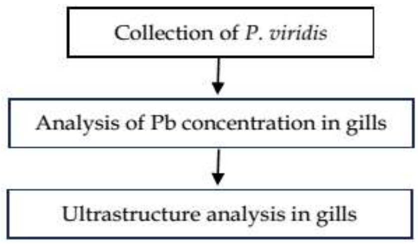

Figure 3. Flow diagram for bioaccumulation and ultrastructural analysis of *P. viridis*

Result and Discussion

Image Gill Ultrastructure Exposed Pb Polluted

Based on AAS analysis, the value of the concentration of heavy metal Pb in the gill tissue in two (Western and Eastern) seasons, and showed no noticeable difference. Based on Table 1 shows that the concentration of Pb on the gill tissue is markedly lowest at 1, 4, and 5 stations, compared to 2 and 3 stations.

Table 1. Pb Accumulation on the Gill Tissue in Western and Eastern Seasons (μg/g)

Content of Pb	Stations				
(X ± SD)	1	2	3	4	5
Eastern Season	0.166 ± 0.016 B	0.210 ± 0.026 A	0.261 ± 0.055 A	0.146 ± 0.017 B	0.160 ± 0.020 B
Western Season	0.161 ± 0.022 B ns	0.205 ± 0.058 A ns	0.212 ± 0.144 A ns	0.131 ± 0.050 B ns	0.158 ± 0.051 B ns

Note: Oneway Anova: Different capital letters (A, B) in the same column indicate significant differences ($p<0.05$) in Pb content between mussel species. T-test, ns= no significant differentce between season in Pb content ($p<0.05$) each species

Based on Table 1 shows that at 1, 4, and 5 stations (Figure 4A) shows that the morphology of the nucleus and mitochondria is in good health, with the nucleus membrane and the intercellular space appearing normal, and the gill histological of mussel from 1, 4, and 5 stations was intact (Figure 4A). Generally, the gill of mussels consisted of two plates. The parallel filaments that make up each plate are joined by cilliary discs. TEM of cells had identified three different types of cells (Figure 4A and 4B) i.e several large mitochondria, flattened epithelial cells covered the first type and with elongated microvilli, mucous cells enclosed between the first one. But we asserted that the results of this study shows that no change surface ultrastructure of nucleus, intracelullar space and mitochondrion of gill in mussel collected from 1, 4, and 5 stations (Figure 4A).

Figure 4B, shows that the ultrastructure morphology of the gills of the green mussel is polluted by heavy metals conditions. The results of the micrograph TEM show that the nucleus membrane is thickened and decayed as a result of contamination of heavy metal into nucleus. Besides, Fig. 4B shows decrease in mitochondria numbers and experiencing a void a fold in the inner membrane of a mitochondrion. Moreover, Fig. 4B shows the ultrastructure changes directed to the arrangement of regularity of gill lamellae and occasional areas avoid of microvilli appeared on some frontal and lateral surfaces. Black granules are detected throughout the cell and in some cases the mitochondrial membrane begins to rot.

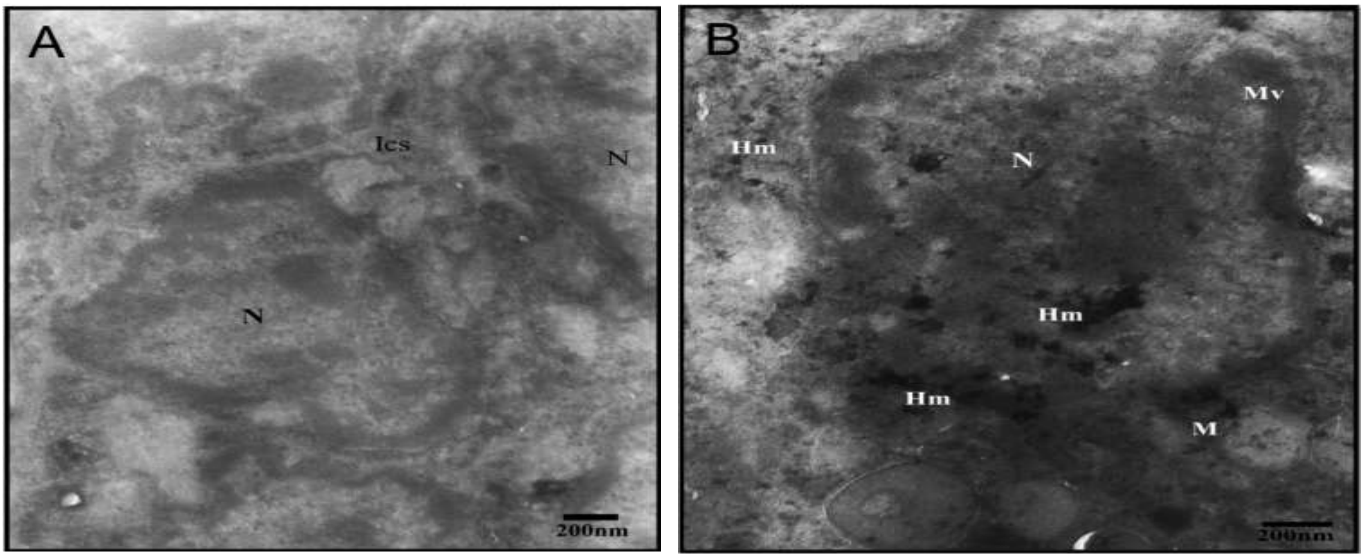

Figure 4. (A). TEM analysis of normal gill tissue in the three (1, 4, and 5) sites collected from Jakarta Bay, showing the nucleus is good health and thin cells with microvilli (mv) cover the apical surface of cells; (B). TEM analysis of gill illustrating the decayed mitochondria (M) and mitochondrial membrane; the gill of green mussel collected from two sites (2, and 3). *Description: Hm: heavy metal; mv: microvilli; N: nucleus; Ics: intracelullar space; M: mitochondria; DM: Damaged mitochondria*

Some important organs of marine organisms such as gills, skin, and intestines act as places of accumulation of many heavy metals. Like other infaunals, the process of taking food in green mussels is carried out by filtering food dissolved in water, and with this method the gills are an organ that has the potential to be highly contaminated with heavy metals (Paruruckumani et al., 2015). The present investigations show that all samples in five stations accumulate Pb heavy metals with different concentrations. Data in Table 1 shows bivalve collected from 2, and 3 stations accumulated Pb heavy metal higher than 1, 4, and 5 stations. This suggests that there has been a significant process of exposed and accumulation of Pb in gills. The analysis results show that the Pb concentration in gills of green mussels contaminated with Pb are different in water, thus confirming the fact that gills are the soft organ that most easily responds to concentration change. Odžak et al. (1994) stated that gills of younger bivalves accumulate more Pb than gills of older individuals, which is probably due to a greater metabolic activity.

Facts on the ground show that there are 13 large rivers in Jakarta Bay that contribute liquid waste to the waters and are exacerbated by the presence of slums and densely populated areas that at any time produce waste from various types of shapes and sources (Prabawa et al., 2021). Based on the location of the sampling location, shows that 2, and 3 sampling stations are most likely influenced by waste inputs from inside and outside Jakarta i.e the Bekasi and Karawang areas which are driven by ocean currents. This is likely to lead to high accumulation of heavy metals which has an impact on ultrastructural changes in green mussel collected from these two stations.

Various circumstances are strongly suspected to be the reason for the difference in Pb accumulation between the study stations and have an impact on ultrastructure changes at the two sampling (2 and 3) stations. In this study, 2 and 3 stations are located relatively close to various industrial areas and other anthropogenic activities, and this is very likely to act as a source of Pb. Therefore, surface runoff and domestic waste from nearby land uses like agriculture, industry, and human settlements are the sources of the heavy metal inputs into the two locations in this study. van der Wulp et al. (2016) and Rumanta (2023b), asserted that high gradients along Jakarta Bay have become inhabited by heavy metal pollutants.

This argument is based on the physiological fact that each tissue of epifaunal and infaunal organisms has a different degree and tolerance of heavy metal absorption, and the biological half-life between one tissue and anothers. Zhang et al. (2017) stated that infaunal organisms has a slow physiological ability to regulate heavy metals in its environment. However, there is an adaptation mechanism of infaunal species to minimize the concentration of heavy metals in soft tissue with the mechanism of calcium ion substitution from soft tissue to hard tissue (shell). Zhang et al. (2017) reported that bivalves accumulate heavy metals slowly throughout life, and eliminate these heavy metals very slowly and can only be done optimally when clean seawater. Pb can be absorbed in the body of organisms such as shellfish in two ways i.e diet exposure, and water exposure by the surface of the gills (Sonawane, 2015). Bivalve are part of aquatic biota which are often used as bioindicators of Pb in the waters, because Pb are included in the highest trophic level and human protein source. If bivalve that accumulate Pb are consumed by humans, then these Pb can accumulate in the human's body. Thus, the excessive number of Pb can endanger human life (Cahyani et al., 2017; Khairuddin, Yamin, et al., 2021).

Figure 4B shows that the Pb has entered and reached important organelles from green mussel and has caused changes and damage to the ultrastructures of some cell organs. It is clearly visible in the cristae voids in the mitochondria of cells that experience cristae voids. The results of this ultrastructure show that there has been serious damage to the epithelium from the gills leading to serious tissue dysfunction consequently producing a damage effect at the cellular organization level. Besides, in Figure 4B showed a thickening of the nuclear membrane. The process of thickening of the nuclear membrane is thought to be a response to heavy metal intake trying to enter the nucleus. Several studies reported that the gills of bivalves were a better organ for biomonitoring than the hepatopancreas. Because they are highly sensitive to pollutants, bivalves are frequently used in marine ecotoxicology to assess seawater quality. Gills are frequently polluted because they serve as the primary interface between organisms and their surroundings (Zannella et al., 2017).

The cells have many microvilli and play an important role in the uptake of substances or substances from the aquatic environment. This is different in Figure 3B, it appears that there are changes in structure and a decrease in the number of microvilli as a result of exposure to Pb. The results of this study accordance with Khan et al. (2018) shows that changes in the structure of microvilli and a decrease in the number of microvilli in *Anodonta cygnea* (Linea, 1876) bivalve exposed heavy metals. In this study, a decrease in microvilli was observed in the middle and front zones of the filaments. This might suggest that cells collapse structurally as a result of ingesting undesired particles, or it might suggest a technique to stop these particles from being ingested. A loss in microvilli causes a reduction in absorption and, most likely, physiological alterations that the body must make up for in some other way.

Aquatic organisms tested after exposure to heavy metals on an experimental scale, showed tissue damage as an impact of functional reactions that revealed the workings of the toxic (Alina et al., 2012; Khairuddin & Yamin, 2021; Rahmawati et al., 2017). At this level, the gill are considered the main targets of contaminants because involved in many important cellular and metabolic functions of aquatic organisms i.e respiration, osmoregulation, and excretion, while still interacting with the external environment, and are very sensitive to changes in water quality (Henry et al., 2012). Paruruckumani et al. (2015) stated that in marine organisms exposed to heavy metals will cause changes cellularly in some organella i.e generally the nucleus located in the center usually shows a slight heterochromatin. Moreover, Paruruckumani et al. (2015) asserted that there are changes in mitochondria smooth endoplasmic reticulum (SER), rough endoplasmic reticulum (RER), Peroxisomes and Golgi complex were scattered in the cytoplasm. Bivalve exposed to the Pb heavy metal will directly cause changes in the ultrastructure of several parts including epithelial removal, hyperplasia, epithelial cell hypertrophy, and partial fusion of some secondary lamellae. All such changes are a defense mechanism against the intake of contaminants. Effects of heavy metals on gills showed lamellar degeneration, epithelial lifting and necrotic changes in intercellular epithelial cells (Chavan et al., 2014).

Conclusion

We concluded that AAS analysis showed that Pb concentration values in gill tissue in both seasons did not show significant differences at stations 1, 4, and 5 compared to stations 2 and 3. The results of ultrastructural analysis showed thickening of the nuclear membrane and a decrease in the number of microvilli as a cellular response. against the entry of the heavy metal Pb into cells. In addition, the results of this study show inflammation, a decrease in the number of mitochondria, and causing cristae to be empty.

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Author Contributions

Conceptualization, M.R. and R.M.K.; methodology, R.M.K. and L.H.; validation, M.R. and R.M.K.; formal analysis, R.M.K.; investigation, M.R., and R.M.K.; resources, R.M.K. and L.H.; data curation, M.R.: writing—original draft preparation, R.M.K and M.R.; writing—review and editing, R.M.K. and L.H: visualization, and M.R. and R. M. K. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest
The authors declare no conflict of interest regarding this article.

References

Ali, H., Khan, E., & Ilahi, I. (2019). Environmental Chemistry and Ecotoxicology of Hazardous Heavy Metals: Environmental Persistence, Toxicity, and Bioaccumulation. *Journal of Chemistry*, *2019*, 1–14. https://doi.org/10.1155/2019/6730305

Alina, M., Azrina, A., Mohd Yunus, A. S., Mohd Zakiuddin, S., Mohd Izuan Effendi, H., & Muhammad Rizal, R. (2012). Heavy metals (mercury, arsenic, cadmium, plumbum) in selected marine fish and shellfish along the straits of malacca. *International Food Research Journal*, *19*(1), 135–140. Retrieved from https://rb.gy/6ydg1i

Attaallah, A., Marchionni, S., El-Beltagy, A., Abdelaziz, K., Lorenzini, A., & Milani, L. (2020). Cell cultures of the Manila clam and their possible use in biomonitoring and species preservation. *The European Zoological Journal*, *87*(1), 624–641. https://doi.org/10.1080/24750263.2020.1827052

Baum, G., Kusumanti, I., Breckwoldt, A., Ferse, S. C. A., Glaser, M., Dwiyitno, Adrianto, L., van der Wulp, S., & Kunzmann, A. (2016). Under pressure: Investigating marine resource-based livelihoods in Jakarta Bay and the Thousand Islands. *Marine Pollution Bulletin*, *110*(2), 778–789. https://doi.org/10.1016/j.marpolbul.2016.05.032

Cahyani, N., Batu, D. T. F. L., & Sulistiono, S. (2017). Heavy Metal Contain Pb, Hg, Cd and Cu in Whiting Fish (Sillago sihama) Muscle in Estuary of Donan River, Cilacap, Central Java. *Jurnal Pengolahan Hasil Perikanan Indonesia*, *19*(3), 267. https://doi.org/10.17844/jphpi.v19i3.15090

Chavan, V. R., & Muley, D. V. (2014). Effect of heavy metals on liver and gill of fish Cirrhinus mrigala. *International Journal of Current Microbiology and Applied Sciences*, *3*(5), 277–288. Retrieved from https://www.cabidigitallibrary.org/doi/full/10.5555/20143201401

Georgescu, B., MIERLITA, D., STRUTI, D., KISS, H., & BOUARU, A. (2017). Metabolic, Bioproductive and Reproductive Effects of Aquatic Exposure to Cadmium in Dish- A Review. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Animal Science and Biotechnologies*, *74*(1), 1. https://doi.org/10.15835/buasvmcn-asb:12198

Hamed, S. S., Radwan, E. H., & Saad, G. A. (2014). Impact of Selected Environmental Pollutants on the Ultrastructure of the Gills Coastal Zones, Egypt. *Open Journal of Ecology*, *04*(14), 907–917. https://doi.org/10.4236/oje.2014.414076

Henry, R. P., Lucu, Č., Onken, H., & Weihrauch, D. (2012). Multiple functions of the crustacean gill: osmotic/ionic regulation, acid-base balance, ammonia excretion, and bioaccumulation of toxic metals. *Frontiers in Physiology*, *3*, 431. https://doi.org/10.3389/fphys.2012.00431

Khairuddin, K., & Yamin, M. (2021). Analysis of Cadmium (Cd) and Lead (Pb) Heavy Metal Content in Shell and Mangroves at Bima Bay. *Journal of Science and Science Education*, *2*(1), 58–61. https://doi.org/10.29303/jossed.v2i1.726

Khairuddin, K., Yamin, M., & Kusmiyati, K. (2021). Analisis Kandungan Logam Berat Tembaga (Cu) pada Bandeng (Chanos chanos forsk) yang Berasal dari Kampung Melayu Kota Bima. *Jurnal Pijar Mipa*, *16*(1), 97–102. https://doi.org/10.29303/jpm.v16i1.2257

Khan, M. I., Khisroon, M., Khan, A., Gulfam, N., Siraj, M., Zaidi, F., Ahmadullah, Abidullah, Fatima, S. H., Noreen, S., Hamidullah, Shah, Z. A., & Qadir, F. (2018). Bioaccumulation of Heavy Metals in Water, Sediments, and Tissues and Their Histopathological Effects on Anodonta cygnea (Linea, 1876) in Kabul River, Khyber Pakhtunkhwa, Pakistan. *BioMed Research International*, *2018*, 1–10. https://doi.org/10.1155/2018/1910274

Mustafa, S. A. (2020). Histopathology and heavy metal bioaccumulation in some tissues of Luciobarbus xanthopterus collected from Tigris River of Baghdad, Iraq. *Egyptian Journal of Aquatic Research*, *46*(2), 123–129. https://doi.org/10.1016/j.ejar.2020.01.004

Odžak, N., Martinčić, D., Zvonarié, T., & Branica, M. (1994). Bioaccumulation rate of Cd and Pb in Mytilus galloprovincialis foot and gills. *Marine Chemistry*, *46*(1-2), 119–131. https://doi.org/10.1016/0304-4203(94)90050-7

Paruruckumani, P. S., Maha Rajan, A., Ganapiriya, V., & Kumarasamy, P. (2015). Bioaccumulation and ultrastructural alterations of gill and liver in Asian sea bass, Lates calcarifer (Bloch) in sublethal copper exposure. *Aquatic Living Resources*, *28*(1), 33–44. https://doi.org/10.1051/alr/2015003

Phuong, T. T. M. (2014). *Bioaccumulation of heavy metals in Nha Trang bay, Khanh Hoa Province Viet Nam*. Retrieved from https://rb.gy/571jmc

Prabawa, F. Y., Adi, N. S., Pranowo, W. S., Sukoraharjo, S. S., Gautama, B. G., & Suhelmi, I. R. (2021). Strategy on marine debris reduction in Indonesia: A review and recommendation. *IOP Conference Series: Earth and Environmental Science*, *925*(1).

https://doi.org/10.1088/1755-1315/925/1/012027

Rahmawati, E., Dewi, D. C., & Fauziyah, B. (2017). Analisis Kadar Logam Tembaga (Cu) pada Permen Secara Spektrofotometri Serapan Atom (SSA). *Journal of Islamic Pharmacy*, *1*(1), 11. https://doi.org/10.18860/jip.v1i1.4179

Rumanta, M. (2019). The potential of Rhizophora mucronata and Sonneratia caseolaris for phytoremediation of lead pollution in Muara Angke, North Jakarta, Indonesia. *Biodiversitas Journal of Biological Diversity*, *20*(8), 2151–2158. https://doi.org/10.13057/biodiv/d200808

Rumanta, M. (2023a). Bioaccumulation of Lead (Pb) content in three species bivalves in Jakarta Bay, Indonesia. *Journal of Biodiversity and Environmental Siences*, *22*(2), 51–57. Retrieved from https://innspub.net/bioaccumulation-of-lead-pb-content-in-three-species-bivalves-in-jakarta-bay-indonesia/

Rumanta, M. (2023b). Cadmium pollution in waters and commercial Shellfish in Jakarta Bay. *International Journal of Biosciences (IJB)*, *22*(2), 260–268. https://doi.org/10.12692/ijb/22.2.260-268

Shin, S. R., Kim, H. J., Park, J. J., Shin, Y. K., & Lee, J. S. (2022). Light and electron microscopy studies of siphon and siphonal sheath formation in the infaunal bivalve Tresus keenae (Bivalvia: Mactridae). *Micron*, *161*, 103343. https://doi.org/10.1016/j.micron.2022.103343

Sonawane, S. M. (2015). Effect of Heavy Metals on Gills of Fresh Water Bivalve Lamellidens marginalis. *IOSR Journal of Environmental Science Ver. I*, *9*(9), 2319–2399. https://doi.org/10.9790/2402-09910511.

van der Wulp, S. A., Damar, A., Ladwig, N., & Hesse, K.-J. (2016). Numerical simulations of river discharges, nutrient flux and nutrient dispersal in Jakarta Bay, Indonesia. *Marine Pollution Bulletin*, *110*(2), 675–685. https://doi.org/10.1016/j.marpolbul.2016.05.015

Yaqin, K., Fachruddin, L., & Rahim, N. F. (2015). Studi kandungan timbal (Pb) kerang hijau, Perna viridis terhadap indeks kondisinya. *Jurnal Lingkungan Indonesia*, *3*(6), 309–317. Retrieved from https://rb.gy/f975s2

Younis, A. M., Mohamed, E., Nafea, A., & Nafea, M. A. (2015). Heavy metals and nutritional composition of some naturally growing aquatic macrophytes of Northern Egyptian Lakes. *Journal of Biodiversity and Environmental Sciences*, *6*(3), 16–23. Retrieved from http://www.innspub.net

Zannella, C., Mosca, F., Mariani, F., Franci, G., Folliero, V., Galdiero, M., Tiscar, P. G., & Galdiero, M. (2017). Microbial Diseases of Bivalve Mollusks: Infections, Immunology and Antimicrobial Defense. *Marine Drugs*, *15*(6), 182. https://doi.org/10.3390/md15060182

Zhang, B., Fang, C. D., Xu, J. H., & Cao, H. J. (2017). Depuration of Cadmium from Blue Mussel (Mytilus edulis) by Protein Hydrolysate–Fe2+ Complex: The Role of Metallothionein. *Journal of Food Science*, *82*(11), 2767–2773. https://doi.org/10.1111/1750-3841.13939