BIODIVERSITAS
Volume 23, Number 11, November 2022
Pages: 5942-5950

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d231148

Metallothionein level in the larva of *Cheumatopsyche* sp. and the relationship between heavy metal concentration in Bone River, Gorontalo, Indonesia

MIFTAHUL KHAIR KADIM[1,2,♥], ENDANG YULI HERAWATI[3], DIANA ARFIATI[3], ASUS MAIZAR S. HERTIKA[3,♥♥]

[1]Doctoral Program, Faculty of Fisheries and Marine Science, Universitas Brawijaya. Jl. Veteran, Malang 65145, East Java, Indonesia
[2]Department of Aquatic Resources Management, Fisheries and Marine Science Faculty, Universitas Negeri Gorontalo. Jl. Jendral Sudirman No. 6, Gorontalo 96138, Gorontalo, Indonesia. Tel./fax.: +62-435-821125, ♥email: miftahulkhairkadim@ung.ac.id
[3]Department of Aquatic Resource Management, Faculty of Fisheries and Marine Science, Universitas Brawijaya. Jl. Veteran, Malang 65145, East Java, Indonesia. Tel./fax.: +62-341-551611, ♥♥email: asusmaizar@ub.ac.id

Manuscript received: 21 September 2022. Revision accepted: 14 November 2022.

Abstract. *Kadim MK, Herawati FY, Arfiati D, Hertik AMS. 2022. Metallothionein level in the larva of* Cheumatopsyche *sp. and the relationship between heavy metal concentration in Bone River, Gorontalo, Indonesia. Biodiversitas 23: 5942-5950.* This study aims to confirm that the larvae of *Cheumatopsyche* sp. contain a metal-binding protein, metallothionein, in their body and correlate it with heavy metal concentrations in the larval tissues and sediments in Bone River. Pb, Cd and Hg concentrations in sediment, tissue and Metallothionein (MT) levels were analyzed in several areas with different metal contamination. The Pb concentration in the sediment averaged 33.6 ppm; Hg 0.17 ppm; Cd 0.125 ppm. Total metals concentration in the whole body of *Cheumatopsyche* sp. ranged from 0.424 to 0.781, while the MT content ranged from 4637 to 6930 $ng.g^{-1}$. The results revealed that the larvae could accumulate different metals and be more effective in accumulating Hg than Pb and Cd, regardless of the level of metal concentration in the sediment. Metal concentrations measured in *Cheumatopsyche* sp. showed a significant correlation with metals in the sediment. Metallothionein levels throughout the larval body showed differences in different areas of metal contamination and this correlated with the concentrations of Pb, Cd, and Hg measured in the larval tissue, thus reflecting their bioavailability in the environment. The results also showed that even under exposure to low metal concentrations, MT was still detectable in *Cheumatopsyche* sp. and proved to be a good predictor of metal accumulation.

Keywords: Accumulation, Bone River, *Cheumatopsyche*, heavy metal, metallothionein

INTRODUCTION

Anthropogenic activities can produce pollution that possibly leads to the degradation of the aquatic environment, especially in rivers (Zhuang et al. 2018); such problems are determined by the life of the biota in that area (Barbieri et al. 2020). One of these pollutants is heavy metal, according to Liu et al. (2018), anthropogenic activities are the leading cause of heavy metal pollution in waters. Heavy metals, e.g., Lead (Pb), Cadmium (Cd), and Mercury (Hg), derived from chemical pollutants are toxic and dangerous, so they have potentially damaging impacts on both the aquatic organisms and the ecological environment (Islam et al. 2015, Fuoco and Giannarelli 2019).

One of the largest rivers in Gorontalo Province is Bone River. The Bone River Corridor is a water conservation facility for the surrounding area because it provides various water needs (managed by local water supply utility), ranging from clean water and agriculture to tourism activities. This river is also part of the Bogani Nani Wartabone National Park area. According to Balihristi data from 2008-2011, land use is a potential threat to the Bone River ecosystem. Most anthropogenic activities around the waters of Bone River come from settlements, agriculture, industry, and sand and gold mining. These activities can lead to organic and heavy metal contamination. Previous research on water quality, especially in the middle, downstream and estuary areas of Bone river by Pateda et al. (2017) and Koniyo (2020), reported that the Bone River indicated the occurrence of heavy metal pollution. Lihawa dan Mahmud (2012) also reported that mercury levels were high in water and sediments in the tributaries of the river's watershed, where gold tailings activity was present. The existence of activities along the Bone River segment can disrupt the ecological balance of aquatic organisms that live in it and can disrupt human health.

Heavy metals are the most dangerous pollutants due to their toxic, carcinogenic, biomagnifying, and bioaccumulative properties. Heavy metals accumulate a lot in sediments, while the bottom substrate of the waters is a place for macrozoobenthos to live. As a result, the sediments are prone to heavy metal contamination (Bere et al. 2016; Ouma et al. 2019). In addition, accumulating heavy metals from sediments and aquatic organisms can be an essential pathway for heavy metal transfer (Pebriani et al. 2022). To date, monitoring of health and water quality in Bone River is still limited to analysis using physico-chemical parameters. Monitoring with a biota approach (biomonitoring) has also been carried out but still needs a more in-depth study to obtain results that describe the actual condition of the river.

The macrozoobenthos community is commonly applied to evaluate organic matter contamination from anthropogenic activities (Everall et al. 2019). It has begun to be used as a monitoring tool for heavy metal pollution (Oremo et al. 2019). In ecological functions in river ecosystems, macrozoobenthos play a vital role. Macrozoobenthos from the aquatic insect group is a natural food source for aquatic biota at other trophic levels. In addition, aquatic insects are central to overhauling organic matter in the waters. Some aquatic insects can tolerate, survive, and accumulate metals in the waters at various levels. One of the insects is Trichoptera larvae, Hydropsychidae. Hydropsychidae, relatively tolerant of metals and the level of contamination in Trichoptera larvae (water insects), is estimated to reflect heavy metal pollution in different streams and river habitats, allowing this species to be an indicator of metal pollution (Ryan et al. 2018; Tszydel et al. 2021).

Water pollution levels can be identified using bioindicator organisms or biomarkers (Hook et al. 2014). Metallothionein (MT) is a specific biomarker for heavy metals (Wong and Stillman 2020). MT is known as a biomarker of heavy metal contamination in aquatic organisms. Several groups of aquatic invertebrates such as mollusks, crustaceans and annelids possess a metal-binding protein, metallothionein, and can induce it mainly by Cu, Zn or non-essential metals Cd and Hg (Amiard et al. 2006). Different levels of metallothionein are also associated with other metal-binding proteins. Widiastuti et al. (2019a) state that the concentration of metal contaminants influences the concentration of MT in organisms. High metal concentrations in cells increase metallothionein. Many studies have shown the induction of MT by metals in vertebrates and invertebrates due to metal contamination. This remarks the beginning of the application of MT as a biomarker of metal pollution in nature. Therefore, MT is a recommended biomarker in environmental and biological monitoring that reflects the presence of metal pollution.

In a previous study, we evaluated the use of MT as a biomarker of heavy metal contamination (Hertika et al. 2018, 2020, 2021), including the certain relationship between every heavy metal (Cd, Pb, Hg) and MT levels in *Crassostrea* sp. acquired from a coastal environment in East Java, Indonesia. However, studies on the use of MT as a biomarker, especially in the Hydropsychidae family, are still limited, indicating the urgency to conduct in-depth studies. This study explored the relationship between MT in *Cheumatopsyche* sp. with heavy metals in the Bone River. It is expected that the study will help analyze East Java's coastal management policy strategies to minimize pollution of the coastal environment.

MATERIALS AND METHODS

Study area

This research was conducted in Bone River (Figure 1), Bone Bolango Regency and Gorontalo City, Gorontalo Province, Indonesia. The river is classified as a large river with water flow rates ranging from 40.28-143.9 $m^3.s^{-1}$. Sampling was conducted from April to June 2022 when the river water discharge was relatively low or not raining. Observation stations were determined purposively.

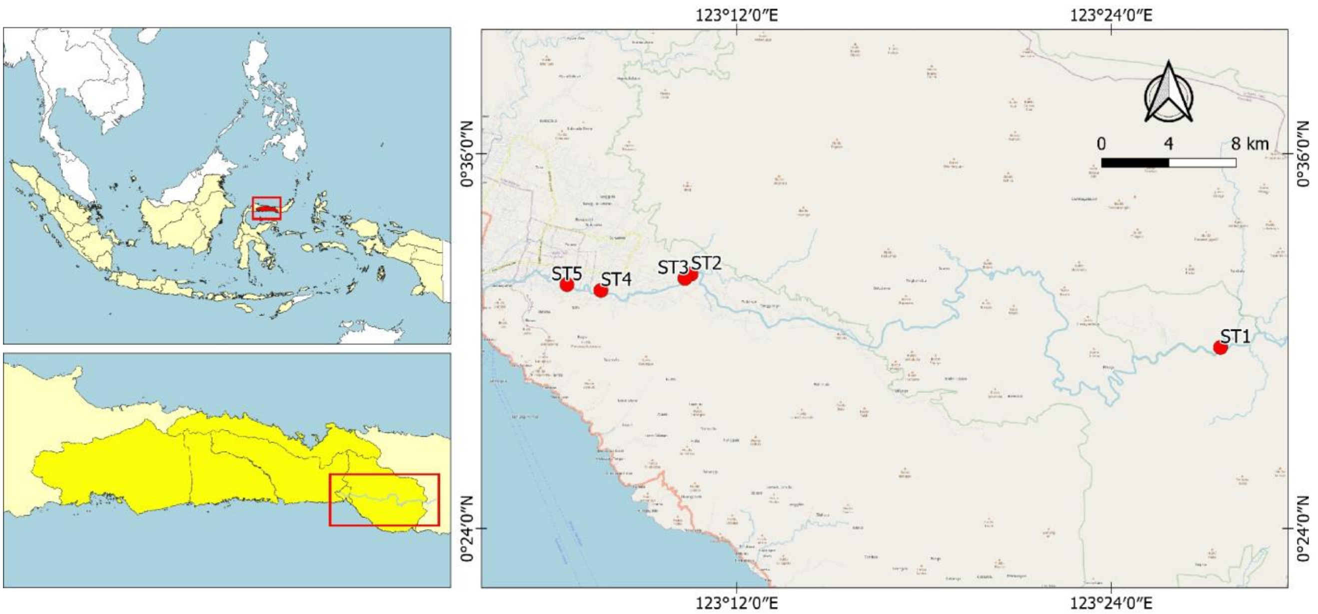

Figure 1. Location of the Bone River in Gorontalo Province, Indonesia. The red dot (•) indicates the sampling area/station (St): St1 is geographically located at 0°29'48.07"N 123°27'30.57"E; St2 at 0°31'0.25"N 123°11'46.59"E; St3 at 0°32'0.99"N 123°10'20.59"E; St4 at 0°31'37.63"N 123° 7'38.89"E and St5 at 0°31'48.83"N 123° 6'33.66"E

Experimental design and sampling procedure

Initially, 12 observation points were identified based on ecological conditions (preferably riffle areas) and anthropogenic activities to evaluate the degree of heavy metal contamination, especially Pb, Cd, and Hg. Since some of these areas displayed similar levels of contamination, five (St1-St5) observation areas were selected considering the contamination gradient, the possible presence of Hydropschidae larvae, and anthropological factors that could potentially be a source of metal contamination. For this reason, different metal levels were expected. St1 is in the upstream area of the Bone River, located in Pinogu Village with a low level of anthropogenic activity and is used as a reference site (control site).

Samples of larvae were taken by employing the kicking technique using a hand net with dimensions of 200 to 400mm wide, 2-3m high, 100-200mm shoulder (reinforcement), and 500 μm mesh size with a maximum range of 10m. Hydropschidae larvae were collected manually by looking for rocks in the sampling area with a maximum range of 100m. Sampling is designed as follows two sites/area dan six replications/site: 5 area × 2 sites × 6 replicates= 60 samples. Sediment sampling was carried out using a modified soil sample ring from a parallon pipe, and a 250g of soil sample was taken (Widiastuti et al. 2019b). Additional environmental variables were measured at each sampling location: dissolved oxygen, pH and temperature. This parameter is measured in the field at a depth of ±4 cm from the bottom surface with a special probe (Hertika et al. 2018). For organisms, the larvae of *Cheumatopsyche* sp. were sorted and then identified using a binocular microscope with a magnification of 8-32 times. The sorted larvae were collected in 10 mL bottles and only the larvae of *Cheumatopsyche* sp. with a length of 1-1.5 cm were analyzed (Figure 2). Ideally, the sample is complete when sufficient individuals have been collected for three replications in each area. In fact, the number of individuals collected is controlled by availability, mainly to meet the sample requirements for observations by the AAS method.

Laboratory analysis

Sediment contamination and metal level in larva Cheumatopsyche

Cheumatopsyche sp. larvae samples that have been sorted and identified are collected until they reach a weight of 1g. Heavy metal concentrations (Pb, Cd and Hg) in sediment and *Cheumatopsyche* sp. were observed using the Atomic Absorption Spectrophotometer (AAS) method. The number read on the spectrophotometer is expressed as the value of the heavy metal concentration of the sample. The sediment concentrations were reported in ppm dry weight (dw), and larva concentrations were reported in ppm wet weight (ww).

Metallothionein determination

Briefly, a 100 mg sample of *Cheumatopsyche* sp. was rinsed with PBS solution. The tissue was then crushed and homogenized with fresh lysis buffer (the present work used PBS solution) with a ratio of 1:9 (w:v 1:9, e.g., 900 μL lysis buffer was added in 100 mg tissue sample). The homogenate was centrifuged at 10,000×g for 5 minutes to obtain a supernatant containing MT. Zebrafish MT1 (Metallothionein 1) ELISA Kit (ELK Biotechnology, Cat: ELK9073) was used in this study. The preparation procedure follows the guide on the kit, following the steps: Prepare seven wells and one well for standard and blank consecutively. Add 100 µL each of the samples and standard working solution into the appropriate wells, cover it with the plate sealer, then incubate at 37ºC for 80 minutes. Remove the solution from all wells. Aspirate the liquid, using 200 µL of 1 × wash solution to wash up each well then leave it for 1-2 minutes. Eliminate the residual liquid from all wells altogether by tearing the plate onto absorbent paper. Repeat the aspirating process and wash three times. After the final wash, remove any leftover wash buffer by aspirating or decanting. Turn over the plate and then blot it against absorbent paper. Add to every well as much as 100 µL of Biotinylated Antibody, cover wells with the plate sealer, then incubate it at 37ºC for 50 minutes. Rinse three times. Add 100 µL of Streptavidin-HRP to each well, cover the wells with the sealer and incubate at 37ºC for 50 minutes. Duplicate the aspiration and wash it five times. Add TMB Substrate Solution to each well as much as 90 µL then cover it with the new sealer. Incubate at 37ºC for 20 minutes. The solution will turn blue with the supplement of TMB Substrate Solution. Add stop reagent as much as 50 µL to each well. The liquid will become yellow by the addition procedure. The absorbance was analyzed using an ELISA reader at 450 nm then the result was converted using a standard curve to get the MT value.

Data analysis

Data from sediment was analyzed using the contamination factor (CF), geoaccumulation index (Igeo). The bioconcentration factor (BCF) is described as the ability of an organism to accumulate Pb, Cd, and Hg elements from its environment (van der Oost et al. 2003). In addition, data from sediment and larva contamination and MT level in each area were analyzed using Permanova, where each station was used as the main fixed factor to see a different significance. When test results show statistically significant differences, pairwise between regions are compared. Grades lower than 0.05 were considered significantly different. Furthermore, the relationship among the concentrations of Pb, Cd and Hg in the larval body of *Cheumatopsyche* sp. on MT levels was determined using multiple regression. Variable Y is MT content, X1 is Pb content, X2 is Cd content, and X3 is Hg content. Data analysis was performed using SPSS version 22.

RESULTS AND DISCUSSION

Water quality parameters

Environmental parameter data at each station is presented in Table 1. This study shows that most physicochemical parameters fluctuate in all sampling stations. The type of riffle flow allows oxygen diffusion so that the DO at all stations is >7.5 mg/L. DO is classified as good with a threshold value that is in the minimum limit stated in the Regulation of Indonesia Government No. 22 of 2021. The river water temperature ranges from of 23.67 to 31°C, and the pH is 6.6. The temperature impacts the solubility of heavy metals. An increase in water temperature causes an increase in the solubility of heavy metals (Mo et al. 2013). When the pH of the waters is high, heavy metals in seawater will settle to the bottom (Yona et al. 2018) and potentially be uptake by benthic organisms. At least this difference can provide a little picture regarding differences in metal concentrations in sediment, larvae and MT levels.

Sediment heavy metal content

The concentrations of heavy metals (Pb, Cd and Hg) observed at five stations can be seen in Figure 3. The average concentration of Pb is higher than that of Cd and Hg.

Based on Figure 1, the highest concentrations of Pb and Cd are in St3 with values of 44.2 ppm and 0.147 ppm, respectively, while the highest concentration of Hg is in St2 with a value of 0.213 ppm. Both Pb, Cd and Hg had the lowest concentrations at St1 with values of 24.1, 0.071 and 0.145 ppm, respectively Cadmium (Cd) became the heavy metal with the lowest average concentration during sampling. Pb generally has the highest average concentration value of 33.6 ppm, followed by Hg (0.17 ppm) and Cd (0.125 ppm). The Pb concentration at St2-St5 in this study was higher than the standard quality value OSQG LEL (Ontario Sediment Quality Guidelines-Lowest Effect Level) and CCME TEL (Canadian Council of Ministers of the Environment-Threshold Effect Level). Still, it did not exceed the standard value of ANZECC (Australian and New Zealand Environment and Conservation Council). The concentration of Hg has exceeded the quality standard values of ANZECC (at St2, St3), CCME TEL (in all stations), and OSQG LEL (at St2). At the same time, the concentration of Cd is still under the quality standards of the three standards. The total measured metal concentration in the sediment per area is shown in Figure 4.

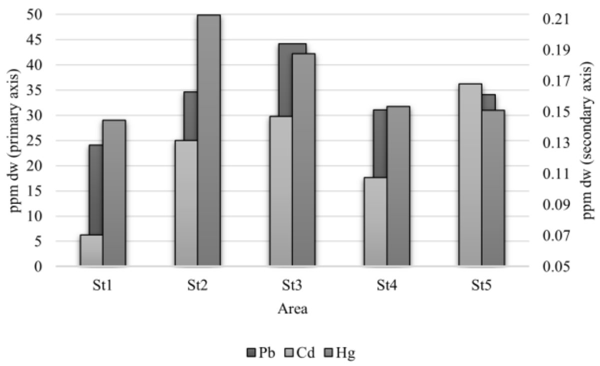

Figure 3. Measured concentrations of heavy metals, expressed in ppm dry weight (dw), in the Bone River sediment. Limits based on ANZECC, OSQG, CCME for Pb (primary axis) respectively: 50, 31, 30.2, for Cd and Hg (secondary axis) respectively: 0.6 and 0.7; 0.2 and 0.13 ppm

Table 1. Environmental parameter data at each station.

Parameters	Study areas									
	St1		St2		St3		St4		St5	
	Mean	(sd)	Mean	(sd)	Mean	(sd)	Mean	(sd)	Mean	(sd)
Temperature (°C)	23.67	0.06	28.67	0.99	31.00	0.75	29.10	0.36	29.57	0.15
DO (ppm)	7.84	0.01	7.63	0.11	7.62	0.12	7.70	0.08	7.52	0.01
pH	6.67	0.01	6.64	0.06	6.63	0.06	6.67	0.06	6.68	0.07

Figure 2. *Cheumatopsyche* sp. (Class Insecta, Order Trichoptera, Family Hydropsychidae, Genus Cheumatopsyche) collected in Bone River, Gorontalo, Indonesia. A. lateral view of the right side; B. genus identifier (black arrow)

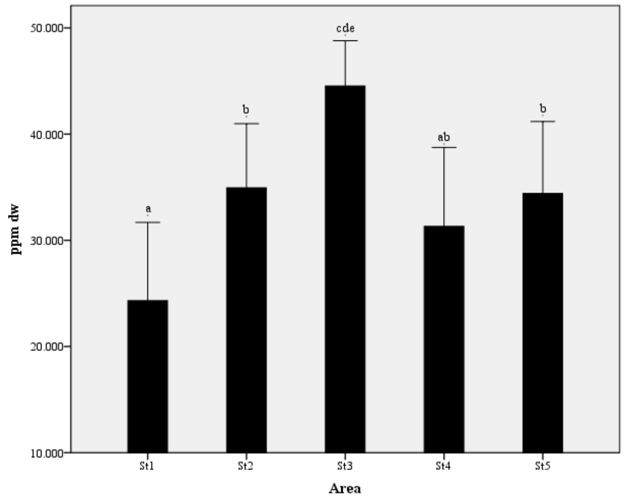

Figure 4. The concentration of total metals (Pb, Cd and Hg) in the sediment in the five sampling areas. Letters (a-d) indicate a significant difference ($p<0.05$) between areas

The measurement outcomes show a contamination gradient along the study area, increasing from St1 to St3, then decreasing at St4 and increasing again at St5. These results also reveal that the respective metal proportions in all areas are maintained. Statistical analyses performed on total metal concentrations in sediments revealed significant differences between study stations (Figure 4), refuting the null hypothesis of no significant difference in contamination between sediments from different areas. Significant differences ($p<0.05$) among stations are indicated by different letters (a-d). Table 2 below presents the value of the contamination factor and the geoaccumulation index of Pb, Cd and Hg metals.

Lead (Pb) has the highest average contamination factor (CF) and geoaccumulation index (I_geo), i.e., 0.805 and 0.337, respectively, followed by Hg (Cf: 0.322; I_geo: 0.085) and Cd (Cf: 0.25; I_geo: 0.084). Interpretation of CF value refers to Hakanson (1980) in Liao et al. (2022), where if the value of CF<1 means the level of contamination is low. If the value of 1≤CF<3, the level of contamination is moderate; if the value of 3≤CF<6 means the level of contamination is high; the value of CF>6 means the level of contamination is very high. The mean value of CF Pb, Cd and Hg shows a number smaller than 1 (CF<1). This means that the sediment at all observation stations is in the category with low contamination levels.

I_geo value interpretation refers to 7 classes Muller (1979) in Edward (2020), i.e., class 0 (not polluted) value I_geo<0; class 1 (not contaminated to moderately contaminated) value 0<I_geo<1; class 2 (medium polluted) value 1<I_geo<2; class 3 (moderate to heavily polluted) value 2<I_geo<3; class 4 (sufficiently polluted) value 3<I_geo<4; class 5 (severely polluted) value 4<I_geo<5; and grade 6 (extremely polluted) the value of I_geo>5. The mean value of I_geo Pb, Cd and Hg in this study was greater than 0 but less than 1 (0<I_geo<1) or included in class 1, meaning that the sediment at all observation stations was categorized as lightly polluted.

Heavy metal analysis in *Cheumatopsyche* sp.

Heavy metal concentrations (Pb, Cd and Hg) in this study were measured in all body parts of the larvae of *Cheumatopsyche* sp. found at each observation station. The measurement results are presented in Table 3.

Table 2. Contamination factor value and metal geoaccumulation index Pb, Cd and Hg

Station	CF-Pb	CF-Cd	CF-Hg	igeo-Pb	igeo-Cd	igeo-Hg
1	0.578	0.141	0.274	0.242	0.047	0.072
2	0.829	0.263	0.402	0.347	0.088	0.107
3	1.059	0.294	0.355	0.444	0.098	0.094
4	0.744	0.215	0.291	0.312	0.072	0.077
5	0.817	0.336	0.286	0.342	0.112	0.076
Mean	0.805	0.250	0.322	0.337	0.084	0.085

Table 3. Heavy metal concentrations (Pb, Cd and Hg) of the larvae of *Cheumatopsyche* sp.

Station	*Cheumatopsyche* (ppm)			BCF		
	Pb	Cd	Hg	Pb	Cd	Hg
1	0.209	0.082	0.228	0.009	1.156	1.574
2	0.326	0.262	0.461	0.009	1.989	2.169
3	0.446	0.392	0.479	0.010	2.663	2.552
4	0.352	0.255	0.448	0.011	2.367	2.915
5	0.356	0.352	0.365	0.010	2.095	2.414
Mean	0.338	0.268	0.396	0.010	2.054	2.325
Limits*/BCF Category	0.2	0.1	0.5	low	low	low

*BPOM No.5 in year 2018

Hydropsychidae, in general, has started to be applied as bioindicator candidates to identify heavy metal concentrations in rivers. Several studies have reported the use of this family as a pollutant monitoring tool, especially heavy metals Tszydel et al. (2016, 2021) and (Rubio-Gracia et al. 2022). Table 3 shows that heavy metals such as Pb, Cd and Hg accumulated in the body of *Cheumatopsyche* sp. The highest heavy metal values in the larvae of *Cheumatopsyche* sp. were all obtained at St3, with a concentration of 0.479 ppm Hg, followed by Pb (0.446 ppm) and Cd (0.392 ppm). The total accumulation of metal concentrations measured in all body parts of the larvae of *Cheumatopsyche* sp. along the study area is presented in Figure 4. In *Cheumatopsyche* larvae, metal accumulation was lower in the less contaminated area (St1) and higher in St3, then decreased in St4 and increased again in St5. The level of metal concentration was measured in the larvae of *Cheumatopsyche* sp. not significantly different between St2 and St4 (Figure 5).

The results revealed that the metal concentration throughout the body reflected the metal concentration observed in the sediment (Figures 4 and 5). The correlation between the metal concentrations of Pb, Cd and Hg in the sediment with the total body load of the analyzed larval samples is presented in Table 4. For all tested correlations, the p-value was always lower than 0.05, meaning that all correlations were statistically significant. Furthermore, multiple linear regression analysis showed that the concentration of heavy metals in sediments also had a significant ($p<0.05$) on total body load with an effect of 91% and resulted in the equation $Y= 0.027 + 0.011(X1) + 1.128(X2) + 0.659(X3)$. This equation shows that if there is an increase in Pb (X1) of one ppm, it will increase the bioconcentration of larvae by 0.011 ppm. Similarly, suppose there is an increase in Cd (X2) level by one ppm. In that case, it will increase the bioconcentration of larvae by 1.128 ppm and an increase in Hg (X3) levels by one ppm will increase the bioconcentration of larvae to about 0.659 ppm.

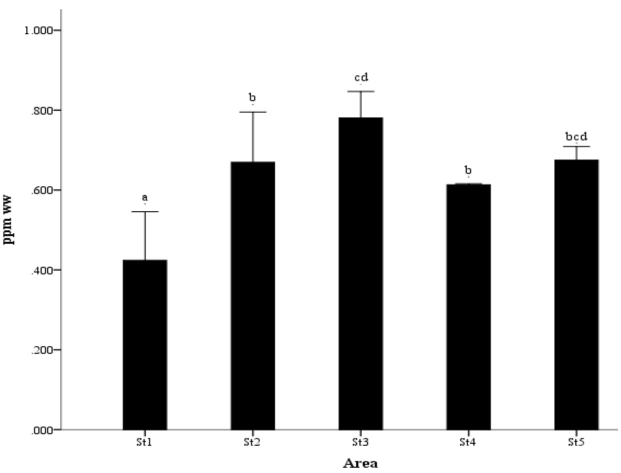

Figure 5. Total metals (Pb, Cd, Hg) concentration in whole-body of Larvae *Cheumatopsyche* sp. Letters (a-d) indicate a significant difference ($p<0.05$) between areas

In this study, measurements of the bioconcentration factor (BCF) were carried out. The value of this factor can be used to express the level of bioconcentration by calculating the toxin concentration in an organism with levels in the surrounding environment that will produce a ratio (van der Oost et al. 2003). The higher the ratio, the more intense the toxin bioconcentration. In this case, the higher the metal content in the biota, the higher the BCF value, the higher the organisms that accumulate heavy metals (Rahayu et al. 2020). The BCF calculation results show that mercury has a higher bioconcentration factor value (Table 3). This indicates that the larvae of *Cheumatopsyche* sp. more effective in accumulating Hg than Pb and Cd. In general, BCF values indicate that the larvae of *Cheumatopsyche* sp. are in the category of low accumulative (BCF<100).

The measurement results also showed that the concentration of Pb and Cd accumulated in the body of *Cheumatopsyche* sp. value had exceeded the number stipulated by Indonesian Food and Drug Authority or BPOM Regulation No. 5 of 2018. On the other hand, although Hg accumulates in the larval body, the concentration value still does not exceed the set figure. The measured concentration values, both in sediments and in larvae, indicate fluctuations due to differences in the characteristics of the waters of each observation station, both physical and chemical properties (Edward 2020; Yang et al. 2020), as well as the ability of *Cheumatopsyche* sp. in accumulating every kind of metal (Tszydel et al. 2016). In this study, the metal concentration in the sediment increased from St1 to St3. Furthermore, the gradient decreased at St4 and increased again at St5. The same pattern occurred in the concentration of metals in the larval body. In contrast to Pb and Cd, the Hg gradient pattern on St3-St5 in the sediment and in the larvae decreased. This gradient pattern may only indicate the degree of bioavailability, not metal concentrations in sediment. Although the concentration of Pb in the sediment was much higher than that of Hg and Cd, the concentration of Pb was only able to accumulate about 1% in the body. On the other hand, the concentration of Hg and Cd in the sediment accumulates about 2× in the body of the biota.

MT levels in *Cheumatopsyche* sp.

MT levels were measured using the ELISA method on all parts of the body of *Cheumatopsyche* sp. The measurement results can be seen in Figure 6. In this study, although the distribution of the measured metal concentration values in the sediment and in the body of *Cheumatopsyche* sp., measured using the AAS method, showed fluctuations, also the level of sediment CF and larval BCF was low, but the MT levels measured at each station showed an almost similar pattern. The MT content gradient of the samples obtained increased from St1 to St3, then decreased from St4 to St5. The MT level at St1, the reference site, has the lowest value of 4637 $ng.g^{-1}$. These results indicate that MT can still be a good biomarker for metal contamination in these larvae under low environmental exposure. A study by Freitas et al. (2012) reported the same in macroinvertebrate species, *Diopatra neapolitana* and *Cerastoderma edule*. MT can reflect the level of metal bioaccumulation in various species of benthic organisms.

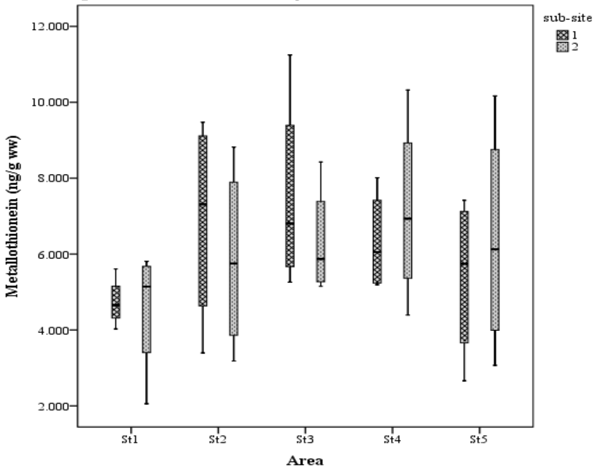

Figure 6. Metallothioneins expressed in ng.g^{-1} ww, in whole-body of larvae of *Cheumatopsyche* sp.

Metal binding protein (MT) expression in each individual or species to respond to the presence of heavy metal pollution is different, depending on the species, organ, and age (Amiard et al. 2006). In the present work, the level of MT levels in the whole body of *Cheumatopsyche* sp. showed a relatively lower value when compared to other macroinvertebrate groups such as clams (Hertika et al. 2018), Bivalvia and Polichaeta (Freitas et al. 2012) but still higher than tubifex (Widiastuti et al. 2019). Girgis et al. (2019) state that differences in metallothionein levels in body tissues of aquatic biota contaminated with heavy metals were also influenced by an increase in the amount of metallothionein mRNA in tissues exposed to heavy metals in response to different heavy metal flow rates in the tissues.

River organic matter and heavy metals can accumulate in the body of Trichoptera larvae (Let et al. 2022), and in *Cheumatopsyche* sp. can accumulate in the digestive organs and cause tissue damage which is indicated by a change in the surface area of the lumen (Yoga 2014). Heavy metals can impact aquatic organisms' physiology (Kadim and Arfiati 2022). Changes in the surface area of the lumen in the tissue are a mechanism to deal with the presence of large amounts of heavy metals and how to get rid of them through the process of cell decay (Morgan et al. 2002). Heavy metal detoxification was performed by synthesizing MT as a form of self-defense and maintaining body homeostasis (Kemp et al. 2017). Hertika et al. (2018) reported a positive correlation among the levels of MT with metals Pb, Cd and Hg. Heavy metal pollutants cause systemic damage to organisms and induce MT production. When heavy metals accumulate in the body, the synthesis of MT can reach its maximum level. Thus, this biomarker can monitor environmental pollution by heavy metals.

The relationship between the content of heavy metals (Pb, Cd and Hg) with the content of MT (quantitative) in the larvae of *Cheumatopsyche* sp.

As mentioned in the previous discussion, the contamination factors of Pb, Cd, Hg in the sediment and the bioconcentration factor of *Cheumatopsyche* sp. are still in the low category. However, the concentration can still induce metallothionein synthesis in body tissues, so MT levels are detected at the measurement time. This corresponds to Dewi et al. (2015), stating that low, concentrations of metals (such as Cd, Hg, or Pb) have a relatively high ability to induce metallothionein as a detoxification mechanism, preventing metal accumulation in the body. The present work showed that, in areas contaminated with high heavy metals, the larvae of *Cheumatopsyche* sp. showed an increase in MT content in counter to higher levels of metal contamination. This revealed that the MT content within these larvae reflects the level of metal accumulation in the organism.

Heavy metal concentrations have a significant relationship with MT levels. Amiard et al. (2006) stated that regression analysis could be used to identify contributing parameters of MT, such as contaminants, heavy metals, and natural factors. The results of the statistical analysis showed that the concentration of heavy metals in the tissues of *Cheumatopsyche* sp. larvae has a significant effect ($p>0.05$) on MT levels (Table 4), so multiple linear regression analysis was performed. The test results showed a significant value ($p<0.05$), this indicates that the concentration of Pb, Cd and Hg in the larval body influences MT levels with the resulting equation $Y= 1.795 + 6.963 (X1) – 2.813 (X2) + 6.874 (X3)$. This equation shows that an increase in Pb (X1) of one ppm increased levels of MT by 6963 ng.g^{-1} and an increase in Hg (X3) levels of 1 ppm would increase levels of MT to around 6874 ng.g^{-1}. On the other hand, an increase in Cd (X2) levels by one ppm will reduce MT levels by 3813 ng.g^{-1}. In previous studies, the levels of MT in the tissues of the aquatic invertebrates *Crassostrea iredalei* and *Crassostrea glomerata* showed a significant association with the concentrations of Cd, Hg and Pb metals (Hertika et al. 2018). Metallothionein binds dissolved metals in the tissue so that biologically the correlation between MT and metals must be higher to dissolve, preventing the accumulation (Freitas et al. 2012). Thus, the obtained association between MT and metals in the larval tissues of *Cheumatopsyche* sp. is biologically meaningful.

Table 4. Heavy metal concentrations (Pb, Cd and Hg) of the larvae of *Cheumatopsyche* sp.

Sampel	*Cheumatopsyche* sp.				
	Pb	Cd	Hg	tma_ Cheu*	MT
Sedimen					
Pb	0.867	-	-	0.912	0.673
Cd	-	0.834	-	0.803	ns**
Hg	-	-	0.631	0.595	ns**
Cheumatopsyche sp.					

Pb	-	-	-	-	0.851
Cd	-	-	-	-	0.738
Hg	-	-	-	-	0.915

Note: *Total metal accumulation in *Cheumatopsyche* sp.; **not significant

The use of biomarkers in evaluating pollution in waters has begun to be developed in aquatic organisms. One of them is using specific biomarkers such as MT to determine the level of heavy metal police. MT is considered a potential biomarker and has been found and quantified in the tissues of several aquatic organisms. The metal binding protein (MT) accumulation in the tissue at the measurement time can indicate heavy metal contamination.

Previous works reported that MT could be induced by essential metals Cu and Zn and non-essential metals Cd, Ag and Hg in vertebrates and invertebrates, but their induction varies. These variations are intraspecific and interspecific and are caused by various environmental and physiological reasons. MT is central to metabolic management and detoxification of non-essential Cd and Hg metals in excess intracellularly (Amiard et al. 2006). Therefore, MTs can be used as biomarkers in a well-designed environmental monitoring program (Hook et al. 2014; Hertika et al. 2021; Kadim and Risjani 2022). Based on this study, heavy metals in the sediment affect the MT levels in the larvae of *Cheumatopsyche* sp. so that it can be used as a good candidate for the investigation of heavy metal pollutant levels through the detection of MT levels.

ACKNOWLEDGEMENTS

We would like to thank the Ministry of Education, Culture, Research, and Technology, Indonesia for funding this research through a doctoral dissertation proposal grant scheme 2022.

REFERENCES

Amiard JC, Amiard-Triquet C, Barka S, Pellerin J, Rainbow PS. 2006. Metallothioneins in aquatic invertebrates: Their role in metal detoxification and their use as biomarkers. Aquat Toxicol 76 (2): 160-202. DOI: 10.1016/j.aquatox.2005.08.015.

Barbieri MV, Peris A, Postigo C, Moya-Garcés A, Monllor-Alcaraz LS, Rambla-Alegre ME, de Alda ML. 2020. Evaluation of the occurrence and fate of pesticides in a typical Mediterranean delta ecosystem (Ebro River Delta) and risk assessment for aquatic organisms. Environ Pollut 274: 115813. DOI: 10.1016/j.envpol.2020.115813.

Bere T, Dalu T, Mwedzi T. 2016. Detecting the impact of heavy metal contaminated sediment on benthic macroinvertebrate communities in tropical streams. Sci Total Environ 572: 147-156. DOI: 10.1016/j.scitotenv.2016.07.204.

Dewi NK, Purwanto, Sunoko HR. 2015. Metallothionein in the fish liver as biomarker of cadmium (Cd) pollution in Kaligarang River Semarang. J People Environ 21 (3): 304-309. DOI: 10.22146/jml.18557.

Edward E. 2020. Penilaian pencemaran logam berat dalam sedimen di Teluk Jakarta. Depik 9 (3): 403-410. DOI: 10.13170/depik.9.3.16800. [Indonesia]

Everall NC, Johnson MF, Wood P, Paisley MF, Trigg DJ, Farmer A. 2019. Macroinvertebrate community structure as an indicator of phosphorus enrichment in rivers. Ecol Indic 107: 105619. DOI: 10.1016/j.ecolind.2019.105619.

Freitas R, Costa E, Velez C, Santos J, Lima A, Oliveira C, Figueira E. 2012. Looking for suitable biomarkers in benthic macroinvertebrates inhabiting coastal areas with low metal contamination: Comparison between the bivalve *Cerastoderma edule* and the Polychaete *Diopatra neapolitana*. Ecotoxicol Environ Saf 75 (1): 109-118. DOI: 10.1016/j.ecoenv.2011.08.019.

Fuoco R, Giannarelli S. 2019. Integrity of aquatic ecosystems: An overview of a message from the South Pole on the level of persistent organic pollutants (POPs). Microchem J 148: 230-239. DOI: 10.1016/j.microc.2019.04.076.

Girgis SM, Mabrouk DM, Hanna MI, Abd-ElRaouf A. 2019. Seasonal assessment of some heavy metal pollution and Metallothionein gene expression in cultured *Oreochromis niloticus*. Bull Natl Res Cent 431 (43): 1-8. DOI: 10.1186/S42269-019-0167-X.

Hertika AMS, Kusriani K, Indrayani E, Nurdiani R, Putra RBDS. 2018. Relationship between levels of the heavy metals lead, cadmium and mercury, and metallothionein in the gills and stomach of *Crassostrea iredalei* and *Crassostrea glomerata*. F1000 Res 7: 1239. DOI: 10.12688/f1000research.14861.1.

Hertika AMS, Kusriani K, Indrayani E, Putra RBDS. 2021. Density and intensity of metallothionein of *Crassostrea* sp. as biomarkers of heavy metal contamination in the Northern coast of East Java, Indonesia. Egypt J Aquat Res 47: 109-116. DOI: 10.1016/j.ejar.2021.04.006.

Hertika AMS, Kusriani K, Indrayani E, Yona D, Putra RBDS. 2020. Metallothionein expression on oysters (*Crassostrea cuculata* and *Crassostrea glomerata*) from the southern coastal region of East Java. F1000 Res 8: 56. DOI: 10.12688/f1000research.17381.1.

Hook SE, Gallagher EP, Batley GE. 2014. The role of biomarkers in the assessment of aquatic ecosystem health. Integr Environ Assess Manag 10 (3): 327-341. DOI: 10.1002/ieam.1530.

Islam MS, Ahmed MK, Raknuzzaman M, Habibullah AM, Islam MK. 2015. Heavy metal pollution in surface water and sediment: A preliminary assessment of an urban river in a developing country. Ecol Indic 48: 282-291. DOI: 10.1016/j.ecolind.2014.08.016.

Kadim MK, Arfiati D. 2022. Effects of pollutants on physiological of river macroinvertebrates: A review. Environ Sci 18 (1): 65-76. DOI: 10.20527/es.v18i1.12980.

Kadim MK, Risjani Y. 2022. Biomarker for monitoring heavy metal pollution in aquatic environment: An overview toward molecular perspectives. Emerg Contam 8: 195-205. DOI: 10.1016/J.EMCON.2022.02.003.

Kemp M, Wepener V, de Kock KN, Wolmarans CT. 2017. Metallothionein induction as indicator of low level metal exposure to aquatic macroinvertebrates from a relatively unimpacted river system in South Africa. Bull Environ Contam Toxicol 99 (6): 662-667. DOI: 10.1007/S00128-017-2201-8.

Koniyo Y. 2020. Analisis kualitas air pada lokasi budidaya ikan air tawar di Kecamatan Suwawa Tengah. J Technopreneur 8 (1): 52-58. DOI: 10.30869/jtech.v8i1.527. [Indonesia]

Let M, Černý J, Nováková P, Ložek F, Bláha M. 2022. Effects of trace metals and municipal wastewater on the Ephemeroptera, Plecoptera, and Trichoptera of a stream community. Biology 11 (5): 648. DOI: 10.3390/BIOLOGY11050648.

Liao J, Cui X, Feng H, Yan S. 2022. Environmental background values and ecological risk assessment of heavy metals in watershed sediments: A comparison of assessment methods. Water 14 (1): 51. DOI: 10.3390/W14010051/S.

Lihawa F, Mahmud M. 2012. Sebaran Spasial dan Temporal Kandungan Merkuri Pada Lokasi Pertambangan Emas Tradisional. Pusat Studi Lingkungan Hidup dan Kependudukan, Universitas Negeri Gorontalo, Gorontalo. [Indonesia]

Liu J, Liu YJ, Liu Y, Liu Z, Zhang AN. 2018. Quantitative contributions of the major sources of heavy metals in soils to ecosystem and human health risks: A case study of Yulin, China. Ecotoxicol Environ Saf 164: 261-269. DOI: 10.1016/j.ecoenv.2018.08.030.

Mo HH, Yoo D, Bae YJ, Cho K. 2013. Effects of water temperature on development and heavy metal toxicity change in two midge species of *Chironomus riparius* and *C. yoshimatsui* in an era of rapid climate change. Entomol Res 43 (2): 123-129. DOI: 10.1111/1748-5967.12014.

Morgan AJ, Turner MP, Morgan JE. 2002. Morphological plasticity in metal-sequestering earthworm chloragocytes: Morphometric electron microscopy provides a biomarker of exposure in field populations. Environ Toxicol Chem 21 (3): 610-618. DOI: 10.1002/ETC.5620210321.

Oremo J, Orata F, Owino J, Shivoga W. 2019. Assessment of heavy metals in benthic macroinvertebrates, water and sediments in River Isiukhu, Kenya. Environ Monit Assess 191 (11): 646. DOI: 10.1007/s10661-019-7858-5.

Ouma KO, Otieno SA, Sharma RR. 2019. Effects of selected heavy metals on morphology of *Oreochromis niloticus* and *Clarias gariepinus* along Ruiru River, Kenya. Bonorowo Wetl 9: 92-107. DOI: 10.13057/bonorowo/w090204.

Pateda SM, Arifin YI, Kasim VN. 2017. Mapping of health disorders related to mercury on community around the Bone River, Gorontalo Province. Intl J Sci Basic Appl Res 36 (4): 83-93.

Pebriani MA, Barus TA, Syafruddin I. 2022. Fish diversity and heavy metal accumulation of Pb, Cu and Zn after Mount Sinabung Eruption in Benuken River, North Sumatra, Indonesia. Biodiversitas 23 (1): 187-194. DOI: 10.13057/biodiv/d230124.

Rahayu DR, Anggoro S, Soeprobowati TR. 2020. Metal concentrations and bio-concentration factor (BCF) in surface water and economical fish species from Wadaslintang Multipurpose Dam, Wonosobo, Indonesia. IOP Conf Ser Earth Environ Sci 593: 012024. DOI: 10.1088/1755-1315/593/1/012024.

Rubio-Gracia F, Argudo M, Zamora L, Clements WH, Vila-Gispert A, Casals F, Guasch H. 2022. Response of stream ecosystem structure to heavy metal pollution: Context-dependency of top-down control by fish. Aquat Sci 84 (2): 1-17. DOI: 10.1007/S00027-022-00849-4.

Ryan SC, Belby C, King-Heiden TC, Haro RJ, Ogorek J, Gerrish GA. 2018. The role of macroinvertebrates in the distribution of lead (Pb) within an urban marsh ecosystem. Hydrobiol 8271 (827): 337-352. DOI: 10.1007/S10750-018-3785-7.

Tszydel M, Błońska D, Jóźwiak P, Jóźwiak M. 2021. SEM-EDX analysis of heavy metals in anal papillae of *Hydropsyche angustipennis* larvae (Trichoptera, Insecta) as a support for water quality assessment. Eur Zool J 88 (1): 718-730. DOI: 10.1080/24750263.2021.1931490.

Tszydel M, Markowski M, Majecki J. 2016. Larvae of *Hydropsyche angustipennis* (Trichoptera, Hydropsychidae) as indicators of stream contamination by heavy metals in Łódź agglomeration. Zootaxa 4138 (1): 127-138. DOI: 10.11646/zootaxa.4138.1.5.

van der Oost R, Beyer J, Vermeulen NPE. 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: A review. Environ Toxicol Pharmacol 13 (2): 57-149. DOI: 10.1016/S1382-6689(02)00126-6.

Widiastuti IM, Hertika AMS, Musa M, Arfiati D. 2019. Mercury absorption in *Tubifex* Sp. worm contaminated with metal washing waste. Pollut Res 38 (3): 575-583.

Widiastuti IM, Hertika S, Musa M, Arfiati D. 2019. Superdioxide dismutase (SOD) and metallothionein (MT) *Tubifex tubifex* at the acute mercury exposure. IOP Conf Ser Earth Environ Sci 236 (1): 012101. DOI: 10.1088/1755-1315/236/1/012101.

Wong DL, Stillman MJ. 2020. Metallothioneins, in Reference Module in Chemistry, Molecular Sciences and Chemical Engineering. Elsevier, Netherlands.

Yang HJ, Jeong HJ, Bong KM, Kang TW, Ryu HS, Han JH, Na EH. 2020. Organic matter and heavy metal in river sediments of southwestern coastal Korea: Spatial distributions, pollution, and ecological risk assessment. Mar Pollut Bull 159: 111466. DOI: 10.1016/j.marpolbul.2020.111466.

Yoga GP. 2014. Kerusakan Jaringan dan Produksi Sekunder Larva Trichoptera pada Perairan yang Tercemar Merkuri. [Distertation]. Institut Pertanian Bogor, Bogor. [Indonesian]

Yona D, Arif ZFM, Hidayati N. 2018. Spatial distribution of heavy metals in the surface sediments of the Southern Coast of Pacitan, Indonesia. Indones J Chem 18 (1): 137-144. DOI: 10.22146/IJC.22400.

Zhuang Q, Li G, Liu Z. 2018. Distribution, source and pollution level of heavy metals in river sediments from South China. Catena 170: 386-396. DOI: 10.1016/j.catena.2018.06.037.